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방사선과 화학물질의 상호작용에 의한 생체영향 연구

Efficiency of Interaction between Various Radiation and Chemicals

주관연구기관

한국원자력연구소

공동연구기관

폴란드핵물리연구소

과 학 기 술 부

제 출 문

과학기술부 장관 귀하

본 보고서를 "방사선과 화학물질의 상호작용에 의한 생체영향 연구"의 보고서로 제출합니다.

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주관연구기관명 : 한국원자력연구소 (KAERI, Korea)

주관연구책임자 : 김 진 규 (Jin Kyu Kim)

연 구 원 : 송 희 섭 (Hi Sup Song)

연 구 원 : 김 재 성 (Jae Sung Kim)

연 구 원 : 천 기 정 (Ki-Jung Chun)

연 구 원 : 이 영 근 (Young-Keun Lee)

연 구 원 : 이 병 헌 (Byoung Hun Lee)

연 구 조 원 : 신 해 식 (Hae Shik Shin)

연 구 조 원 : 이 경 희 (Kyoung Hee Lee)

초 빙 연 구 원 : 블라디슬라프 페틴 (Vladislav G. Petin)

공동연구기관명 : 폴란드핵물리연구소 (INP, Poland)

공동연구책임자 : 안토니나 체불스카-바실레브스카
(Antonina Cebulska-Wasilewska)

요 약 문

Ⅰ. 제 목

방사선과 화학물질의 상호작용에 의한 생체영향 연구

Ⅱ. 연구개발의 목적 및 필요성

폴란드핵물리연구소는 미국, 일본, 유럽 등지의 원자력 선진국들과 강한 협력체제를 유지하고 있을 뿐 아니라 방사선생물학 분야에 관한 오랜 연구경험을 보유하고 있다. 따라서 한국원자력연구소와 폴란드핵물리연구소 간의 공동연구를 통하여 선험적 연구기술을 공유하고 공동으로 개선·발전시키는 노력을 기울이는 한편 방사선생물학에 관련된 응용 및 첨단 연구기술을 선진화함에 연구목적을 두고 있다.

특히 폴란드핵물리연구소가 오랜 경험을 가지고 있는 방사선지표생물 연구분야의 연계실험으로 상호보유기술을 검증하고 협력체제를 확립하였다. 두 기관이 각각 보유한 방사선 조사시설 및 연구시설과 인력을 활용한 상호보완적 연구를 수행하는 것은 양 기관에 도움이 되었다.

Ⅲ. 연구개발의 내용 및 범위

본 국제 공동연구에는 다음과 같은 연구개발 수행 내용이 포함되었다.

1. 방사선 및 화학물질의 영향 분석을 위한 TSH 생물지표 응용
2. 중성자와 붕소화합물이 TSH 돌연변이에 미치는 영향
3. 방사선에 의해 유발되는 TSH 돌연변이에 대한 살충제의 효과
4. 감마선 및 살충제가 사람 림프구 손상에 미치는 영향
5. 사람 림프구 DNA에 미치는 방사선과 B 및 Gd 화합물의 영향
6. 복합적 상승작용에 관한 수학적 해석
7. 상승작용에 관한 일반 규칙성 해석
8. 방사선 및 화학물질의 상승작용에 있어서 결정인자 분석

Ⅳ. 연구개발 결과

1. 방사선 및 화학물질의 영향 분석을 위한 TSH 생물지표 응용

한국원자력연구소의 X-선, ^{252}Cf 선원 그리고 폴란드 선원의 중성자를 조사하여 *Tradescantia* 4430 클론의 수술털 세포에 유발되는 유전자 돌연변이, 치사돌연

변이 및 세포주기인자의 선량-반응 관계를 분석하였다. 본 공동실험은 한국원자력연구소와 폴란드핵물리연구소의 실험조건하에서 자주달개비 TSH 세포를 대상으로 보론화합물 처리에 따른 ^{252}Cf 중성자의 상대생물효과비 (RBE)를 규명할 수 있는지 여부를 가리기 위한 파일로트 규모의 대전-크라쿠프 연계실험을 실시하였다.

2. 중성자와 붕소화합물이 TSH 돌연변이에 미치는 영향

Tradecantia 4430 클론의 수술털 세포에서 중성자에 의해 유발되는 각종 돌연변이를 통하여 붕소화합물에 의한 방사선생물효과 증감여부를 분석하였다. 정상화서 및 붕소화합물이 전처리 화서를 공기중에서 ^{252}Cf 중성자를 0~0.2 Gy 조사한 다음 유전자돌연변이와 치사돌연변이를 분석하였다. 중성자의 상대생물효과비 (RBE) 산정을 위한 기준자료는 정상화서에 X선 0~0.5 Gy 조사한 실험군의 분석값을 사용하였다. 정상세포의 유전자돌연변이 유발에 있어서 중성자의 최대 RBE는 7.2로 나타났다. 붕소화합물을 전처리에 의해 중성자에 의한 치사돌연변이 유발의 RBE 최대값은 6.2에서 34.3로 증가되었으며 자사세포 유발의 RBE 최대값은 1.6에서 5.6으로 각각 증가하였다. 본 결과를 통하여 세포에 붕소화합물을 전처리할 경우 치사돌연변이 유발에 대한 중성자의 방사선생물효율을 크게 증대시킬 수 있음이 실험적으로 입증되었다.

3. 방사선에 의해 유발되는 TSH 돌연변이에 대한 살충제의 효과

살충제와 방사선이 자주달개비 수술털 돌연변이에 미치는 복합적 영향을 평가하였다. 포트에서 생육된 *Tradescantia* 4430 식물체에 코발트 선원으로부터 0.3, 0.5, 1.0 및 2.0 Gy의 감마선을 조사하였다. 살충제는 감마선 조사 24시간 전에 농업용으로 사용되는 파라치온 유제를 살포하였다. CT 실험군과 Pa+γ 실험군에 있어서의 돌연변이 빈도가 증가한 고조기간은 방사선 조사 후 7~11일로 돌연변이 빈도는 방사선량 증가에 따라 선형적으로 증가하는 반응관계를 보였다. CT 실험군에서의 선량반응식 기울기 (증가계수)는 5.99 (r^2=0.988)이었으나, Pa+γ 실험군의 증가계수는 3.43 (r^2=0.981)으로 CT 실험군의 값에 비해 현격히 낮았다. 즉 Pa+γ 실험군이 CT 조사군에 비하여 방사선에 의한 돌연변이 유발 증가율이 43% 억제됨을 확인할 수 있었으며 이는 살충제 전처리가 방사선에 의한 세포유전물질 손상을 오히려 저감시키는 효과를 나타낸 것이기 때문이다. 이 같은 실험결과는 두 가지 불리한 자극이 복합적으로 작용할 때 나타나는 일반적인 상승작용과는 상반되는 결과이며 오히려 파라치온 전처리에 의해 식물체가 보다 강한 자극에 대비할 수 있도록 유도되는 적응반응이나 방사선효과에 대한 변화작용이 나타난 것으로 해석된다.

4. 감마선 및 살충제가 사람 림프구 손상에 미치는 영향

병해충을 막기 위해 농업용 살충제가 광범위하게 사용되고 있다. 농약사용에 따른 생물학적 위해가 우려되며 농약이 또 다른 환경유해요인과 인체에 상승적으로 작용할 경우 농업재해로 이어질 수 있다. 다양한 인자에 의한 DNA 손상을 감지하는데 유용한 단세포겔전기영동법 (SCGE)을 이용하여 살충제와 방사선에 의한 사람 림프구 DNA 손상을 평가하였다. 각기 다른 농도로 살충제를 10분간 전처리한 림프구와 정상 림프구에 0~2.0 Gy의 방사선으로 조사한 다음 DNA 손상도를 평가하였다. DNA 가닥 절단에 대한 표식인 tail moment의 증가는 감마선에 대해서 뚜렷한 선량-반응 관계를 나타내었다. SCGE 분석법을 통한 평가결과, 권장 사용농도 이상의 살충제는 림프구에 대한 유전독성을 나타내었을 뿐 아니라 방사선과 함께 상승작용을 일으켜 림프구 DNA 손상을 더욱 증가시키는 것이 확인되었다.

5. 사람 림프구 DNA에 미치는 방사선과 B 및 Gd 화합물의 영향

단세포겔전기영동 분석법을 이용하여 붕소 및 가돌리늄 화합물을 처리한 림프구 DNA의 방사선 반응을 분석하였다. 사람 말초혈액으로부터 분리한 림프구에 붕소 및 가돌리늄 화합물을 10분간 전처리하거나 또는 그대로 ^{60}Co 감마선을 0, 1, 2 및 4 Gy를 조사하고 단세포겔전기영동을 실시하였다. 분석결과 붕소 화합물 (50 nM or 250 nM of ^{10}B)은 림프구 DNA의 방사선민감도를 낮추는 것으로 밝혀졌다. 반면에 가돌리늄 화합물 (50 nM of ^{157}Gd)은 선량증가에 따른 방사선감수성의 증대를 초래했으며, 이 같은 효과는 4 Gy 조사군에서 가장 뚜렷하였다 ($p<0.001$). 또한 보론 화합물과 가돌리늄 화합물을 1 : 1 혼합하여 전처리한 경우도 림프구 DNA의 방사선민감도의 감소가 나타났다.

6. 복합적 상승작용에 관한 수학적 해석

방사선과 고온처리에 의한 생물손상의 상승작용을 설명할 수 있는 수학적 방법론을 설정하기 위한 연구를 수행하였다. 이 해석 방법론은 각개 요인에 의해 유발된 준손상 (sublesion)은 생물체에 치명적이지 않지만 준손상 간의 상호작용에 의하여 부가적인 손상 (lesion)의 유발에 근거하고 있다. 설정된 모델을 이용하여 각 요인에 의해 생성된 치명적 손상 비율에 대한 상승작용의 의존성, 그 최대값 및 조건 그리고 조사 선량률에 대한 상승효과의 의존성 등을 예측할 수 있다. 두 strain의 효모에 대한 파장 254 nm 자외선 및 고온 (45~57.5℃)을 동시에 처리하였을 때 나타나는 실험결과를 이용하여 모델예측 값을 검정한 결과, 본 모델이 타당함이 입

증되었다.

7. 상승작용에 관한 일반 규칙성 해석

이온화 방사선, 자외선, 초음파 및 몇몇 화학물질 등이 고온과 동시에 작용하였을 때 나타나는 상승작용의 규칙성을 도출하였다. 이를 위하여 박테리아, 효모세포를 이용한 실험자료는 물론 바이러스, 박테리아 포자 및 포유동물의 배양세포를 이용한 광범위한 실험결과를 이론적으로 분석하였다. 고온조건이 또 다른 불리한 요인과 함께 작용할 때 생물체가 보이는 반응에 일반적 규칙이 있음이 밝혀졌다. 즉, 각 요소들의 특정 농도 또는 강도에 대하여 최대 상승작용을 구현할 수 있는 특정한 온도범위가 반드시 존재한다는 것이다. 이러한 최적 온도범위를 벗어날 때는 상승효과의 감소가 초래된다. 물리적 요인의 강도나 화학적 요인의 농도가 상승효과에 크게 영향을 주며, 요인의 강도나 농도가 낮을수록 낮은 온도를 사용하는 것이 최대 상승 효과를 유발시킨다.

8. 방사선 및 화학물질의 상승작용에 있어서 결정인자 분석

이온화 방사선과 다른 물리·화학적 요인이 동시에 작용 시의 연구결과들을 통해 물리·화학적 요인의 강도(농도)가 낮을수록 낮은 온도범위에서 최대의 상승효과가 나타난다는 사실이 입증되었다. 이는 생물권에 존재하는 낮은 강도의 유해환경인자들이 상호 복합적으로 작용하여 상승작용을 일으킬 수 있음을 시사한다. 따라서 특정 환경 내에서의 생물학적 위해도 (risk) 평가시 반드시 유해 환경인자들 간의 상승작용이 고려되어야 한다.

Ⅵ. 공동연구결과의 활용계획

KAERI-INP 공동연구를 통하여 방사선생물학, 핵의학, 인간 모니터링, 환경생물학 분야 등에 폭넓게 응용되는 제반 연구기법을 확립하였다. 특히 기관간 연계실험을 수행함으로써 두 기관이 보유한 연구장점을 최대로 활용할 수 있었을 뿐 아니라 한국원자력연구소 보유기술의 수준을 검증할 수 있었다. 본 공동연구를 통해 획득된 연구결과들은 SCI에 등재된 유명학술지에 공동 논문으로 게재될 것이며, 이는 한·폴 간 과학기술분야 관계증진에 크게 기여할 것이다. 또한 TSH 돌연변이 분석기술, SCGE 기법 및 상승작용 해석기술 등은 개선을 거쳐 한국-폴란드간의 호혜적 협력관계 발전을 위한 기술근거로 계속해서 활용될 것이다.

SUMMARY

I. Title of Project

Biological Efficiency of Interaction between Various Radiation and Chemicals

II. Objectives of Research and Division of Role for Cooperation

Korea Atomic Energy Research Institute (KAERI) is aiming to acquire and develop further the technologies in *Tradescantia* assay and the single cell gel electrophoresis assay application to radiation and environmental biology through the cooperation between KAERI and INP since the Henryk Niewodnizcanski Institute of Nuclear Physics (INP, Poland) has the plentiful experiences of research on radiation biology and its related field. Related research facilities and personnels of the two institutes are involved in parallel research and are subject to do, if necessary, joint experiments on major topics.

III. Work Scopes of Research

The cooperative research consisted of the following work scopes.

1. Application of TSH bioindicator for studying the biological efficiency of radiation
2. Relative biological efficiency of californium-252 neutrons in the induction of gene and lethal mutations in TSH cells normal and enriched with boron compound
3. Effect of pesticide on radiation-induced mutations in TSH cells
4. Interaction of radiation with pesticide on DNA damage in human peripheral blood lymphocytes
5. Radiomodifying effect of boron and gadolinium compounds in human peripheral blood lymphocytes
6. Mathematical description of synergistic interactions
7. General regularities of synergistic interactions
8. Determinant of synergistic interaction between radiation, heat and chemicals in cell killing

IV. Results of Research

1. Application of TSH bioindicator for studying the biological efficiency of radiation

The effectiveness of neutrons in the induction of various abnormalities in the *Tradescantia* stamen hair (TSH) normal or boronated cells of the clone heterozygous for the flower color was assessed. Dose response relationships for various endpoints (gene and lethal mutations and cell cycle alterations) in somatic cells of *Tradescantia* plants clone 4430 (T-4430), were established for mixed fast and thermal neutrons from isotopic source of ^{252}Cf source. The effectiveness of neutrons in relation to X-rays in the induction of various abnormalities in the *Tradescantia* stamen hair cells of the clone heterozygous for the flower color was assessed. Dose response relationships for various endpoints in somatic cells of T-4430 and clone 02, were established previously for X-rays, and 5.6 MeV neutrons from U-120 cyclotron in Krakow [1]. This was a pilot experiment to check if it is possible to establish the RBE values for ^{252}Cf irradiated TSH cells, with and without boron ion pretreatment, in conditions of mutual Taejon-Krakow experiment.

2. Relative biological efficiency of californium-252 neutrons in the induction of gene and lethal mutations in TSH cells normal and enriched with boron compound

The effectiveness of neutrons from a californium-252 source in the induction of various abnormalities in the *Tradescantia* clone 4430 stamen hair cells (TSH-assay) were studied. Special attention was paid to check whether any enhancement is visible in effects caused by process of boron neutron capture in the cells enriched with boron ions. Inflorescences, normal or pretreated with chemicals containing boron, were irradiated in the air with neutrons from a ^{252}Cf source at KAERI, Taejon, Korea. Following the culturing according to standard procedures screening of gene and lethal mutations in somatic cells of stamen hairs have been done, and dose response relationships were plotted. To estimate the relative biological effectiveness (RBE) of the beam under the study, numbers of *Tradescantia* inflorescence without chemical

pretreatment were irradiated with various doses of X-rays. The ranges of radiation doses used for neutrons were 0~1.0 Gy and for X-rays 0~0.5 Gy. A visible increase in a maximal RBE values was observed in present studies, which for the induction of gene mutations were estimated as 11.1 comparing the value 5.6 in the studies reported earlier. Presented results showed an increase, statistically significant, in biological efficiency of radiation from the ^{252}Cf source in samples pretreated with boron containing chemicals. Inflorescence pretreated with borax responded to neutrons differently. Although, for the induction of gene mutations no significant difference was observed, though, in case of cell lethality the values of RBE have changed from 5.5 to 34.7 and from 1.6 to 5.6 for two various lethal end points respectively. Comparison was done of two independent experimental studies on alteration of dose-response curves due to changes in the post-exposure treatment of the cuttings.

3. Effect of pesticide on radiation-induced mutations in TSH cells

To investigate the combined effect of radiation and pesticide on *Tradescantia* somatic cell mutations, potted plants of *Tradescantia* 4430 on which parathion had been sprayed evenly 24 hours before irradiation. Radiation doses were 0.3, 0.5, 1.0 and 2.0 Gy of gamma-ray. The plants irradiated only with the gamma-ray radiation were used as control groups (CT). Pink mutation frequency increased linearly proportional to the radiation dose and the peak interval of elevated mutation frequencies appeared during 7~11 days after irradiation in both CT and Pa+γ groups. The slope of dose-response curve in CT was 5.99 (r^2=0.988), while it was 3.43 (r^2=0.981) in Pa+γ. It seemed that parathion pretreatment had a protective effect against radiation-induced cell damages since it decreased the slope value by 43%. It is suggested that an adaptive response or radiomodification could be induced in irradiated stamen hair cells by parathion pretreatment.

4. Interaction of radiation with pesticide on DNA damage in human peripheral blood lymphocytes

Agricultural pesticides may cause certain biological risks since they are widely used to eradicate pests. Agricultural disasters may arise even from the

possibility of their synergistic interaction with other harmful environmental factors. The effect of pesticide on radiation-induced DNA damage in human blood lymphocytes was evaluated by the single cell gel electrophoresis (SCGE) assay. The lymphocytes, with or without pretreatment of the pesticide, were exposed to 0~2.0 Gy of ^{60}Co gamma ray. Significantly increased tail moment, which was a marker of DNA strand breaks in SCGE assay, showed an excellent dose-response relationship. The present study confirms that the pesticide has the cytotoxic effect on lymphocytes and that it shows the synergistic interaction with radiation on DNA damage as well. The results may have a role of providing biological information necessary for the prevention of agricultural disaster.

5. Radiomodifying effect of boron and gadolinium compounds in human peripheral blood lymphocytes

The modification of radioresponse in human lymphocytes pretreated with boron or gadolinium compound was studied by assessing the DNA damage using the single cell gel electrophoresis, the comet assay. The lymphocytes from the human peripheral blood were irradiated with 0, 1, 2 and 4 Gy of gamma rays from a ^{60}Co isotopic source without or with pretreatment of boron or gadolinium compound for 10 minutes at 4℃. Following the processes such as slide preparation, cell lysing, unwinding and electrophoresis, neutralization, staining, and analytic steps, gel electrophoresis was performed. The results show that pretreatment with boron compound (50 nM or 250 nM of ^{10}B) is effective in reducing the radiosensitivity of the lymphocyte DNA. In contrast, pretreatment with gadolinium compound (50 nM of ^{157}Gd) led to a dose-dependent increase in the radiosensitivity, which was the most prominent with a dose of 4 Gy ($p<0.001$). Furthermore, when the lymphocytes were pretreated with a mixture (1:1) of boron (250 nM) and gadolinium (50 nM) compounds, the reduced radiosensitivity was also observed.

6. Mathematical description of synergistic interactions

A new mathematical model for synergistic interaction of lesions produced by ultraviolet (UV) light and high temperature has been proposed. The model suggests that synergism is expected from the additional lethal lesion arising

from the interaction of sublesions induced by both agents. These sublesions are considered noneffective after each agent taken alone. The model predicts the dependence of synergistic interaction of the ratio of lethal lesions produced by every agent applied, the greatest value of the synergistic effect as well as the conditions under which it can be achieved, and the dependence of synergistic effect on UV light fluence rate. These predictions of the model have been tested for simultaneous combined action of UV light (254 nm) and heat (45~57.5℃) on two strains of wild-type diploid yeast cells of *Saccharomyces cerevisiae.* The theory appears to be appropriate and the conclusions valid.

7. General regularities of synergistic interactions

Synergistic interaction of the simultaneous action of hyperthermia with ionizing radiation, ultraviolet light, ultrasound and some chemical agents has been analysed using experimental data obtained by authors with bacterial and yeast cells and published by others for viruses, bacterial spores and cultured mammalian cells. Some general non-trivial rules in responses of these cellular systems to the combined action of hyperthermia with one of the other inactivating agents were revealed. For every constant intensity or concentration, there was a specific temperature that maximizes the synergistic interaction of hyperthermia with another agent employed. Any deviation of temperature from the optimal one resulted in a reduction of the synergy. The intensity of the physical factors or the concentration of chemical agent strongly influenced the synergy: the lesser intensity of the agent applied, the lesser temperature under which the treatment occurred should be used to obtain the highest or some definite value of synergism.

8. Determinant of synergistic interaction between radiation, heat and chemicals in cell killing

Experimental data obtained for simultaneous action of ionizing radiation with different physical or chemical agents on various cellular systems evidence that the lesser the intensity of physical factor or the concentration of chemical agents, the smaller the temperature that has to be used to provide the highest or a definite level of synergistic interaction. On this basis, it is inferred that the synergism may take place at small intensities of harmful environmental factors

existing in the biosphere. Hence, the assessment of health or environmental risks should take into account the synergistic interaction between harmful agents.

V. Plan for Use of the R & D results

KAERI and INP have established wide variety of research techniques applicable to radiation biology, nuclear medicine, human monitoring, and environmental biology through the cooperative project. The joint experiment, in special, made it possible to utilize the merits of both institutes and to test and verify KAERI's current technology level. All results of the cooperative research will be jointly published in high standard scientific journals listed in the science citation index (SCI), which can make the role of fundamental basis for the improved relationship between Korea and Poland. Research skills such as TSH assay, SCGE assay and synergism assay developed through joint research will be further elaborated and will be continuously used for the collaboration two institutes.

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제 1 장 서 론

제1절 공동연구 추진 배경

1994년 12월 10일 한국에서 개최되었던 한·폴 과학기술장관회담에서는 이듬해인 1995년 5월 폴란드 바르샤바에서 제1차 한·폴 과학기술 공동위원회를 개최하기로 합의하였으며 8개 제안과제에 대하여 공동연구를 추진하기로 합의함으로써 한국과 폴란드간의 과학기술 분야의 공동연구 기틀이 마련되었다.

한국원자력연구소 (KAERI)와 폴란드원자물리연구소 (INP) 두 기관은 방사선 지표생물 연구는 물론 방사선생물학, 방사선 환경분야 및 핵의학에 이르는 폭넓은 분야에 걸쳐 공동관심사가 있음을 여러 가지 경로를 통하여 상호 확인한 바 있다. 특히 폴란드핵물리연구소 소장 (Prof. Andrzej Budzanowski) 및 국제협력 관련 부서에서는 한국원자력연구소와의 공동연구에 적극성을 보이고 있을 뿐 아니라 관련 정부기관인 Polish Atomic Energy Agency (장관 Dr. Niewodniczanski)도 한국과의 공동연구에 각별한 관심을 가지고 있다.

제1차 한·폴 과학기술 공동위원회 개최에 따른 후속조치로서 1995년 12월 한국원자력연구소와 폴란드핵물리연구소 (KAERI-INP) 간의 공동연구에 관한 약정 (MOU)을 체결하였다. 이를 근거로 '지표생물을 이용한 방사선량 감시'에 관한 국제공동연구를 1995년~1998년에 걸쳐 수행한 바 있다.

한편 두 연구기관간 공동연구가 호혜적이라는 점에 인식을 같이하고 1998년 '방사선과 화학물질의 상호작용에 의한 생체영향 연구'를 MOU의 보조약정 (Annex II)에 추가함으로써 본 공동연구를 시작하게 되었다.

체결된 약정에 따라 공동관심 분야에 대한 양 기관의 독립적 연구수행을 원칙으로 하며 필요에 따라 양 기관이 공동 참여하는 연계실험을 실시하는 방식으로 공동연구개발을 수행하였다. 한국원자력연구소는 본 공동연구를 통해 폴란드핵물리연구소의 연구시설과 숙련된 인력을 공동 활용하는 동시에 장기간 축적된 방사선생물학 관련기술을 빠른 기간 내에 습득할 수 있는 기회로 삼을 수 있었다. 연구원 교환, 공동실험 수행 등의 과정을 거쳐 습득된 기술은 한국적 실정에 맞도록 개선된 기술로 발전시켜 실용화에 접근할 수 있는 응용기술로서 자리잡을 수 있을 것으로 판단된다.

제2절 공동연구의 목적 및 범위

본 공동연구는 한국원자력연구소와 폴란드핵물리연구소가 각각 보유하고 있는 강점기술을 상호 공유하고 공동으로 발전시키는 한편 기관간 병립적이며 상호보완적 실험연구를 수행하여 그 결과를 공동으로 활용하는데 그 목적이 있다.

방사선과 화학물질의 상호작용을 연구함에 있어 ^{60}Co 감마선, X-선 및 ^{252}Cf 중성자는 물론 synergistic interaction을 해석하기 위한 범주에는 열 (hyperthermia), 자외선 (ultraviolet light) 및 초음파 (ultrasound)를 포함시켰다. 의료목적으로 관심의 대상이 되고 있는 붕소화합물과 가돌리늄 화합물을 실험대상 화학물질에 포함시켰으며 농업적으로 널리 사용되고 있는 살충제의 세포독성 (cytotoxicity) 및 돌연변이 유발성 (mutagenecity)를 파악하고 방사선과 복합적으로 작용하였을 때 나타나는 생체영향을 실험적으로 분석하였다. 다음의 실험기법을 이용하여 연구를 수행하였다.

▷ TSH assay ; 자주달개비 수술털 (*Tradescantia* stamen hair) 세포에 나타나는 돌연변이를 생물말단점 (biological end-point)으로 삼아 방사선과 화학물질의 복합적 작용을 분석하였다.

▷ Comet assay ; 사람의 말초혈액 림프구를 분리하여 방사선과 화학물질의 영향을 실험하였으며 림프구 DNA 손상정도를 단세포전기영동 (SCGE; single cell gel electrophoresis)을 수행하여 분석하였다.

▷ Synergism assay ; 포유동물 배양 세포주 (culture cell line)를 포함하여 다양한 세포계 및 생물체에 대한 실험결과를 분석하였다. 특히 synergistic interaction의 이론적 해석을 위한 모델설정, 최대 상승작용을 영향을 미치는 주효인자 검출 및 규칙성을 규명하기 위한 실험 대상으로는 광범위한 가혹실험조건에 잘 견디며 실험을 위한 배양·처리 절차가 까다롭지 않으면서도 단순한 eukaryotic cell인 효모세포를 주로 이용하였다.

한·폴 (KAERI-INP) 양측은 지금까지의 공동협력이 방사선생물학, 방사선환경학 및 방사선의학 등 다양한 분야의 호혜적 협력관계에 도움을 준다는 것에 인식을 같이하고 있으며 다양한 분야로의 공동연구 확대를 희망하고 있다. 특히 폴란드 강점기술분야인 방사선생물학, 방사화학 관련 기술을 단기간에 습득하여 국내기술로 발전·정착시키는 것이 기대될 뿐 아니라 연구결과의 국제유명학술지 논문 공동게재를 통하여 국내 방사선 관련 분야의 국제적 위상을 제고시킬 수 있을 것으로 기대된다.

제3절 상대국과의 공동연구 역할 분담 체계

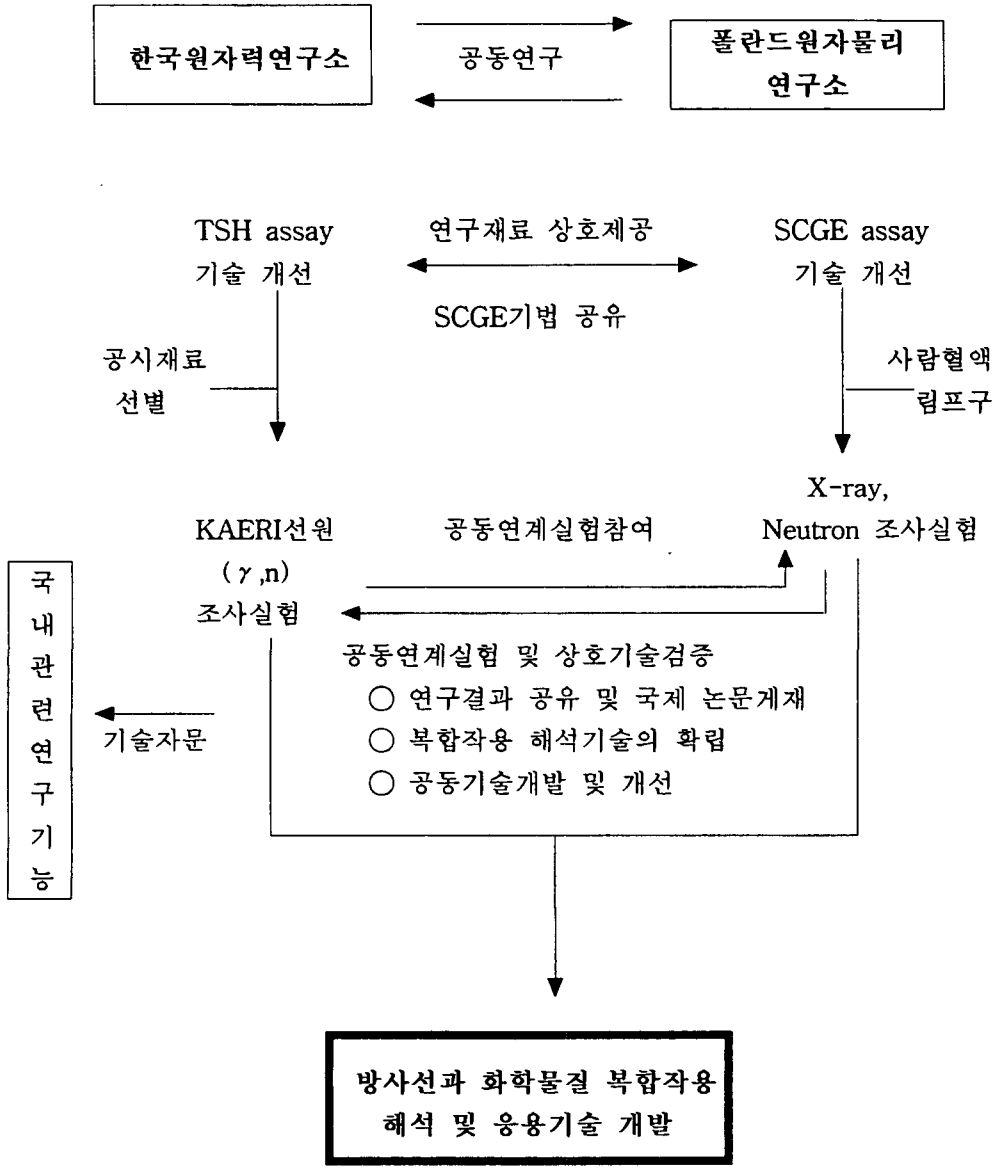
TSH assay에 사용된 연구용 식물체의 stock culture는 폴란드원자물리연구소의 자동화 온실에서 입수한 T-4430를 이용하였다. 다량의 공시식물체가 필요한 경우 조직배양을 통해 대량 번식한 식물체를 이용하였는데 이 경우 유전적 순수성에 대한 검증 과정을 반드시 거친 후 사용하였다.

SCGE assay에 사용된 사람 말초 혈액은 한국원자력연구소의 두 명의 자원자(남, 46세, 28세)로부터 채혈하여 사용, 공동실험의 경우도 KAERI 측의 혈액시료 또는 분리후 초저온 보관된 림프구 시료를 사용하였다.

방사선 조사시설의 경우 폴란드핵물리연구소의 X선 발생장치를 이용하였으며 한국원자력연구소의 보유시설인 국가 2차 표준 방사선장 시설의 X선 발생장치와 ^{252}Cf 중성자 선원을 이용한 조사실험을 수행하였다. 또한 고선량과 대규모 시료조사실험에는 한국원자력연구소의 ^{60}Co 감마선원 (선원강도 150 TBq, Panoramic Irradiator, Atomic Energy of Canada Ltd.)을 이용하였다.

폴란드핵물리연구소는 X선과 ^{252}Cf (AGE 보유 선원) 중성자에 대한 제반 실험을 담당하였으며 공동연계실험의 경우 TSH 돌연변이 검경분석 및 혈액 림프구 SCGE assay에 5인의 연구인력을 실험필수 기간중 투입하였을 뿐 아니라 TSH assay처럼 후속 분석이 필요한 경우의 인력도 투입하였다. 공동실험에 소요되는 소모성 경비는 실험연구의 work scope 별로 나누어 각자 부담하였다.

[공동 연구 체계]


한국원자력연구소
공동연구
폴란드원자물리 연구소
TSH assay 기술 개선
연구재료 상호제공
SCGE기법 공유
SCGE assay 기술 개선
공시재료 선별
사람혈액 림프구
X-ray, Neutron 조사실험
KAERI선원 (γ,n) 조사실험
공동연계실험참여
공동연계실험 및 상호기술검증
○ 연구결과 공유 및 국제 논문게재
○ 복합작용 해석기술의 확립
○ 공동기술개발 및 개선
기술자문
국내관련연구기능
방사선과 화학물질 복합작용 해석 및 응용기술 개발

제4절 공동연구기관 및 연구책임자의 연구수행능력

1. 공동연구기관

ㅇ 기관명 : 헨리크 니보드니차인스키 핵물리연구소
(The Henryk Niewodniczanski Institute of Nuclear Physics)
ㅇ 주 소 : ul. Radzikowskiego 152, 31-342 Kraków, Poland.
ㅇ 전 화 : 48-12-637-00 40
ㅇ 전 송 : 48-12-637-54 41
ㅇ 개 관

폴란드 남부 지방에 위치한 고도인 크라쿠프에 위치하고 있는 핵물리연구소는 1955년 현직 폴란드 원자력청 장관의 부친인 Prof. Henryk Niewodniczanski에 의해 설립되었다. 공식 명칭은 설립자의 이름을 따라 The Henryk Niewodniczanski Institute of Nuclear Physics로 불린다. 연구소원은 약 400여명으로서 유럽에서는 중대형에 속하는 연구소로서 1998년 현재 연구실 (department 또는 group) 및 관련 부서 (section 또는 division/center)를 포함하여 26개에 달하는 연구 및 지원조직을 보유하고 있다.

특히 본 공동연구의 상대 부서인 방사선환경생물연구실 (DREB)은 실장인 Prof. Antonina Cebulska-Wasilewska를 비롯한 20 여명의 연구인력을 보유하고 있을 뿐 아니라 방사선생물학 연구에 필요한 각종 연구시설을 갖추고 있다. 자주달개비 식물체의 유지 및 증식에 필요한 대형 자동화 유리온실과 각종 환경조건의 인위적 조절이 가능한 대형 배양설비 (growth facility)는 본 연구에 있어 크게 활용된 시설들이다. 1997년까지 중성자 조사 및 암환자 진료에 활용되었던 U-120 cyclotron을 폐쇄하고, 1998년에는 새로운 X-ray 조사시설을 완비하여 더욱 개선된 연구환경을 조성해 나아가고 있다. 그 외에 방사선생물학적 실험을 수행하기 위한 다수의 SCGE 분석 관련 설비를 보유하고 있으며 기타 시설로 실험동물 사육시설, 현미경실, 저온실 등을 갖추고 있다.

2. 공동연구책임자의 연구수행능력

ㅇ 성 명 : Prof. Antonina Cebulska-Wasilewska, Dr. hab.
ㅇ 직 위 : Head, Department of Radiation and Environmental Biology

○ 연구능력

방사선환경생물연구실은 Prof. Antonina Cebulska-Wasilewska의 주관 하에 20년 이상에 달하는 방사선생물학연구 수행을 통하여 습득한 연구기술을 다량보유하고 있다. 특히 방사선지표생물인 자주달개비 연구에 있어서는 전세계적인 중심 역할을 수행하여 오고있다. 따라서 미국의 BNL 연구소, 네덜란드 Leiden 대학의 연구진들도 INP의 연구에 참여함으로써 원자력 선진 각국으로부터 그 연구능력을 인정받고 있다. 또한 유럽에서 다국적 과제 (NATO, UN 및 EC projects)로 수행하는 여러 가지 방사선관련 연구에 주도적으로 또는 적극적으로 참여하여 활약함으로써 INP의 연구능력을 인정받고 있다. 특히 미국의 Western Illinois 대학, 오스트리아 Vienna 대학의 Cancer and Tumor Research Institute, 네덜란드 Petten의 Netherlands Energy Research Foundation, 독일의 Ulm University, 체코의 Institute of Experimental Botany 등과 수행하고 있는 폭넓은 협력연구는 INP의 연구능력을 확인할 수 있는 객관적 증거로 볼 수 있다.

제 2 장 국내외 기술개발 현황

제1절 TSH assay를 이용한 국내 연구 현황

한국원자력연구소에서는 자주달개비 식물체의 방사선 감수성에 대한 실험적 자료를 확보하기 위한 방사선 지표생물 연구노력을 기울여 왔다. 방사선 지표생물중 선진국에서 주로 연구에 이용하는 T-4430, T-02, KU 9 클론을 확보하여 증식시키고 있으며 이들을 이용한 방사선 종류별 반응관계 규명은 물론 환경요인, 농약살포 등에 의한 복합적 반응영향에 관한 실험연구가 계속적으로 수행되고 있다. 지금까지의 연구개발 실적으로는 각종 방사선의 저선량 영역에서의 TSH 변이 반응성 판별기법 확립, 자주달개비 생물지표의 감마선, X선 및 중성자에 대한 선량-반응 관계 수립, TSH 체세포 돌연변이에 있어서의 californium-252 중성자의 생물학적 효과비 검증, 환경인자 (photoperiod, diurnal temperature difference 등)와 방사선의 복합작용에 의한 영향 해석 등을 들 수 있다 [1-8].

Kwon *et al.* (1981)은 한국원자력연구소 금곡농장의 감마육종장에 선원으로부터 일정한 거리 간격별로 자주달개비를 식재하여 선량에 따른 수술털세포의 돌연변이율을 측정하였다 [9]. 방사선장내의 선량범위는 3.6 mR/day에서 182 R/day였으며 105 R/day 이상의 고선량율을 제외하고는 전 실험범위에 걸쳐 선량이 증가함에 따라 돌연변이율도 비례적으로 증가함을 밝힌 바 있다. 또한 3.6 mR/day의 저선량율의 조건하에서도 T-4430의 돌연변이를 감지할 수 있었으며 이로써 과수나 관상수의 돌연변이 유발에도 감마선장을 이용할 수 있다는 결론을 얻었다. 이 연구보고가 국내에서는 처음 시도된 방사선 지표생물에 대한 연구이다.

최근에는 환경내 위해요소가 증대되기 시작하면서 환경재해 예방에 관한 관심이 증가하고 있다. TSH 생물지표를 이용한 원자력 시설 주변 환경방사선의 생물학적 안전성 평가, 살충제 등 농업재해 관련 요인 및 그 영향에 관한 생물학적 정보획득 수단으로 활용하기 위한 연구가 진행되고 있다 [10-11].

제2절 TSH assay를 이용한 외국 연구 현황

미국에서는 BNL 연구소를 중심으로 방사선 지표생물로 *Tradescantia*를 선정하고 이에 대한 연구를 활발히 수행해 왔다. BNL 연구소는 실험재료의 생물학적 변이를 배제하기 위하여 수종의 자체 클론을 만들어 사용하고 있으며 많은 나라에서 이 BNL 클론들이 이용되고 있다. 특히 TSH 돌연변이계를 이용하여 원자로 중성자의 생물학적 효과비 (RBE)를 해석하는 등 중성자포획치료법과 연관된 응용실험을 수행한 사례도 있다 [12].

일본은 쿄토대학교에서 개발된 KU 클론을 연구재료로 이용하고 있다. 우라와현의 사이타마 대학교에서는 최근까지 지속적인 연구를 진행하고 있다. 최근의 연구는 저선량에 의한 체세포 돌연변이율 변화에 대하여 초점이 맞춰져 있으며 한편으로는 방사선 이외의 환경내 돌연변이원에 의한 자주달개비 체세포돌연변이 반응 및 상승작용에 관한 연구도 활발하다. Ichikawa와 Nakata (1975)는 하마오카 원전 주변에 자주달개비를 식재한 후 시험 가동기간 및 가동정지 기간중의 환경방사선에 의한 돌연변이율의 차이를 측정한 바 있다 [13].

Cebulska-Wasilewska *et al.* (1992)은 체르노빌 원전 사고에 따른 대기오염 및 그 영향을 평가하기 위하여 T-4430을 이용하였다 [14]. 폴란드 크라쿠프 지역에서 사고이후 11일 경과 시점부터 자주달개비 수술털 세포의 돌연변이를 감지할 수 있었다. 수술털 백개당 평균 돌연변이 빈도는 1986년에 0.43, 1987년에는 0.21을 나타내어 시간 경과에 따른 공기오염 방사선 물질의 감소추세를 반영하였다. 대부분의 대형 원자력 사고시 방사능 오염의 범위가 주변에 설치된 방사능 감시망 범위를 훨씬 초과하여 나타나기 때문에 이러한 경우의 천연감시망으로서의 대안 가능성이 제시되는 경우로 볼 수 있다.

한국원자력연구소와 폴란드핵물리연구소가 협력을 시작한 1994년 이후 TSH assay를 이용한 공동연구 결과도 다수 보고되어 있다[4, 15-17]. 이같은 공동연구의 결과는 TSH 생물지표를 이용한 각국의 연구에 긍정적 영향을 미칠 것으로 기대된다.

제3절 SCGE assay를 이용한 국내외 기술개발 현황

1. SCGE assay 기술 발전의 배경

단일세포 전기영동 (SCGE; single cell gel electrophoresis) 기법은 혜성분석 (comet assay)이라고도 불리며 각각의 세포에서 DNA 손상을 직접 가시화하는 전기영동 기술로서 1984년에 Ostling과 Johanson에 의해서 처음으로 소개되었다 [18]. 이 기술은 낮은 선량의 방사선이 조사된 세포를 슬라이드 상의 얇은 아가로즈 겔에 끼워 넣어 세포막의 분해, 전기영동, 그리고 형광 염료로 염색하는 단계를 거친다. 전류가 하전을 띠고 있는 DNA를 핵으로부터 잡아당김으로써 완화된 DNA와 깨진 DNA 절편들은 이동시키게 된다. '혜성' 같은 모양에서 이름 붙여진 이 이미지가 DNA 손상정도를 결정하는 척도로 이용된다. Ostling과 Johanson은 전기영동이 이뤄지는 동안 head로부터 떨어져 나오는 DNA의 양이 실제로 조사된 방사선량과 관계가 있음을 관찰하였다.

초기의 Ostling과 Johanson의 중성적 방법은 DNA 초나선 (supercoiling)에서 나타나는 한가닥 절단 (SSBs; single-strand breaks)을 민감하게 검지할 수 있었다. 그러나, 이 연구자들에 의해 사용된 세포막 분해 조건은 모든 단백질을 제거하는데 비효과적이었다. 그러므로, 이것은 세포막 분해 조건이 아닌 방사선의 주요 영향 때문에 DNA 초나선이 상실되고 DNA의 '후광' 방출로 나타내어 졌고 SSB의 존재를 민감하게 감지해 낼 수 있었다. 그러나, 더욱 확실한 세포막 분해 조건들을 사용하였을 때 세포의 단백질이 95% 이상이 상실되어 깨어진 duplex 분자가 이동할 수 있게 되었다. 초기에 두 실험실에서는 독립적으로 변성 조건을 달리하여 DNA SSBs를 측정하는데 적용하였다 [19,20]. 첫 번째 실험실 [20]은 적은 strand breaks 측정상의 민감도를 최대화하는데 집중한 반면에 두 번째 실험실은 약품이나 방사선에 대한 민감성이 다양하게 나타나는 소 세포군을 구분 감지하는데 주력하였다 [19].

SSBs는 일반적으로 높은 pH 조건하에서 실시되는 alkaline unwinding [21-23], alkaline elution [24], 그리고 alkaline sucrose sedimentation [25,26] 등과 같은 방법으로 측정되었다. 원리는 단지 알칼리에 의해서 DNA 염기 쌍이 해리되어 단일 가닥에서 나타나는 불연속성을 감지할 수 있다는 것이었다. 중성 pH에서의 long

duplex molecule의 연속성은 SSB에 의해서 영향을 받지 않는다. 그러나, 이런 중성 조건의 SSBs는 핵 안에서 DNA의 초나선과 조밀한 응축 (packaging) 때문에 DNA의 반응에 심오한 영향을 미칠 수 있다. 진핵 세포 (eukaryotic cells)의 DNA 분자들은 (길이 50-100 cm) 직경 5-10 ㎛의 핵 안에 맞도록 105 배로 응축되어야 만 한다. SSBs는 초나선 구조의 완화를 유도할 수 있는 출발점이라는 점에서 중요성을 가지고 있는 이유는 초나선 구조의 완화를 통해서 가시적인 시험이나 DNA sedimentation 분석 등의 방법으로 DNA 손상을 감지할 수 있기 때문이다. 즉 혜성 분석에서 사용된 절차와 그것이 만들어내는 결과들은 초나선 완화와 alkaline unwinding의 조합 또는 초나선의 완화 자체에 의해 나타난다고 할 수 있다.

DNA에서의 가닥절단을 측정하는 여러 가지 분석법들이 개발되어 왔으며 이들 각각은 특정한 장단점들을 가지고 있다 [21,27]. SCGE assay는 single cell suspension 상태로 얻을 수 있는 각종 진핵세포를 대상으로 하여 DNA 손상과 수복의 세포간의 차이점을 감지할 수 있다는 점과 극도로 작은 세포의 샘플을 (from 1 to 10,000 cells) 필요로 하며 하루 만에 결과를 획득할 수 있다는 점에서 특히 가치가 있는 기술이다. 또한, 혜성분석은 DNA 손상의 측정을 촉진하고 강화하는 이미지 분석을 별도로 수행한다고 하여도 분석수행의 비용이 매우 저렴하다. SCGE 분석법의 개발 초기에 Olive와 그의 동료 연구자들은 이 방법으로 분석된 단일 세포에서의 손상 감지 민감도가 세포군내의 평균 손상을 측정하는 다른 분석법의 민감도와 유사하다는 점을 보고한 바 있다 [28]. 그러나, 근래의 보고서에서는 다른 방법들에 비해 DNA 손상을 감지하는 민감도가 탁월함이 객관적으로 입증되고 있다. SCGE 기술의 민감성은 UV 방사선 조사 후 exogenous UV endonuclease로 세포를 배양함으로써 UV 방사선 조사의 결과로 생기는 DNA 가닥절단을 알칼리 SCGE와 alkaline unwinding에 의해서 조사함으로써 확인될 수 있었다. 그 결과, SCGE assay에서 109 dalton당 0.1 DNA breaks만큼의 적은 양도 감지가 가능하였다 [29]. Schmezer와 그의 동료 연구자들이 alkaline elution 방법과 비교하였을 때도 SCGE의 민감도가 탁월함을 알아냈다.

지금까지 SCGE assay는 산화적 손상, UV, 이온화방사선에 대한 감수성을 평가하는 데 소량의 사람 림프구 시료를 사용하여 적용되어 왔다 [20,28,30,31]. 림프구는 사람의 면역체계에서 중요한 역할을 담당하고 있기 때문에 림프구의 상태는 사

람의 건강을 나타내는 척도로 사용될 수 있다.

지난 수년 동안에 혜성분석에 대해 많은 관심이 증대되어 왔고 특히 근래에는 많은 논문에서 이 분석기술을 적용한 결과들이 발표되었으며 또한 새로운 분야에 적용하기 위한 연구와 개선이 급진전되고 있다. 혜성분석의 독특한 특징은 각각의 세포에서 DNA 손상의 정도를 직접 보여주기 때문에 한 세포군 안의 모든 세포들이 같은 정도의 손상을 받았는지를 설명하는 것이 가능하다. 방사선이 조사되는 동안 또는 어떤 물질이 처리되는 동안에 저항성의 차이에 기인한 이종 반응을 나타내는 각 세포군을 인식하는 것도 가능하기 때문에 특유의 처리절차에 대한 종양 반응의 예견에도 적용할 수 있다. SCGE 분석법은 또한 다양한 실험적 조건들 하에서 DNA 손상과 수복을 연구하는데 사용되어 왔다. 이 기술은 유전자 독성 연구와 DNA 수복의 기전을 해석하기 위한 수단으로 자리잡고 있다.

2. SCGE assay의 발전 및 적용

Rydberg와 Johanson [32]은 X선 조사 후에 알칼리 분해된 세포핵의 비율이 선량에 비례하여 점차적으로 증가되는 것을 관찰하였다. 그들은 단일 세포에서 DNA 손상을 정량적으로 분석하였다. 세포를 슬라이드 상의 아가로스 (agarose)에 끼워 넣어 알칼리 조건하에서 분해한 후 세포들을 중성화시킨 다음 아크리딘 오렌지 (AcOr; acridine orange)로 염색하여 DNA 손상의 정도를 측정하였다. 측광계 (photometer)를 사용하여 DNA 한가닥 절단을 나타내는 빨간 형광에 대한 DNA 두 가닥 절단 (DSBs; double-strand breaks)을 나타내는 녹색의 비율을 측정함으로써 손상도를 정량하였다. 1984년에 Ostling과 Johanson [18]은 electrophoretic microgel 기술을 개발함으로써 각각의 세포에서 DNA 손상을 감지하는 민감성을 향상시켰다. 세포들은 슬라이드상의 아가로스에 끼워지고 고농도에서 detergents와 salts에 의해서 lysing시켰다. 그 후 5 V/cm로 중성 조건하에서 전기영동을 수행하였다. 그 후 양극 방향으로 뻗어 가는 tail DNA를 AcOr로 염색하였다. X선 3 Gy까지의 선량에 비례하여 중심과 비교하여 tail에서 더 많은 형광 염색도를 관찰할 수 있었다. Ostling과 Johanson은 SSBs에 의해 초나선 DNA가 풀려 DNA가 중심으로부터 나오는 것이라고 설명했다.

알칼리 조건과는 매우 다른 중성 SCGE 기법을 사용한 Olive와 그의 동료 연구

자들은 [33] DSBs가 겔안에서 DNA 단편들을 만들어내는 원인이라고 주장했고 5 Gy의 X선은 세포당 약 200 DSBs에 상응하는 손상을 일으킨다는 것을 검출 결과를 통하여 확인하였다.

일반적으로 적용되는 SCGE 기술은 Singh과 그의 동료들이 Ostling과 Johanson의 방법을 높은 pH에서 전기영동을 수행하는 절차로 변형시킨 것이다. 그럼으로써, DNA 가닥절단뿐만 아니라 알칼리 취약부위 (alkali-labile sites)도 밝힐 수 있게 되었다. 브롬화에티디움 (EtBr; ethidium bromide)으로 염색한 후에 핵으로부터 양극쪽으로 나타나는 DNA의 신장된 형상은 세포의 손상 정도를 나타낸다. 손상된 각각의 세포의 DNA-strand breaks들은 형광으로 노출되는 head와 tail의 길이와 밀도를 가진 혜성의 양상으로 나타난다. 손상 받지 않은 세포들은 꼬리를 가진 혜성의 모습을 나타내지 않으며 단지 혜성분석에 있어서 머리부분으로 정의되고 있는 원래의 모양을 유지하는 핵 (intact nuclei) 만을 나타낸다.

SCGE assay는 다음과 같이 다양한 분야에 적용이 가능하며 점차 그 응용분야가 확장되고 있다. 국내에서는 Kim *et al.* (1999)이 SCGE assay를 적용하여 복숭아 추출물이 가지는 방사선 방어효과를 측정한 보고 이후 활발한 연구가 진행중이다 [97].

▷ 방사선 생물학
▷ 자외선에 의한 DNA 손상 평가
▷ 알킬화 물질에 의한 DNA 손상 평가
▷ 산화적 손상 평가
▷ 유전 독성학
▷ 세포의 자사 (apoptosis) 연구
▷ 생체 DNA 손상 감시 (biomonitoring)
▷ 방사선을 조사한 식품의 검지
▷ 방사선효율 변화물질의 효과 분석 등

제4절 Synergism 연구 등 관련분야의 연구개발 환경 변화

1. 국내 관련분야의 환경변화

국가 복지 및 국민보건 향상을 위해서는 원자력 발전 분야뿐만 아니라 비발전 분야에 대한 투자와 연구개발이 균형적으로 이뤄져야 한다는 인식이 자리잡아 가고 있는 실정이다. 특히 산업 및 의료 분야에 있어서 방사선의 긍정적 이용이 증가되고 있으며 다양한 용도로 사용되는 화학물질 또한 질적 양적 팽창을 거듭하고 있다.

산업 및 의료 분야에 있어서 방사선의 긍정적 이용이 증가되고 있으며 다양한 용도로 사용되는 화학물질 또한 질적 양적 팽창을 거듭하고 있다. 방사선에 민감한 생물지표 또는 생물말단 중에서 사람 림프구와 TSH 세포는 돌연변이 반응성면에서 많은 유사도를 가지고 있기 때문에 두 가지 실험계를 병립적, 복합적으로 이용함으로써 다양한 유해물질, 화학물질과 이온화방사선의 상호작용에 의한 영향을 정량적으로 평가할 수 있음이 연구개발 결과로 나타나고 있다.

복잡한 현대 사회의 구조는 생물체에 위해를 유발할 수 있는 복합적 요인의 pool을 이루고 있기 때문에 생체영향의 평가를 위해서는 다면평가기법이 개발되어야 한다. 여러 가지 요인들이 상승적으로 작용하여 나타나는 synergism을 해석할 수 있는 평가기법의 하나로서 동식물 세포의 DNA 손상을 가시적으로 평가하는 SCGE가 확립되어 가는 단계이다. 복합적 요인에 의한 생물체 위해를 정확하고 빠르게 진단·평가하여 조건별 대응책을 사전에 준비하는 것이 필요하며 이러한 과정에서 생물체 위해 또는 손상을 이론적으로 해석할 수 있는 체계 확립과 분석기술 체계 확립이 동시에 요구되고 있다.

2. 국외 관련분야의 환경변화

동유럽 국가들에 대한 우리나라의 경제·산업적 교류가 현격히 증가하고 있는 것과 때를 같이하여 과학기술분야의 교류·협력의 중요성이 그 어느 때보다 강조되고 있다. 특히 본 연구의 상대국인 폴란드에 대한 우리나라의 경제투자 규모의 급

신장과 이적, 물적 교류의 신장세에 힘입어 폴란드의 우세 분야인 기초과학 분야의 공조 여건이 성숙되고 있다.

한국-폴란드 과학기술공동위원회가 상설 기구에 준하는 협력대화의 채널로 가동되고 있는 점과 1995년 이후 지금까지 이어져온 폴란드원자물리연구소와 한국원자력연구소간의 공동연구 수행 경험은 생명과학 및 환경분야 전반에 걸친 양국간 협력의 기틀을 마련했다고 평가된다.

체코, 영국, 미국, 독일, 오스트리아 등 여러 국가에서 방사선과 화학물질 또는 유해 환경요인에 의해 나타나는 synergistic interaction을 깊이 있게 연구하기 시작했으며 방사선에 민감한 생물지표로서 사람 림프구와 TSH 세포계를 이용한 연구가 그 주류를 이루고 있다. Synergistic interaction에 관한 연구에 있어서 러시아 의학방사선연구센터 (MRRC RAMS)는 뛰어난 연구업적을 가지고 있는 기관이다. 연구대상 생물로는 단순한 eukaryotic cell system인 효모 세포를 주로 이용하고 있으며 이온화 방사선은 물론 비이온화 방사선 (non-ionizing radiation)에 이르는 폭넓은 분야의 물리·화학적 요인들간 상승작용을 연구하고 있다.

제 3 장 연구개발수행 내용 및 결과

제1절 방사선 및 화학물질의 영향 분석을 위한 TSH 생물지표 응용

(Section I. Application of TSH bioindicator for studying the biological efficiency of radiation)

1. Introduction

In this section we present data from the pilot experiment of which aim was to compare relative biological efficiency of two californium-252 sources from Academy of Mining and Metallurgy, Krakow, Poland and KAERI Taejon, Korea. The special attention was paid to check whether in the range of neutron energies available in the exposures any enhancement effects are visible due to process of boron neutron capture in the cells enriched with boron ion. The boron ions emits alpha particles through the process of neutron capture. These alpha particles can therefore give rise to an enhanced impact on surrounding biological systems such as cells and cell organelles. The enhancement in biological efficacy due to boron neutron capture is of great importance for its applicability to clinical purposes which is called "boron neutron capture therapy (BNCT)".

The planned experiments can be divided into the following categories:

- Determination of the dose-response relationships for inducing gene mutations, cell killing and cell cycle inhibition in somatic cells of *Tradescantia* stamen hairs (TSH) by X-rays and neutrons.
- Analysis of relative biological efficiency (RBE) for Cf-252 neutrons.
- Determination of the biological effects in TSH system after neutron irradiation following pretreatment with borax as a chemical containing boron ions ($Na_2B_4O_7.10H_2O$).

The induction of gene mutations in the somatic cells of hetorozygous for the flower color *Tradescantia* clone 4430 will be used as bioindicator for evaluating RBE of neutrons. It is one of the most radiosensitive plant systems known so far; the dose-response curves have been determined down to doses of 0.25 cGy for X-rays and 0.01 cGy for neutrons [80]. The significant features of *Tradescantia* that make it useful for radiation studies, particularly at low dose region, are its extreme sensitivity to radiation and relative ease with which the various genetic points in somatic cells can be reliably scored. The flower buds are sufficiently small to permit neutron irradiation which is uniform with respect to both dose and neutron energy. *Tradescantia* stamen hair is essentially of a single-meristematic-cell nature; because it grows by repeated divisions of terminal and subterminal cells. Therefore, the dose response curves for different genetic end points can be compared with data from single cell systems of other organisms. Depending on the dose level the number of 10,000-100,000 of stamen hairs per one point are examined to determine the pink, single pink and stunted hair frequency.

2. Material and methods

The bioindicator applied for our studies is *Tradescantia* stamen hair (TSH) assay. Hybrid clones of *Tradescantia* have often been used as a biological plant test system based on somatic mutations and cell lethality in the stamen hair cells. They have been used for radiobiological studies extensively in the previous years. The system was primarily developed by A. H. Sparrow and his co-workers at Brookhaven National Laboratory over a number of years. In the case of *Tradescantia* clone 4430, the high sensitivity of plants to radiation is accompanied by high sensitivity to chemicals, as well. *Tradescantia* clone T-4430, interspecific diploid /2n=12/ hybrids originating from the Brookhaven National Laboratory were used in our studies.

2-1. Plant culture

The experimental plants were provided by in vitro culture from the

stock plants of *Trádescantia* clone 4430 transported from the Institute of Nuclear Physics in Krakow where plants were grown in the conditions desciribed elswhere [47]. Shoot meristems which were selected from healthy plants were sterilized in sodium hypochlorite solution and placed into the growth medium [48]. Various growth regulators such as kinetin, BA and 2,4-D were added to the Murashige-Skoog basal medium to attain the maximal growth. Plantlets from the culture were transfered into the greenhouse after certain duration of hardening.

Plants were grown under greenhouse conditions with growing and cultivation procedures were maintained as described by Underbrink *et al.* [49]. An average of 21 cuttings per dose were exposed to both types of radiation under the study and in order to avoid the storage effect [50] cuttings were irradiated not earlier than 24 hours after the cutting. After irradiation cuttings were cultivated in the growth chamber at 18-21℃ with the 18-h day and appropriate humidity for the 90%. Then cuttings were transported into Poland into the growth chamber and gene and lethal mutations were scored from day 7-th to 20-th after the treatment. Flowers in bloom were collected between the 7th day and 20th day after exposure.

2.2 *Tradescantia* stamen hair assay

TSH assay measures were done under stereomicroscopes (x25). Flowers were taken at their full blooming, normally early in the morning on each day and stored in the refrigerator before scoring. Stamen hairs were carefully removed with forceps from the flowers and placed onto the slide glass upon which proper amount of mineral oil was spreaded. Scoring was performed to determine the pink, single pink and stunted cells frequency.

Gene mutations, characterized by single or numerous adjacent pink cells in the hair, were counted as one mutational event (PF). The single cell pink event can be considered as an indicator of mutations induced after the S phase of the cell cycle [51]. A ratio between the number of single pink cells and all pink mutation events (CCF) was considered as a factor indicating cell cycle behavior. Number of hairs containing less than 17 cells in case of clone T-4430 (stunted hairs) was taken as a measure of cell lethality (STF) caused by exposure. The

mean values of mutation frequency calculated for the scoring period and expressed as a number of mutations per 100 hairs were used as a measure of the mutation effect caused by the exposure.

2-3. Chemical pretreatment and irradiation of *Tradescantia* plants.

The chemical introducing boron ion into TSH cells was borax: $Na_2B_4O_7.10H_2O$ supplied by Hayashi Pure Chemicals Company, Osaka. Borax was dissolved in distilled water. The number of cuttings used for each treatment group varied between 20 to 26. After removal of the oldest flower buds (2-4), the 1% concentrated borax solution (20 μl) was applied directly into the inflorescence.

Irradiation of *Tradescantia* plants with mixed thermal-fast neutrons from Cf-252 source was done at KAERI. Total activity of the isotopic source applied was ~$5x10^{-1}$Ci, giving the dose rate of fast neutrons at the 1m distance about 19.3 mSv/h in case of irradiation in the air. Normal and pretreated with borax cuttings were placed in the plastic bags containing approximately about 100 ㎖ of distilled water. For various doses plastic bags with cuttings were situated at the various distances from the source (0.33, 0.50, 0.60 m). The time of irradiation was 3 hours 15 minutes. Irradiations were performed 48 hours after borax pretreatment. In case of X-rays plants were irradiated in the air and in case of ^{252}Cf neutrons they were immersed in distilled water. After irradiation cuttings were transfered into the growth chamber for further cultivation in aerated Hoagland's solution (6x dilution) with 18-h day at 21±1℃ and appropriate humidity [47].

3. Results and discussion

The purpose of this part of the project is to determine relative biological effectiveness for inducing gene and lethal mutations in TSH assay by a series of exposures to X-rays and different doses and various energy neutrons from ^{252}Cf source. The range of neutron energies proposed for studies is extremely interesting part of the LET spectrum for data on biological response because of

importance of those energies for clinical applications of neutron capture.

In this pilot studies the preliminary data for dose-response curves (for normal and boronated cells) were provided. Chemical concentrations, radiation doses and conditions, number of analyzed hairs and cells are presented in Tables 1 and 2. The mean values of mutation frequencies (PF, SPF, STF) were calculated for days 10-th to 20-th of scoring period and these values were used for graphic presentation of the data. Figs. 1-3 present time dependent development of mutation rates induced by various exposures of X-rays and neutrons without and with borax pretreatment, respectively. Fig. 4 and 5 show exposure dependent relationship of the mean values of gene and lethal mutation frequencies induced by X-rays and californium neutrons in TSH cells. Fig. 5 present a simulation of linear dose-response relationship for the gene mutations induced in TSH cells with an assumption that neutron dose increases linearly with decreasing distance from the source. There is a linear dose dependence in the effect induced by neutrons and a slight increase due to borax pretreatment in low doses observed. Visible enhancement in gene mutation frequencies due to pretreatment with borax was observed in cells irradiated with various doses and none of a such enhancement in case of lethal mutations.

The effectiveness of neutrons in relation to X-rays in the induction of various abnormalities in *Tradescantia* stamen hair cells of two clones heterozygous for the flower color were assessed previously [46]. Dose response relationships for various endpoints (gene and lethal mutations and cell cycle alterations) in somatic cells of *Tradescantia* clone-4430 plants (T-4430) were established for X-rays, 5.6 MeV neutrons from U-120 cyclotron in Krakow and fission neutrons from Reactor Center in Petten, Holland. In case of fission neutron comparison studies, neutron and X-ray irradiations were performed simultaneously in Petten.

Dose response relationship for the mutation induction in *Tradescantia* according to the molecular theory of radiation action [52] is described as follows:

$$M = \{1-\exp[-q(\alpha D + \beta D^2)]\}\exp[-(s+p)(\alpha D + \beta D^2)] \qquad [46]$$

where:

M = mutation frequency

D = radiation dose in Gy,

α, β = probabilities per unit dose and square unit that DNA double strand breaks (dsb) are induced in one or two simultaneous energy depositions respectively,

q = probability that the radiation induced DNA dsb lead to a specific mutation,

s = probability that the induced DNA dsb lead to the suppression of a specific mutation,

p = probability that the DNA dsb lead to cell reproductive death.

In the low dose region the values of s, p and β are close to zero and relative biological effectiveness approaches to its maximum value that means [RBE = α_n/α_x]. Our earlier obtained experimental data for radiation induced gene mutations in *Tradescantia* clone 4430 was used to plot the dose-response curves and to calculate the values of alpha coefficient and relative biological effectiveness (RBE). On the base of our former results we can estimate that fission neutrons are more than twice as effective as 5.6 MeV neutrons for gene mutation induction in both *Tradescantia* clones. The RBE values for T-02 clone do not differ statistically significant from those for T-4430 clone, so we could conclude that radiosensitivity of these two clones is similar. Irradiation of *Tradescantia* clone-02 plants with the mixed flux of thermal and fast neutrons from the ^{252}Cf source in Krakow resulted in the time of exposure dependent response relationship both in case of gene and lethal mutations. *Tradescantia* clone-4430 is known as the clone that is very sensitive to chemicals. Irradiation of *Tradescantia* clone-4430 plants with the mixed flux of thermal and fast neutrons from the ^{252}Cf source in Taejon resulted in the weak time of exposure dependent response relationship both in case of gene and lethal mutations and only a slight enhancement in biological effect due to boron-ion pretreatment. These results proved still promising from the clinical point of view of BNCT in spite that we could only find a slight enhancement in biological efficacy of ^{252}Cf radiation by boron neutron capture, which was partly cuased by sparse data.

This experiment was done on a pilot scale without any assurance of the possible data fluctuations invoked by the slight difference in thickness and arrangement of inflorescence samples and of water introduced for thermalization of neutrons during irradiation. Further study on such physical factors mentioned above will make it possible to measure precisely the enhancement in biological efficacy by boron neutron capture.

Although, presented results from the pilot experiment with ^{252}Cf source in KAERI are only preliminary data, with not sufficient statistics yet, but they demonstrate that joint studies are possible and improvement of the precision in irradiation and plant transport conditions may give a promising tool for comparative studies of biological effectiveness of radiation sources.

4. Conclusion

The effectiveness of neutrons in the induction of various abnormalities in the *Tradescantia* stamen hair (TSH) normal or boronated cells of the clone heterozygous for the flower color was assessed. Dose response relationships for various endpoints (gene and lethal mutations and cell cycle alterations) in somatic cells of *Tradescantia* plants clone 4430 (T-4430), were established for mixed fast and thermal neutrons from isotopic source of ^{252}Cf source. The effectiveness of neutrons in relation to X-rays in the induction of various abnormalities in the *Tradescantia* stamen hair cells of the clone heterozygous for the flower color was assessed. Dose response relationships for various endpoints in somatic cells of T-4430 and clone 02, were established previously for X-rays, and 5.6 MeV neutrons from U-120 cyclotron in Krakow [46]. This was a pilot experiment to check if it is possible to establish the RBE values for ^{252}Cf irradiated TSH cells, with and without boron ion preatreatment, in conditions of mutual Taejon-Krakow experiment.

Table 1. Distances, exposures conditions and doses applied in irradiations with neutrons from californium-252 source

Distance [m]	Dose equivalent [mSv]	Irradiation time [min]	Dose [Gy]	Remarks
0.33	6.3	195	0.018	
0.50	6.3	195	0.008	
0.60	6.3	195	0.006	

Table 2. Biological effects measured in *Tradescantia* clone T-4430 after irradiation with californium-252 neutrons

Dose [Gy]	^{10}B [ppm]	NOH	PF/100 hairs ± SD	LMF/100 hairs ± SD
0	-	47296	0.35	nd
0.006	-	4170	0.3±0.2	1.7±1.2
0.008	-	1107	0.8±0.5	nd
0.018	-	3822	0.8±0.4	0.5±0.4
0.006	240	2760	0.7±0.3	4.9±1.7
0.008	240	9895	0.8±0.4	2.3±1.3
0.018	240	3297	1.0±0.4	0.9±0.7

Table 3. Biological effects measured in *Tradescantia* clone T-4430 after X-ray irradiation

Dose (Gy)	NOH	PF/100 hairs ± SD	LMF/100 hairs ± SD
0	47,296	0.35	nd
0.3	4,494	1.7±0.7	4.8±1.6
0.5	6,638	2.4±0.8	3.2±1.4

* nd = not detected

Table 4. Values of α coefficient and RBE estimated from the dose-response curves for gene mutations

Type of radiation	^{10}B[ppm]	α_{PF}	α_{LMF}	RBE_{PF}	RBE_{LMF}
neutrons	-	**0.28**	*	**5.6**	**nd**
neutrons	240	**0.38**	*	**7.9**	**nd**
X-rays	-	**0.05**	*		

* Too few points to be analyzed.

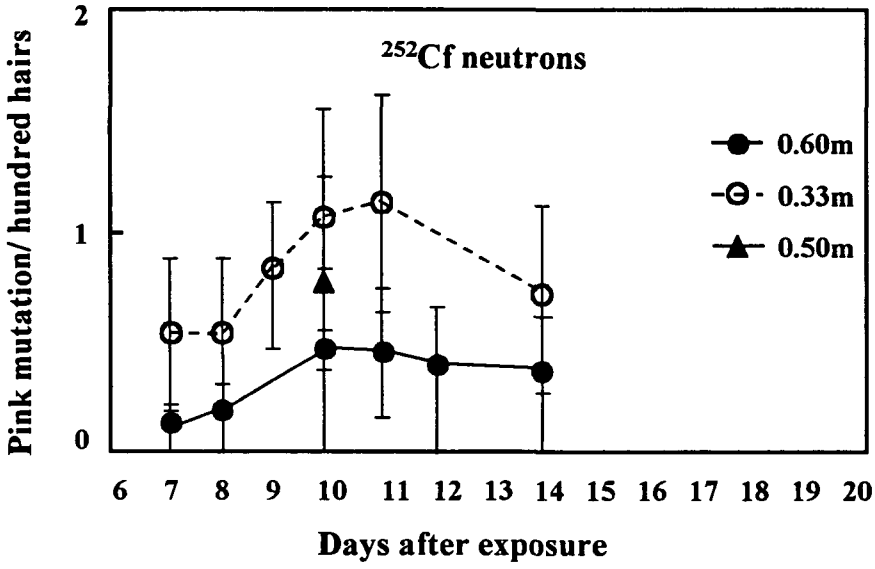


Fig. 1. Daily changes of gene mutation frequencies in stamen hairs of the *Tradescantia* irradiated with neutrons from ^{252}Cf source.

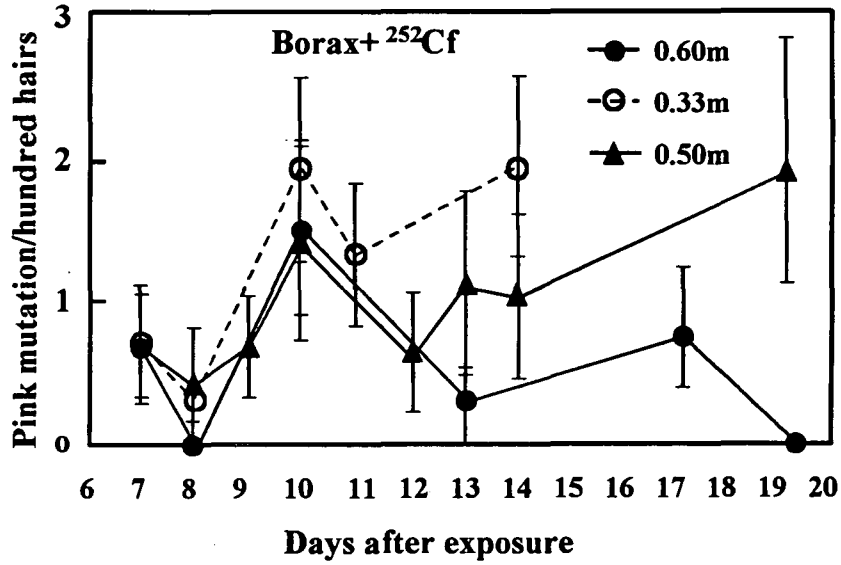


Fig. 2. Daily changes of gene mutation frequencies in stamen hairs of the *Tradescantia* irradiated with neutrons from ^{252}Cf source after borax pretreatment.

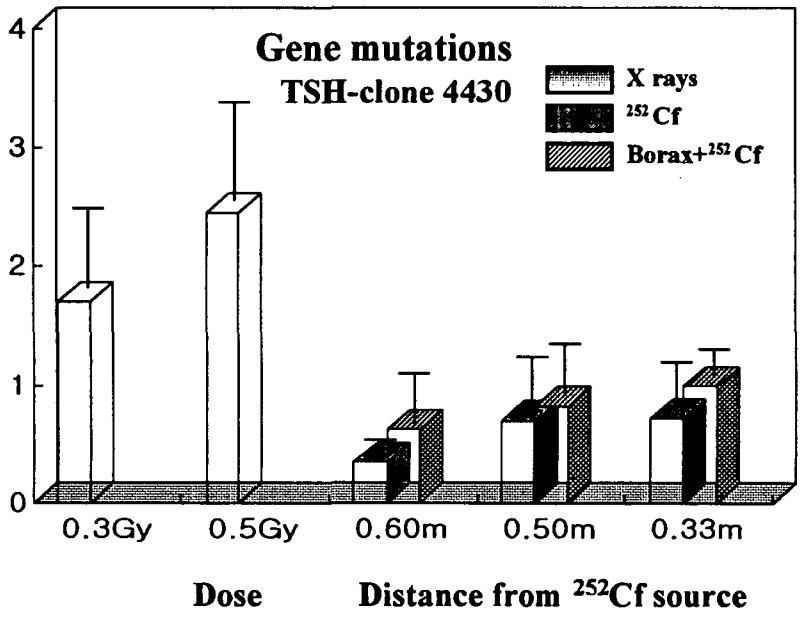


Fig. 3. Dose-response relationship for gene mutation frequencies in stamen hairs of the *Tradescantia* by different experimental schemes.

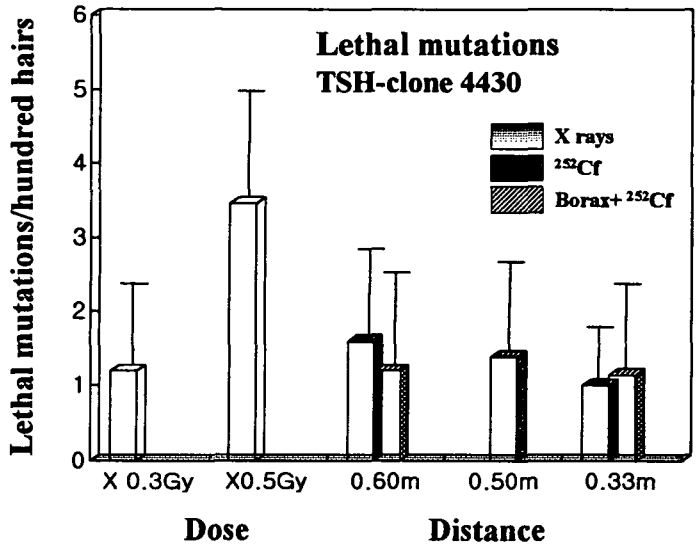


Fig. 4. Dose-response relationship for lethal mutation frequencies in stamen hairs of the *Tradescantia* by different experimental schemes.

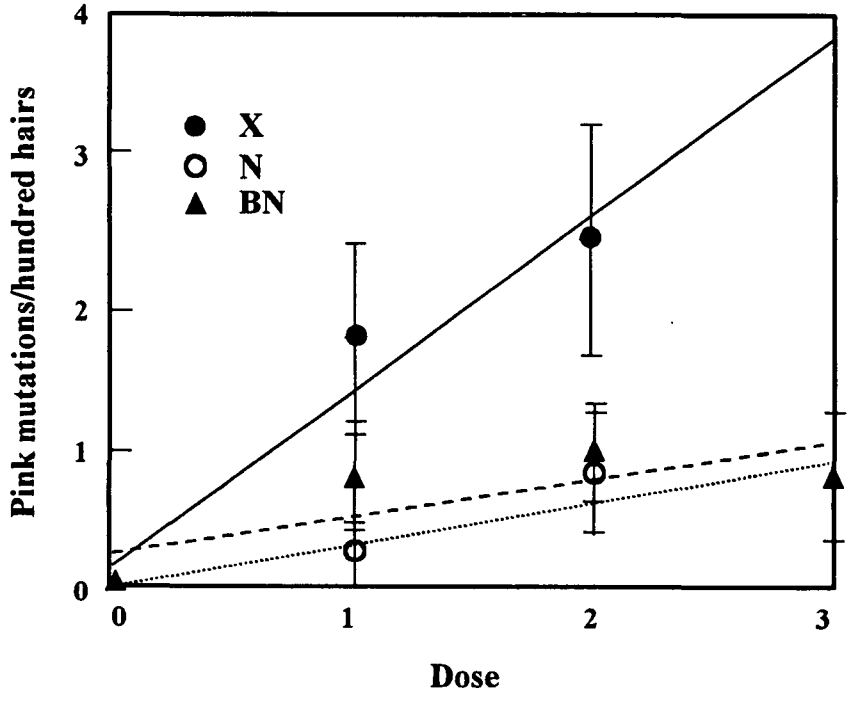


Fig. 5. Linear approximation of the gene mutations induced in stamen hairs of the *Tradescantia by* neutrons(N), X-rays(X) and borax+neutrons(BN).

제2절 중성자와 붕소화합물이 TSH 돌연변이에 미치는 영향

(Section II. Relative biological efficiency of californium-252 neutrons in the induction of gene and lethal mutations in TSH cells normal and enriched with boron compound)

1. Introduction

For the investigation of the mechanisms by which effects of ionizing radiation in living cells are initiated, an important consideration is the comparison of responses caused by this radiation which differs with regard to their ionization density. Part of the difficulty lies in the different mechanisms of energy loss of neutrons of various energies. This is not a particularly serious problem for mono-energetic beams of a high energy neutrons, which lose most of their energy through the production of recoil protons in elastic collisions with hydrogen nuclei. It becomes serious, though, for the broad spectra of fast neutron energies produced by nuclear reactors, and is a big problem for thermal neutrons which lose their energy through capture reactions with various elements [53,54]. Particular importance is the micro-distribution of high cross-section trace elements, especially boron. Recently a tendency is observed to apply low energy neutrons for cancer radiotherapy based on the neutron capture reaction [55,56]. Atoms of boron-10 react with thermal neutrons and then give rise to a particles and lithium nuclei. This reaction is used in boron neutron capture therapy (BNCT). A cross section for this reaction with thermal neutrons is one of the highest known, so that is why fission neutrons are the most interesting for BNCT therapy. A similar spectrum of neutron energy to fission neutrons has the source of Californium-252, so these neutrons are taken for studies.

In radiobiological investigations, plant system based on *Tradescantia* cells (TSH-cells assay) is well known model system used in our studies on biological effects of ionizing radiation [14,16,46,50,57-60], and in environmental monitoring as well [57,58]. However, reported earlier by Sparrow and Underbrink values of

the RBE for various energy neutrons are one of the highest known [61,62], and detected for the energy of neutrons somehow lower than expected on the base of the RBE neutrons energy dependence [54]. The purpose of this work was to determine biological effectiveness of neutrons in the induction of gene and lethal mutations in Trad-SH-cells. Series of exposures to neutrons from an isotopic source of Californium-252 in the relation to X-rays were the sources of radiation. Special attention was also paid to check whether any enhancement in effects caused by pretreatment with boron containing chemicals could be observed.

2. Material and methods

Tradescantia is a member of *Commelinaceae* family. It has several unique features which cause that it is a very good object for radiobiological experiments [57]. Heterozygous for the color of flower *Tradescantia* plants (i.e. clone 02 or 4430) are known as the most radiosensitive plant assay Trad-SH [61,63]. Stamen hair cells can be easily mutated from normal blue color to pink. A relatively short experimental period is required for experiments with this assay. Usually induced mutations are scored during days 11 through 15 after irradiation, because these days, due to highest rate of the cells being irradiated in the most radiosensitive part of the cell cycle, show usually the highest frequency of pink cells that are expression of gene mutation [5,57]. Following irradiation, each young inflorence may result in several flowers in the period of expression of mutations. Each flower bears about 300 stamen hairs, and each stamen hair consists about 20 cells that are progeny of the irradiated cell. So, each flower picked up from irradiated influence and scored during scoring period gives the independent repetition of the distribution of mutation rate in the populations of 300 cells. Each from irradiated cuttings gives the repetition of another set of cell population irradiated under the same experimental conditions. In general, it allows gathering enough data to be statistically significant even in a very low dose of radiation region [56]. The sensitivities of *Tradescantia* stamen hair cells have almost the same range of sensitivity level as mammalian

cells [59]. While other eukaryotic systems may also mutate at these low doses, *Tradescantia* has a distinct advantage because hundreds of thousands of stamen hairs can be scored easily and quickly.

2-1. Biological assay

Irradiated cuttings were taken from the *Tradescantia* plantation in Radiobiology Department of Korean Atomic Energy Research Institute in Taejon. This plantation has grown from a young shoots received by *vitro* culture propagation of meristems of T-4430 that as the stock plants had been cultivated in the Department of Radiation and Environmental Biology of the Institute of Nuclear Physics in Kraków [16,57]. Plants in KAERI were grown under greenhouse conditions where in most of the year natural climate conditions were similar to the conditions described elsewhere [57,61]. On average twenty-five cuttings per dose were exposed to radiation, not earlier than twenty-four hours after cutting to avoid the storage effect [50].

After irradiation the cuttings were cultivated in the growth chamber at 18 -21℃ with the 18-h daylight period and 90% of the relative humidity. The Hoglands solution was changed about every four days. Flowers were harvested at their full blooming, normally early in the morning on each day and stored in a refrigerator until scoring.

Examinations of mutations were done with the use of stereomicroscope (x25). Stamen hairs were carefully removed from the flowers with forceps and placed on the slide glass upon that a proper amount of mineral oil was spread. Scoring was done to find out the mutated cells for determination of:

PF, SPF - gene mutations frequencies characterized by numerous adjacent pink cells or single pink cell in the hair and each of them was counted as one gene mutation event,

LMF - lethal mutations frequencies characterized by stunted hair (defined as a hair in upper third part of stamen containing less than 17 or 12 cells in Trad - 4430 and Trad-02 respectively),

PAF - potential apoptotic cells frequencies characterized by colorless and dwarfed cells hair ends that in Trad-SH cells are indicators of

cellular death.

Fig. 6 shows examples of the time dependent development of mutation rates, detected in Trad-SH, after various exposures. To avoid missing the highest values in mutation frequencies mutation events were scored from day 7-th to 19-th. The mean values of mutation frequency calculated for the scoring period and expressed as the number of mutations in 100 hairs were used as a measure of the mutation effect caused by the exposure.

2-2. Irradiation of Tradescantia plants

Irradiations of *Tradescantia* clone T-4430 cuttings with neutrons from ^{252}Cf were done at KAERI, Taejon, Korea. The average energy (2.02 MeV) of bared neutrons from ^{252}Cf has varied 1.77 - 1.46 MeV at the 0.5 m till 1 m distance from the source, respectively [63]. The total activity of the isotopic source applied was ~3.5 GBq, giving the dose equivalent rate of fast neutrons at the 1 m distance of about 0.5 mGy/h in the case of irradiation in air. Several groups of cuttings were situated at various distances from the source (various dose rates) and irradiated for the same period of 18h. Irradiation in various distances between samples and ^{252}Cf source have resulted in absorbed doses shown in Table 5, that were estimated on the base of the neutron flux distributions in the air [63,64]. In the case of reference radiation the cuttings were irradiated with Phillips Ind.X-ray machine (150 kV, 10 mA), within the dose range 0-0.5 Gy.

2-3. Chemical pretreatment

The purpose of chemical pretreatment was to introduce the boron ions into target cells, and to study whether nuclear reaction of boron neutron capture can enhance the biological efficiency of neutrons in the TSH-cells enriched with boron ions. Each inflorescence (~0.12~0.15 ml of volume) from a group of plants designed for chemical pretreatment was injected one day before irradiation with 20 $\mu\ell$ of boron containing chemical. Borax ($Na_2B_4O_7 \cdot 10H_2O$) supplied by Hayashi Pure Chemical Company, Osaka, in a 1% solution (240 ppm of B-10) was applied as a chemical introducing boron-10 ions. Assuming an even distribution of the solvent in the inflorence it should approximately result in 40

ppm of the maximal final concentration in a target cell.

3. Results

Doses of radiation applied and frequency of flowers in bloom after exposures is shown in the Table 5. In the standard period of mutation scoring (between 11th-16th day) it is visible lower efficiency of blooming after X-ray irradiation. Fig. 6 shows examples of time dependent development of induced mutation rates. In the standard conditions, in the days between 11-15 and 15-19 day after exposures appear the highest mutation rates in cases of gene and lethal mutation frequencies respectively. There is seen certain delay in the period with the highest mutation rates. In response to that scoring period was extended to days from 10th until 19th after irradiation. In the Table 5 is also shown a number of stamen hairs (NoH) analyzed for the presence of mutation events. The NoH value reflects number of cells that had been irradiated and their progenies were analyzed.

Table 6 shows results of the averages from the biological effects frequencies evaluated for the whole scoring period, for the values obtained in the cases of gene (PF and SPF) and lethal (LMF and APF) mutation frequencies induced in this studies by irradiation with neutrons. Table 7, again shows average values, from the whole scoring period, observed for gene (PF and SPF) and lethal (LMF and APF) mutation frequencies induced by irradiation *Tradescantia* with X-rays. Fig. 7a shows dose response relationships for the induction of gene mutations, by X-rays in normal cells and by neutrons in normal or pretreated with borax cells, that were evaluated as average responses measured in the standard period (11-15 day). Fig. 7b shows the same in the cases of responses evaluated as the averages from the whole scoring periods. Dose response relationships drawn after irradiation with californium neutrons or with X-rays were similar for both scoring periods. A similar comparison between results estimated in case of single cell mutation frequencies is shown on Fig. 8a and 8b for two scoring periods respectively. In both cases of gene mutation frequencies (PF and SPF) similar dose dependent increases were observed that resulted in

better description of the neutrons dose response curve by a polynomial fit, with tendency to express saturation level, and linear-quadratic relationship for X-rays. The _ values presented on the figures are linear factors in equations describing relationship evaluated on the base of the least square best fits, the correlation coefficients for two types of fits are also shown on the figure. Saturation, observed for mutation frequencies at higher doses of the neutron dose-response curves, are more visible in cases of dose-response curves obtained for plants pretreated with borax.

In general, lethal mutation events are expressed later than gene mutations, so there was no difference between measurements in standard and extended scoring periods, so why Fig. 9 presents only one set of dose-response curves. Again there are presented dose response relationships observed in plants (normal and boron pretreated) irradiated with neutrons and normal plants irradiated with X-rays. Although results seem to show more scattered values in the case of lethal frequencies, though parameters describing fit goodness are still very high.

Fig. 10 presents dose-response curves for the induction by X-rays and neutrons the hair ends that are consist of colorless dwarfed cells. In Trad-SH cells assay those cells are indicators of cellular death and were used for the determination of potential apoptotic cells frequencies (APF). There are also much greater than in case of gene mutation frequencies differences in the slopes of dose-response curves fitted for plants normal and pretreated with borax solution in both cases LMF and APF. This is known that exposure to radiations might be blocking to some extent the cell cycle. In Trad-SH assay the single pink cell is supposed to present mutation induced in the post DNA synthesis stage [57], so if the ratio between SPF and PF has not changed, this would suggest that distribution of cells in various cell stages had not been affected by radiation exposure. In order to check whether experimental conditions during the X-rays or neutrons exposures have not altered differently the cellular cycles, significance of difference in theirs ratios between single pink cells and all pink mutational events was investigated. Table 8 shows mean values of the cellular cycle factor (CCF= SPF/PF) estimated for various radiation treatments. There is no significant difference between those cell cycle factors in the populations of cells under the study.

4. Discussion

A fairly comprehensive overview on mathematical modeling of biological responses is given by J. Kiefer [65]. Although, according to editors, it was difficult to cover all the aspects in this field, they present modeling of many aspects crucial for cellular and therapeutical radiobiology, starting from cellular radiation action, through the role of energy distributions of charged particles in the mutagenic radiation action, and relative biological effectiveness. One of the models quoted there is of the great values to our studies because describes the dose response relationship on the example of the *Tradescantia* cells [52]. The dose-response relationship for the induction of mutation in *Tradescantia* is according the molecular theory of radiation action described as follows:

$$M = \{1 - \exp [-q (\alpha D + \beta D^2)]\} \exp[-(s + p)(\alpha D + \beta D^2)] \qquad (1)$$

where:

M - mutation frequency,

D - radiation dose in Gy,

α, β - probabilities per unit dose and square unit that DNA double strand breaks (dsb) are induced in one or two simultaneous energy depositions respectively,

q - probability that the radiation induced DNA dsb lead to a specific mutation,

p - probability that the radiation induced DNA dsb lead to cell reproductive death.

s - probability that the induced DNA dsb lead to the suppression of a specific mutation.

At low dose ranges and rates, multiple-break effect yields from low LET radiation became small, and values of s, p and b in the equation (1) were so

close to zero, that the dose-effect curves became linear. On other hand, when cellular processes are altered, the probability of killing, suppression and consequently expression of mutations might be changed and this will result in alteration of values for p, q, and s. Fig. 10 and 11 display how alteration of the probability that radiation induced DNA dsb leads to specific mutation (q), or its suppression and cell killing (s+p) modifies *Tradescantia* dose-response curves. Dose response relationships presented there are obtained by computer simulation of the alteration in values of s, q, p, parameters that had been fitted from our experimental data to the equation (1).

Biological efficiencies relative to X-rays are changing with the dose, or in other way with the level of biological effect at which efficiencies of radiation are compared. However, at very low dose rates the RBE values for neutrons compared to X or γ-rays should become independent of dose and should eventually reach some limiting, largest value defined as $RBE_{max} = \alpha_n / \alpha_x$ [52], where α_n and α_x are the linear factor in the equation describing the dose-response relationship. Estimates of maximal values of RBE of Californium-252 neutrons in the induction of gene and lethal mutations in *Tradescantia*, made on the base of our results are shown in the Table 8. The values of RBEmax estimated from the whole scoring period for the induction of gene mutations by Cf-252 neutrons is slightly higher than values reported for this source previously by Cebulska-Wasilewska *et al.* [16]. Results reported by Cebulska-Wasilewska previously [16] were obtained in two independent experiments and were almost the same 5.6 and 5.8 respectively, comparing the value 7.1 of RBE presented in this section. Both values (5.6 and 5.8) were obtained after an alteration of post-exposure condition due to transportation to laboratory in Poland, that was the same for X-ray and neutrons irradiated plants, and apparently have not affected the estimate of relative biological efficiency. Slightly higher value of RBE =7 might be explain by difference in the post exposure repair conditions. As it is seen form figures 6 and 7, it seems that slopes are more affected by the change of parameter q than s+p. It suggests that variation in the experimental conditions may alter probability of the expression of the specific mutations, and in a consequence RBE. In the results presented in this section the higher value of RBE evaluated from the

period 11-15 should be excluded as is due to different change in the time dependent development (Fig. 6) of the gene mutations induction observed after X-rays and the neutrons beam under the study. The highest PF values induced by X-rays are out of scoring period 11-15, and that affects RBE. Studies by Kim *et al.* [5] also have shown that change in the photoperiod alters the average values of average pink mutation frequency induced by X-rays.

According to Bewley if a single ionization at a vital spot were all was needed to inactivate the cell, the more spread out the distribution of ionizations the more effective would be radiation. For high LET, the RBE would then fall steadily with rising LET value [53,54]. Although, obtained RBEmax value is higher than reported by Cebulska-Wasilewska [16], though it seems to be in good agreement with the expectation based on dependence of the biological efficiency on neutrons energy. The valuated RBE value is higher than the RBE = 4 and reported for more energetic neutrons (5.6 MeV) (Cebulska-Wasilewska et al [16] and lower than values reported for most effective energies. Using somatic mutation test in the stamen hair of *Tradescantia* flowers Davies and Bateman (quoted after Bender [53]) determined the relative effectiveness of 0.65 MeV fast neutrons from the 3H (p,n) 3He reaction as compared with X-rays. At the arbitrary level of 15 mutations per flower, the RBE was about 17.5. Davies and Bateman calculated the maximum RBE to be about 40 (for chronic exposures, where the low LET dose-square component would be minimized. RBE value for pink mutation frequency reported by Underbrink, Sparrow [61] was 11.97 for 0.43 MeV neutrons and 250 kV X-rays.

Comparison between biological effects observed in two groups of plants (untreated or pretreated with a borax solution, and irradiated for the same time and at the same distance from the californium source) showed a slight increase in the gene mutation frequency in pretreated plants. An increase was observed only in the scoring period 11-15 and was statistically insignificant. However, in cases of lethal mutation frequencies RBE are much higher in case of plants pretreated with borax. The RBE values estimated from the comparison of parameters estimated from dose-response curves were almost five times higher and depending on the biological end-point. Significant alterations in RBE values were observed after pretreatment with 40 ppm of B-10 from borax from 5 to

34.5 in case of lethal mutations. Such a high increase in efficiency in lethality might be explained by experimental conditions slightly altered the neutron spectrum, suggesting that losing energy by neutrons inside the sample pre-exposed to boron containing solvent significantly increased the probability of neutron capture and elevated value of lethal damage. It might also be explained by alteration of repair process due to affected cellular environment by infusion of borax into target cells. The last one is also highly probable, as according to molecular theory of radiation biology this process will be associated with an alteration of maximal value in a peak for gene mutation frequency. This could be seen in the modeling of the dose response curve presented on fig. 11a and b, and our dose-response curves presented on fig. 7 and 8. The simulation shown on fig. 11b explains why we cannot see the change in gene mutation frequency due to pretreatment with boron-10 ion. The much higher lethality changes dose response curve, however the slope remains the same.

Comparison of the results from various independent studies presented in this section confirms the existing possibility of influence of external conditions on individual response. This has the particular sense in radiobiology and environmental monitoring [57,63]. Our investigations showed that in each environmental survey it is essential need to see the broadest possible spectrum of biological end-points, and to do in some extent a standardization of the response. Summing up, among plant systems *Tradescantia* is a very important object to use in environmental monitoring as well as in the studies of the ionizing radiation influence on the cells. The aim of this section was to determine the radiobiological efficiency of ^{252}Cf neutrons, and to test how experimental conditions particularly presence of boron-10 ions in the cell can influence the biological response. Presented results showed statistically significant increases in the biological efficacy to induce lethal effects of radiation from the ^{252}Cf source in samples pretreated with boron-containing chemicals, and almost no difference in mutagenic potency. Although the results showed the tendency that was expected, farther studies are needed on the concentrations of boron-10 in the target cells to reach definitive conclusions.

5. Conclusion

The effectiveness of neutrons from a Californium-252 source in the induction of various abnormalities in the *Tradescantia* clone 4430 stamen hair cells (TSH-assay) were studied. Special attention was paid to check whether any enhancement is visible in effects caused by process of boron neutron capture in the cells enriched with boron ions. Inflorescences, normal or pretreated with chemicals containing boron, were irradiated in the air with neutrons from a 252Cf source at KAERI, Taejon, Korea. Following the culturing according to standard procedures screening of gene and lethal mutations in somatic cells of stamen hairs have been done, and dose response relationships were plotted. To estimate the relative biological effectiveness (RBE) of the beam under the study, numbers of *Tradescantia* inflorescence without chemical pretreatment were irradiated with various doses of X-rays. The ranges of radiation doses used for neutrons were 0 - 1.0 Gy and for X-rays 0 - 0.5 Gy. A visible increase in a maximal RBE values was observed in present studies, which for the induction of gene mutations were estimated as 11.1 comparing the value 5.6 in the studies reported earlier. Presented results showed an increase, statistically significant, in biological efficiency of radiation from the 252Cf source in samples pretreated with boron containing chemicals. Inflorescence pretreated with borax responded to neutrons differently. Although, for the induction of gene mutations no significant difference was observed, though, in case of cell lethality the values of RBE have changed from 5.5 to 34.7 and from 1.6 to 5.6 for two various lethal end points respectively. Comparison was done of two independent experimental studies on alteration of dose-response curves due to changes in the post-exposure treatment of the cuttings.

Table 5. Average values from the whole scoring period of the biological effects average values of frequencies of mutation detected in the whole scoring period (PF, SPF, LMF, CDF — pink, single pink, lethal and colorless frequency, respectively) measured in *Tradescantia* clone T-4430 after irradiation with Californium-252 neutrons

Dose (Gy)	B-10 (ppm)	NoH[a]	PF×10^2±S.E.	SPF×10^2±S.E.	LMF×10^2±S.E.	CDF×10^2±S.E.
0	-	8670	0.32±0.07	0.03±0.02	0.26±0.13	0.00
0.01	-	7968	0.84±0.13	0.47±0.10	1.23±0.38	0.13±0.03
0.03	-	6844	2.40±0.26	1.14±0.18	1.13±0.31	0.76±0.10
0.05	-	7899	4.71±0.35	2.23±0.21	2.71±0.47	0.38±0.08
0.10	-	8576	5.53±0.40	2.61±0.49	3.90±0.78	0.57±0.10
0.20	-	8112	10.17±0.45	5.13±0.47	8.79±1.06	1.46±0.19
0	40	2759	0.28±0.14	0.06±0.04	0.00	0.00
0.01	40	6576	1.72±0.19	0.73±0.13	4.82±1.41	0.30±0.08
0.03	40	6106	2.84±0.36	1.71±0.25	8.24±2.25	0.41±0.09
0.05	40	4569	4.31±0.62	2.11±0.28	7.80±1.74	0.56±0.10
0.10	40	4466	4.61±0.48	2.55±0.30	30.63±7.46	2.72±0.47
0.20	40	2640	7.05±0.62	3.26±0.47	21.22±6.72	3.61±0.52

[a] NoH is a number of stamen hairs analyzed for the presence of mutation events.

Table 6. Average values from the whole scoring period for the biological effects measured in *Tradescantia* clone T-4430 after X-ray irradiation[a]

Dose (Gy)	NoH	PF×10^2±S.E.	SPF×10^2±S.E.	LMF×10^2±S.E.	CDF×10^2±S.E.
0	8670	0.32 ± 0.07	0.03 ± 0.02	0.26 ± 0.13	0.00
0.1	4300	1.36 ± 0.31	0.63 ± 0.17	0.69 ± 0.32	0.06 ± 0.02
0.3	4640	3.88 ± 0.48	1.88 ± 0.36	0.83 ± 0.36	0.32 ± 0.07
0.5	5865	7.03 ± 0.44	3.02 ± 0.24	2.98 ± 0.66	0.27 ± 0.06

[a] Abbrebiations are used as in the Table 5.

Table 7. Average values of cell cycle factor (CCF = SPF/PF) and significance (P) of differences between average values of cell cycle factors evaluated after exposures to X-rays and californium

Treatment	CCF±S.D.	Sum of squares	P
X-rays	0.40±0.30	8.1	
^{252}Cf	0.44±0.29	18.3	0.4565
$^{10}B + ^{252}Cf$	0.42±0.33	18.3	0.4565

Table 8. Values of maximal relative biological efficiencies (RBE) estimated for various biological end-points induced in *Tradescantia* 4430 clone after exposure to ^{252}Cf neutrons

Treatment	α_{PF}	RBE_{PF}	RBE_{SPF}	RBE_{LF}	RBE_{CDF}
X-rays	9.59±0.1	-	-	-	-
^{252}Cf	68.31±17.8	7.2±1.94	5.1	6.2	1.6
^{10}B + ^{252}Cf	59.24±17.4	6.2±1.86	5.8	34.3	5.6

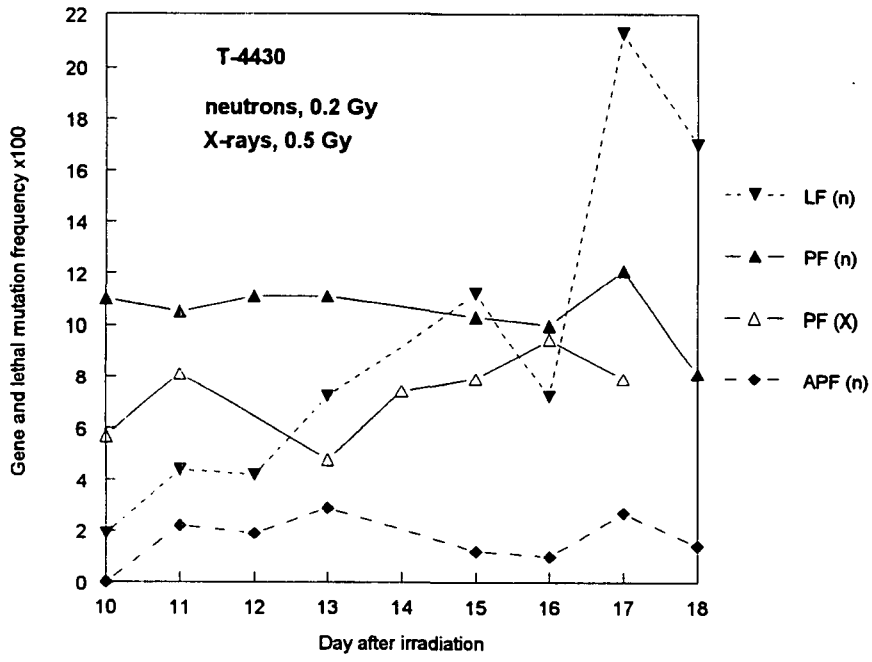


Fig. 6. Time dependent development of gene mutation frequencies (PF) in T-4430 irradiated with neutrons and X-rays. Coefficients α_n and α_x are presented respectively to each dose-response curves are estimated from best fits of those dose-response curves.

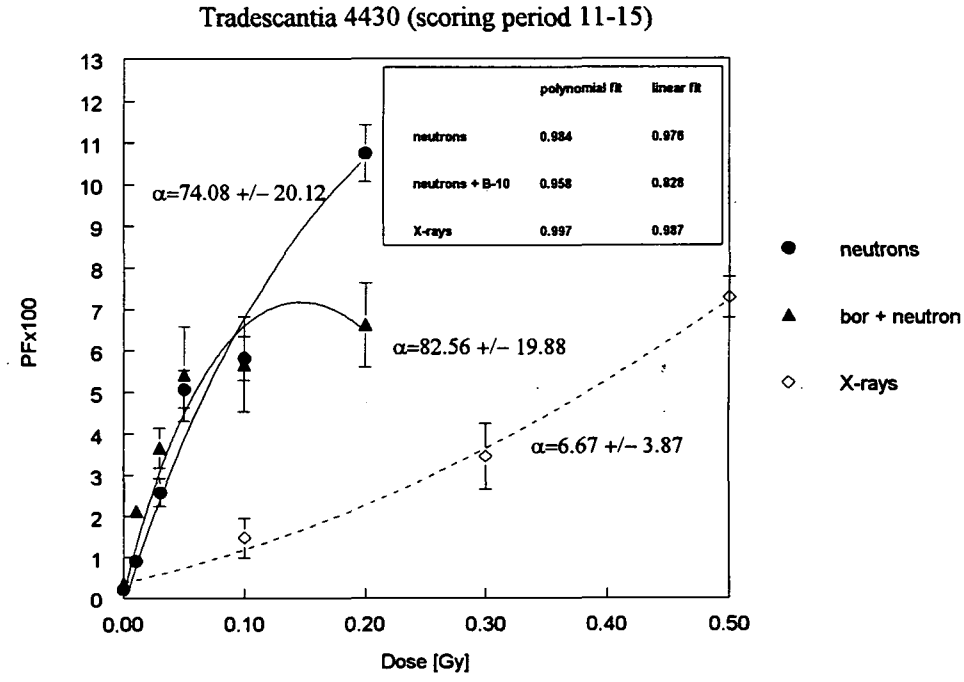


Fig. 7. Gene mutation frequencies (PF) in T-4430 cells irradiated with X-rays and with neutrons after or without borax pretreatment ; average values from the scoring period 11-15th day. Coefficients α_n and α_x are presented respectively to each dose response curves are estimated from best fits of those dose response curves.

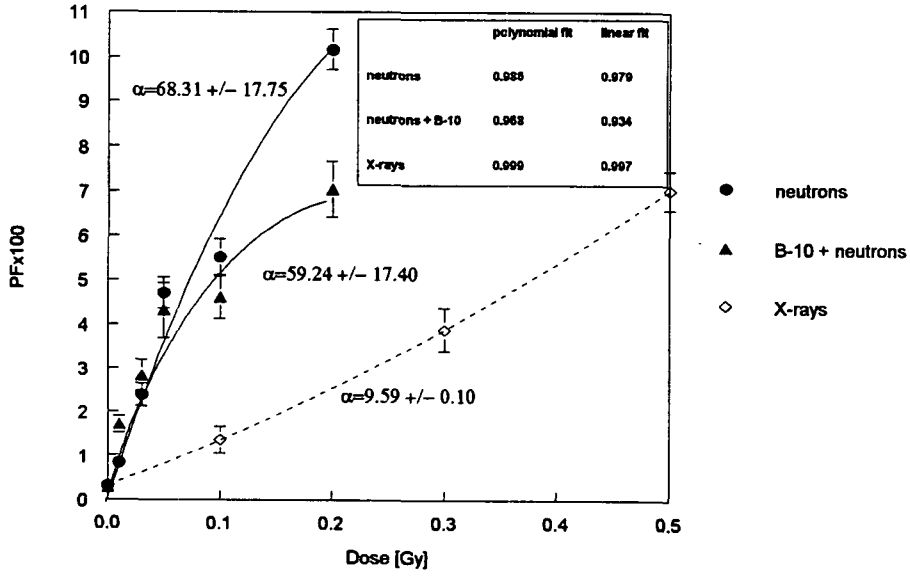


Fig. 8. Gene mutation frequencies (PF) in T-4430 cells irradiated with X-rays and with neutrons after or without borax pretreatment ; average values from the whole period. Coefficients α_n and α_x are presented respectively to each dose-response curves are estimated from best fits of those dose-response curves.

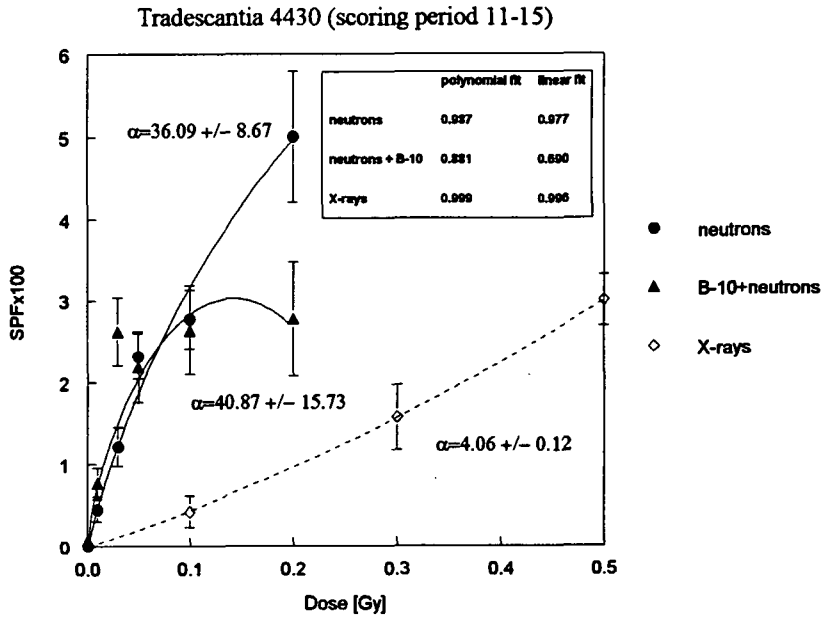


Fig. 9. Single cell gene mutation frequencies (SPF) in T-4430 cells irradiated with X-rays and with neutrons after or without borax pretreatment ; average values from the scoring period 11~15th day. Coefficients α_n and α_x are presented respectively to each dose-response curves are estimated from best fits of those dose-response curves.

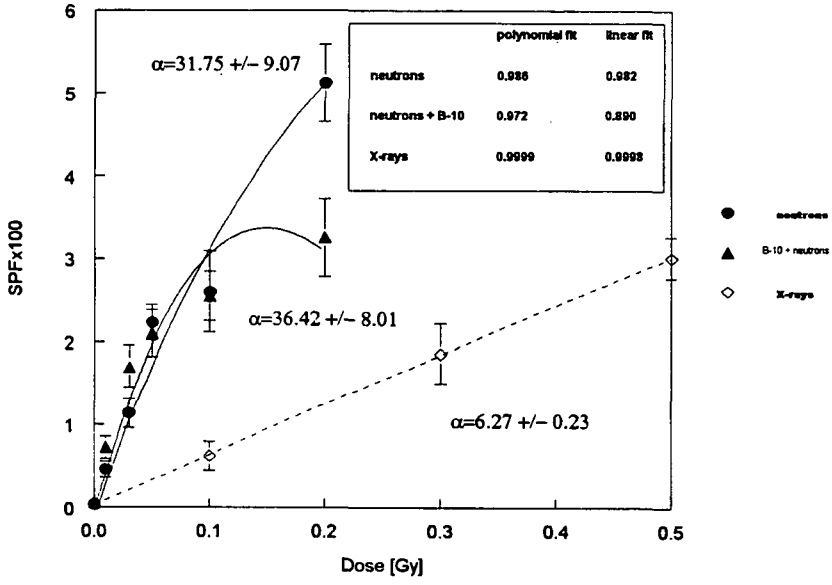


Fig. 10. Single cell gene mutation frequencies (SPF) in T-4430 cells irradiated with X-rays and with neutrons after or without borax pretreatment ; average values from the whole period. Coefficients α_n and α_x are presented respectively to each dose-response curves are estimated from best fits of those dose-response curves.

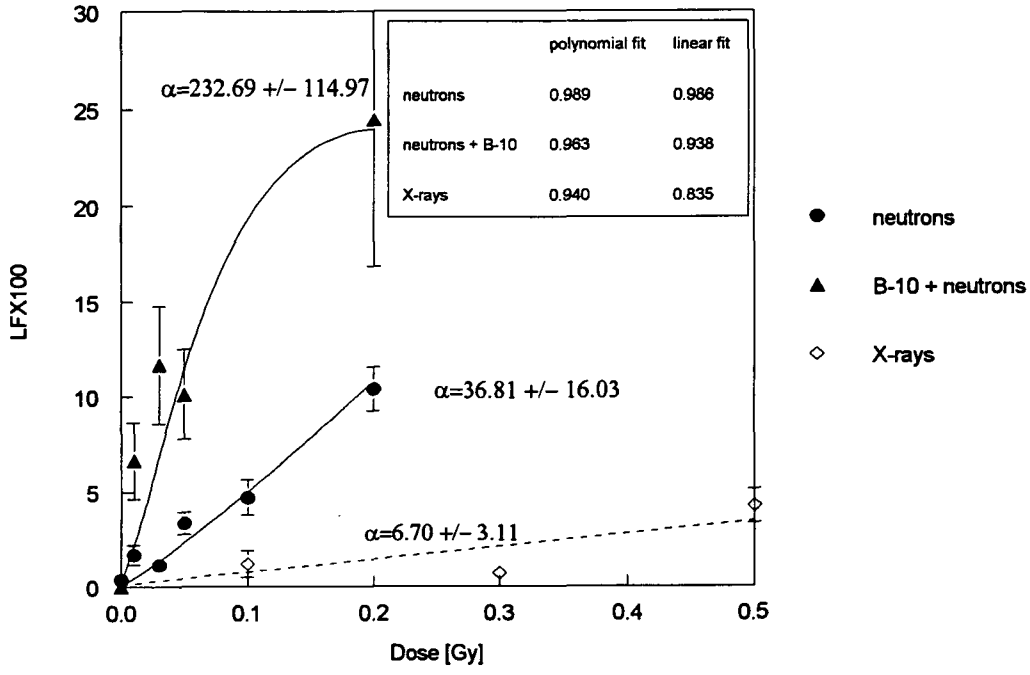


Fig. 11. Lethal mutation frequencies (LF) in T-4430 cells irradiated with X-rays and with neutrons after or without borax pretreatment. Coefficients α_n and α_x are presented respectively to each dose-response curves are estimated from best fits of those dose-response curves.

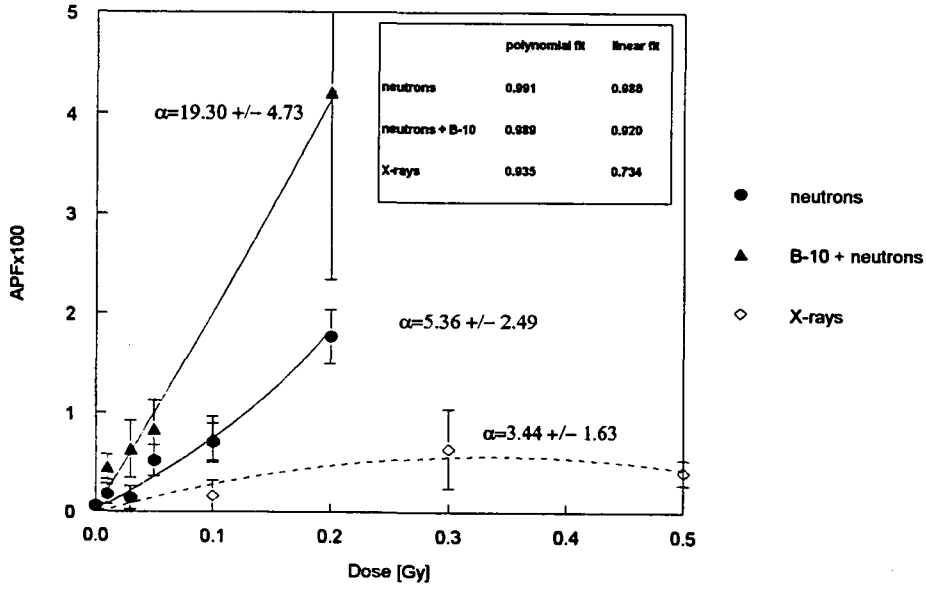


Fig. 12. Potential lethal mutation (apoptotic cells) frequencies (APF) in T-4430 cells irradiated with X-rays and with neutrons after or without borax pretreatment. Coefficients α_n and α_x are presented respectively to each dose-response curves are estimated from best fits of those dose-response curves.

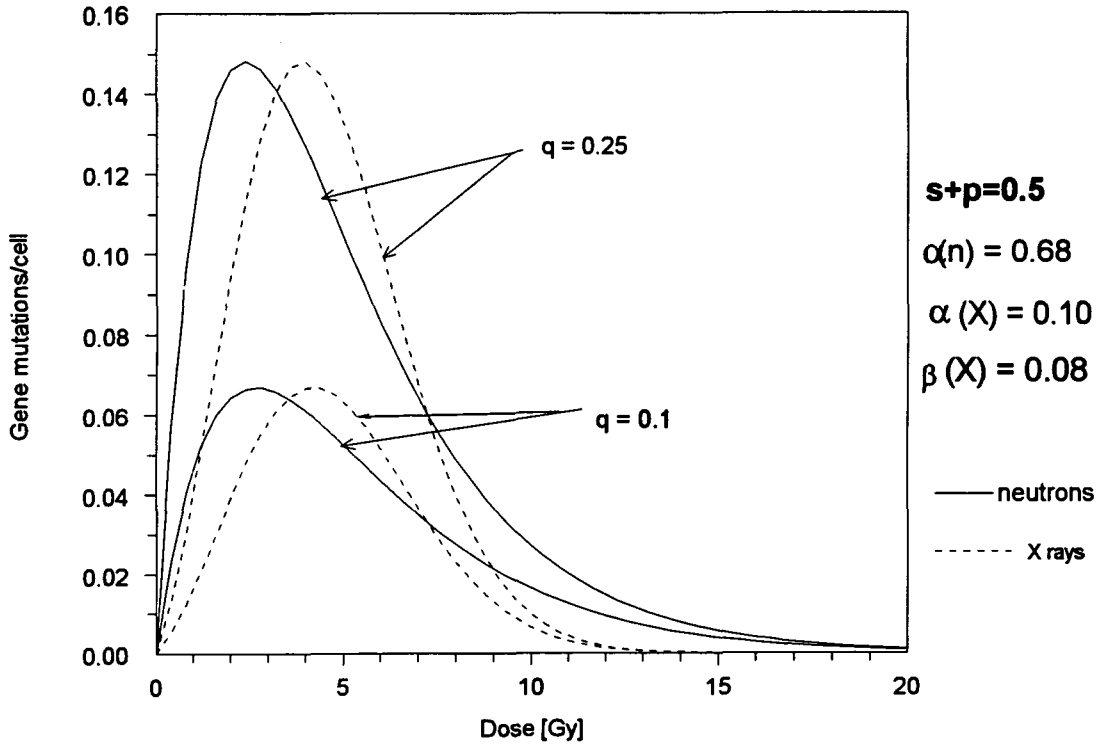


Fig. 13. Simulation of alteration of the probability that radiation induced DNA dsb lead to suppression of a specific mutation (s), or killing.

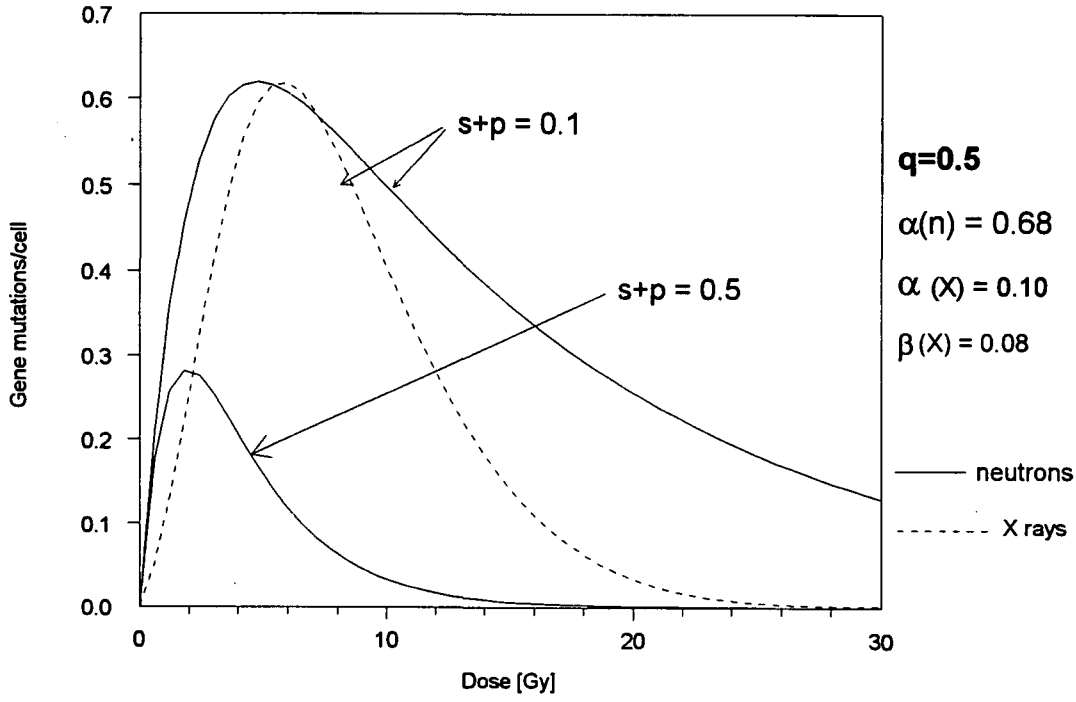


Fig. 14. Simulation of alteration of the probability that radiation induced DNA dsb lead to suppression of a specific mutation (s), or killing.

제3절 방사선에 의해 유발되는 TSH 돌연변이에 대한 살충제의 효과

(Section III. Effect of pesticide on radiation-induced mutations in TSH cells)

1. Introduction

Ionizing radiation can induce physical, chemical and biological changes in organisms, which results in various damage. The fact that radiation-induced damage can be modified by several factors is of great interest in that it is directly related to the protection of human beings. Physical factors can modify the radioresponse of organisms are the kind of radiations, irradiation types, environmental conditions of irradiation. Such biological factors as species, individual, age, metabolism and nutrition can also be modification factors. In addition, various chemicals and natural compounds have been known as radioprotectors or radiosensitizers that are applicable to nuclear medicine or radiation therapy. When the result of interaction between two factors is greater than the sum of the effects of individual factors, it is called synergism [69].

Agricultural pesticides are being widely used for eradication of bacteria, insects and the other organisms harmful to crops. The reason why organophosphorous insecticides have been widely used is their high selective toxicity. The insecticide such as parathion can also give rise to damage to human beings. The impurity and its metabolite of the insecticide are more toxic than the insecticide itself [77,81].

Tradescantia 4430 clone is a interspecific hybrid whose flower color is heterozygous (blue dominant, pink recessive). If the plant is exposed to mutagen, promutagen or ionizing radiation during the development of its inflorescence, distinct type of various gene and lethal mutations in the stamen hair cells can easily be detected. Accordingly *Tradescantia* stamen hair (TSH) assay has been a good tool for mutation study in many countries [71,73,81]. Many studies using TSH bioindicators have been carried out for botanical monitoring of environmental radiation [8], dose-response of neutrons and its RBE study [67],

and studies on the effect of environmental factors on radiation-induced mutations [8].

The present study was designed to experimentally elucidate the combined effect of pesticide with ionizing radiation using TSH bioindicators.

2. Materials and methods

2-1. Experimental plants

Tradescantia 4430 clone was used as experimental plants. The healthy plants grown in the pots were taken into the laboratory for acclimation 24 hours before the experiment. Each experimental group composed of more than 20 pots.

2-2. Treatment of pesticide

Pesticide was commercially available parathion (Cheil Chemical Co., made in 1997). The parathion was diluted to 1 ㎎/ℓ, which is recommended for agricultural purpose. The solution was sprayed evenly over the inflorescence. Irradiation was done 24 hours after the treatment of parathion.

2-3. Irradiation

The plants were exposed in the air to 0.3, 0.5, 1.0 and 2.0 Gy of gamma ray from the ^{60}Co source (source strength 150 TBq, Panoramic Irradiator, Atomic Energy of Canada Ltd.).

2-4. Culture of the plants

After irradiation the plants in the pots were moved into the growth chamber and Hoagland's No. 2 solution (1/6 diluted) was supplied every three days as a fertilizer [70]. The experimental group irradiated only with gamma ray(CT) and the group irradiated after pretreatment of parathion (Pa+γ) were cultured under the same condition (14 light, 20℃, relative humidity 80%, irradiance 293 $\mu E/m^2/sec$).

2-5. Scoring of mutations

Examinations of mutations were done under the stereomicroscope (x25). Stamen hairs were carefully removed from the flowers with forceps and placed on the slide glass upon that a proper amount of mineral oil was spread. Scoring was done to find out the mutated cells for determination of:

PF, SPF - gene mutations frequencies characterized by numerous adjacent pink cells or single pink cell in the hair and each of them was counted as one gene mutation event,

LMF - lethal mutations frequencies characterized by stunted hair (defined as a hair in upper third part of stamen containing less than 17 cells in T-4430).

Mutation frequencies were calculated from the pooled date during the peak interval and expressed as pink mutations/100 hairs.

3. Result and discussion

Pink mutation frequency did not change with time after pesticide spray (Fig. 15). This result means that the recommended concentration of parathion does not cause an increase in mutation events. In all irradiated groups, pink mutation frequencies started to increase seven days after irradiation and reached maximum values 9 to 11 days after irradiation (Figs. 16-18). The maximum value of pink mutation frequency of CT group was much higher than that of Pa+γ group (Fig. 16). From the pooled data during the peak interval (7~11 days after irradiation), pink mutation frequency for each radiation dose was calculated. The pink mutation frequencies showed a linear dose-response relationship (Fig. 19). α, the increase coefficient of pink mutation (slope of the dose-response curve), is 5.99 in CT group. In contrast, α in Pa+γ group is 3.43. This result means that the pretreatment of parathion supressed the radiation-induced pink mutations by 43%.

The dose-response relationship for the induction of gene mutations in *Tradescantia* can be well explained by the molecular theory of radiation action described by Chadwick and Leenhouts (1980) [52]. The dose-response relationship, however, in the range of low dose is generally linear. Accordingly

it can be expressed by the simple linear equation as follow;

$$M = \alpha D + b_0 \quad \text{----------} \quad (1)$$

where,

M = mutation frequency,

α = mutations induced by unit dose,

D = radiation dose (Gy),

b_0 = spontaneous mutation frequency.

The above equation can be easily applied when data from the experiments are not sufficient because it give rise to a minimal decrease in the degree of freedom of data. Taken into consideration the fact that the present experiment was carried out with low radiation doses, the application of the linear equation to explain the dose-response relationship is valid [7].

There have been many reports on the synergistic interaction between two or more stimuli which are unfavorable to the biological systems [68,69,72,76,78,79]. Titenko-Holland *et al.* (1997) reported the possible protection by the low concentration of marathion rather than damage in the micronucleus assay on the human lymphocytes [83]. Furthermore none or little effect of methyl parathion in the induction of mutation was also reported by Sobti *et al.* (1982, [80]) and Kaur & Grover (1983, [75]). Kas'yanenko & Koroleva (1979) reported that chemical mutagen like ethylenimine and pesticides such as granosan, sevin, captan, and morotsin could increase the cell sensitivity [74].

The result of this study showed the decrease in the induction of mutations in TSH cells due to the pretreatment of pesticide. If the kind of stimuli is the same, the adaptive response is possible. Ionizing radiation and pesticide are definitely different stimuli on the biological system. However, the reduced mutation frequency seemed like the result of adaptive response or radioprotection due to the pretreatment of pesticide. It should be further investigated the mechanism of the effect of parathion pretreatment. The result of this experiment is of great importance since it provides the clue for the pesticide effect on the radiation-induced mutations.

4. Abstract

To investigate the combined effect of radiation and pesticide on *Tradescantia* somatic cell mutations, potted plants of *Tradescantia* 4430 on which parathion had been sprayed evenly 24 hours before irradiation. Radiation doses were 0.3, 0.5, 1.0 and 2.0 Gy of gamma-ray. The plants irradiated only with the gamma-ray radiation were used as control groups(CT). Pink mutation frequency increased linearly proportional to the radiation dose and the peak interval of elevated mutation frequencies appeared during 7~11 days after irradiation in both CT and Pa+γ groups. The slope of dose-response curve in CT was 5.99(r^2=0.988), while it was 3.43(r^2=0.981) in Pa+γ. It seemed that parathion pretreatment had a protective effect against radiation-induced cell damages since it decreased the slope value by 43%. It is suggested that an adaptive response or radiomodification could be induced in irradiated stamen hair cells by parathion pretreatment.

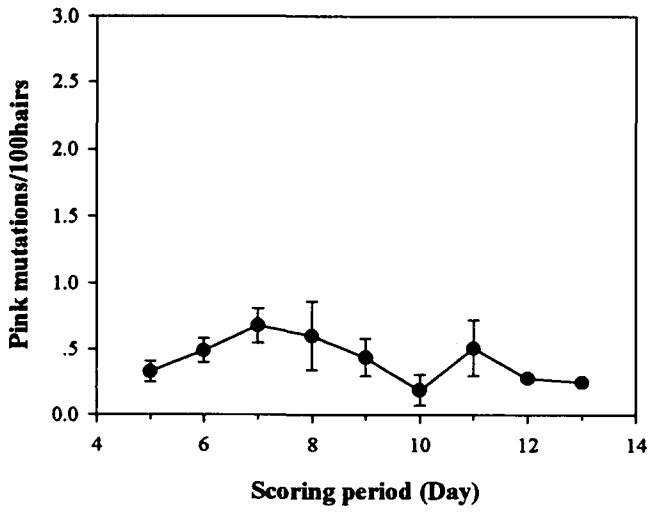


Fig. 15. Daily changes of pink mutation frequencies in stamen hairs of the *Tradescantia* after parathion (1㎎/ℓ) spray onto inflorescences.

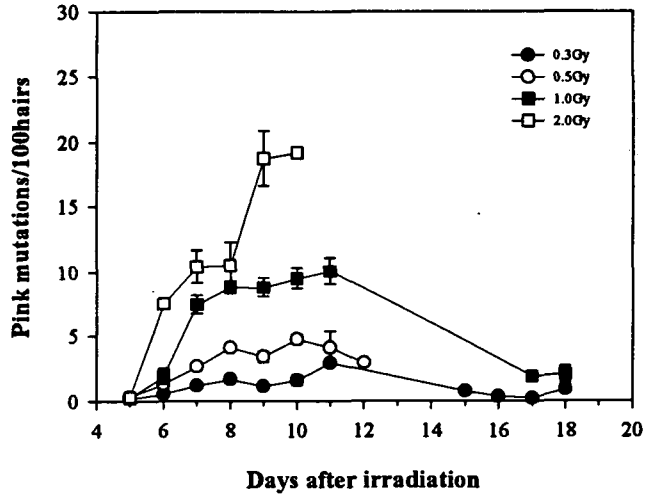


Fig. 16. Daily changes of pink mutation frequencies in stamen hairs of the *Tradescantia* control group irradiated with gamma ray.

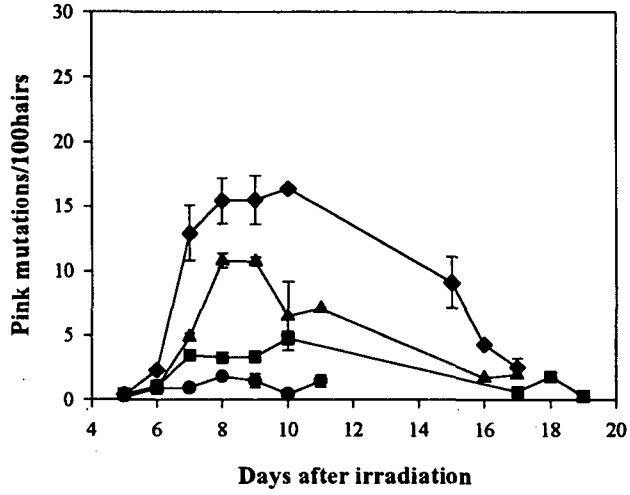


Fig. 17. Daily pink mutation frequencies in TSH cells treated with parathion 24 hour after irradiation with gamma ray (● 0.3, ■ 0.5, ▲ 1.0, ◆ 2.0 Gy).

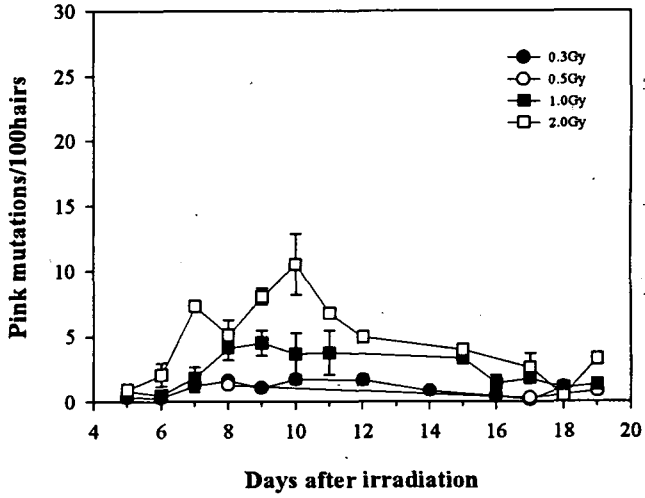


Fig. 18. Daily pink mutation frequencies in TSH cells irradiated with gamma ray 24 hours after parathion treatment.

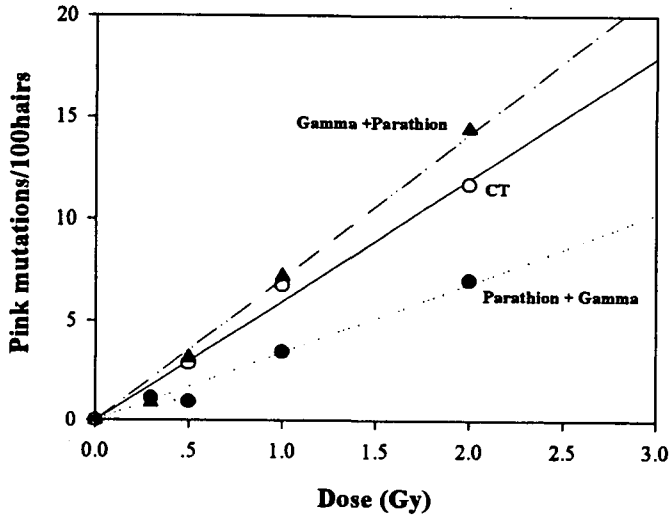


Fig. 19 . Dose-response relationships of pink mutation frequencies induced in stamen hairs of the *Tradescantia*

제4절 감마선 및 살충제의 상호작용이 사람 림프구 손상에 미치는 영향

(Section IV. Interaction of radiation with pesticide on DNA damage in human peripheral blood lymphocytes)

1. Introduction

Agricultural pesticides are being widely used for eradication of bacteria, insects and the other organisms harmful to crops. The organophosphorous pesticides such as parathion and marathion have high toxicity on insects and are also toxic to human beings. The impurity and its metabolite of the insecticide are more toxic than the insecticide itself [77,82]. The toxicity of the insecticide can be one of causes of environmental or agricultural disaster since the use of insecticide tends to increase year by year.

Radiation effects in biological systems is closely related to free radicals which cause damage in DNA or macromolecules [88]. Radiation-induced DNA damage can be changed by various physico-chemical factors [60,86]. Physical factors can modify the radioresponse of organisms are the kind of radiations, irradiation types, environmental conditions of irradiation. Such biological factors as species, individual, age, metabolism and nutrition can also be modification factors. In addition, various chemicals and natural compounds have been known as radioprotectors or radiosensitizers that are applicable to nuclear medicine or radiation therapy. When the result of interaction between two factors is greater than the sum of the effects of individual factor, it is called synergism [88,90].

Comet assay, known as a single cell gel electrophoresis (SCGE), was introduced by Ostling and Johanson (1984) at first and named after the appearance of comet [18]. This assay is considered to be a sensitive methodology for monitoring of genotoxic effects [93,94]. The use of this assay has been greatly increased in many fields [18,20,83,84].

Above all, the alkaline single-cell gel electrophoresis assay has been used as a powerful and sensitive technique for detecting different types of DNA

damages, ranging from DNA single-strand breaks to alkali-labile lesions, in individual eukaryotic cells [20,28,89]. The comets, consisting of heads and tails, reflect the types of DNA damages and the degree of DNA strand breaks. The advantage of the comet assay is that it doesn't require massive amount of cells and that the detection of damage in an individual cells can be made in the very quick and efficient way.

This study aimed to assess the cytotoxicity of pesticide and to analyse the combined effects of pesticide with ionizing radiation on the human blood lymphocytes by using the single cell gel electrophoresis.

2. Materials and methods

2-1. Isolation of lymphocytes

Human peripheral blood samples were obtained from a healthy male donor (28 years old). Lymphocytes were isolated by mixing 100 $\mu\ell$ of heparinized blood and 200 $\mu\ell$ of RPMI 1640 (Sigma) medium with 10% fetal bovine serum (FBS) and kept on ice. Two hundreds microliters of Histopaque 1077 (Pharmacia) was underlaid 300 $\mu\ell$ RPMI 1640 diluted blood. The blood cells were centrifuged at 400 X g for 4 min at 4℃. The obtained ficoll layer was washed in 1 ml cold phosphate buffered saline (PBS, pH 7.4). Viability of cells after isolation, determined using the trypan blue exclusion was ≥98%.

2-2. Treatment of pesticide

Pesticide was commercially available parathion (Cheil Chemical Co., made in 1997). The parathion was diluted to 1, 5, and 10 ㎎/ℓ. The isolated lymphocytes were treated with the parathion solution for 10 minutes at 4℃.

2-3. Irradiation

Irradiation was done at 4℃ with various doses (0, 1, and 2 Gy) of gamma ray from ^{60}Co source (dose rate: 20.1 cGy/min., source strength: 150 TBq, Panoramic Irradiator, Atomic Energy of Canada Ltd.) at Korea Atomic Energy Research Institute (KAERI). Under the same condition of ^{60}Co source,

the normal isolated lymphocytes without pesticide pretreatment were irradiated with the same range of doses.

2-4. Comet assay

The comet assay was performed as described by Singh *et al.* (1988) with minor modifications [20]. All steps were carried out on ice to avoid repairing of damaged cell DNA and under dim light to prevent the occurrence of additional DNA damage. Two hundred microliters of 1% normal melting point agarose (NMA) diluted in distilled water was added at 50℃ to fully frosted microscope slides, and kept for 10 min at room temperature to dry. The coverslips were removed. As a second layer, 100 ㎕ of 0.75% low melting point agarose (LMA) was added together with 1-2 X 10^5 suspended cells and covered with a coverslip. The slides were kept cold for 10 min at 4℃. After removal of the coverslips, 100 ㎕ of 0.75% LMA was added as a third layer and then the slides were again covered with coverslips, kept for 10 min at 4℃ and removed the coverslips. The slides were immersed in a jar containing cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100 and 10% DMSO were added fresh). The slides were kept at 4℃ for at least 1 hr. After the lysis, the slides were placed on a horizontal electrophoresis chamber. The unit was filled with a freshly made alkaline electrophoresis buffer (300 mM NaOH and 1 mM EDTA, pH 13.0) to a level of 0.25 cm above the slides. The cells were exposed to alkali for 20 min to allow for DNA unwinding and expression of alkali-labile sites. For the DNA electrophoresis an electric current of 25 V (0.75 V/cm) and 300 mA was applied for 20 min. After electrophoresis, the slides were placed horizontally and some drip of neutralization buffer (0.4 M Tris, pH 7.4) was added to remove the excess alkali. Finally, 50㎕ ethidium bromide (20 ㎍/ml) was added to each slide, covered with a coverslip, kept in a humidified box. DNA damage was identified using the image analyzing system Kinetics (Version 4.0). The images of 50 randomly selected cells (25 cells from each of two replicate slides) were analyzed from each group from a fluorescence microscope (Olympus BX50) equipped with an excitation filter of 515~560 nm and a barrier filter of 590 nm.

3. Result and discussion

Assessment of DNA damages in lymphocytes is regarded as the typical way of cellular damage detection [84]. The level of DNA strand breaks of normal cells are morphologically different from that of damaged cells in the comet assay. Normal cells show the round shaped nucleoid. In contrast, the comet of damaged cells consists of head and tail [86,89]. In case of the dead cells, their comets are distinct and are called as apoptotic comets (Fig. 20).

The length of the comet tail can be simply used as indication of the level of DNA damages. The tail length, however, can lead to an overestimation of DNA damage when a small fractions of damaged DNA forms an abnormally long tail. To avoid such a shortcoming, we used the tail moment value instead of the simple tail length. The tail moment is defined as the following equation.

$$\text{Tail Moment} = |\,\text{tail mean} - \text{head mean}\,| \times \text{tail \% DNA} / 100 \quad \text{------ (1)}$$

where,

tail mean is the average intensity of fluorescence staining in tail,

head mean is the average intensity of fluorescence staining in head.

The lymphocytes pretreated only with PBS were exposed to gamma-radiation doses from 0 to 2 Gy. The tail moment value in the comet assay reflects the level of DNA damages. As can be seen in Fig. 21, the increases in the tail moments clearly indicate the dose-response relationship of cellular DNA damage with radiation dose. The tail moment of unirradiated cotrol cells was 2.9 ± 0.28 while it was 4.1 ± 0.67, 9.9 ± 0.81, and 15.9 ± 1.73 in 0.1, 0.5, and 2.0 Gy irradiated group, respectively.

Fig. 22 shows the result of SCGE assay on lymphocytes treated with parathion for 10 minutes. The level of DNA damage, expressed as the tail moment, increased with the concentration of parathion. Higher concentrations of parathion induced significant damages in lymphocyte DNA.

When treated with the concentration of 1 mg/ℓ, the recommended concentration for agricultural use, DNA damage was slightly higher than that of

untreated control cells but the difference is statistically not significant. However synergistic effect on DNA damage in synergistic effect in the lymphocytes irradiated with 2.0 Gy of gamma-ray after pretreatment of 1 mg/ℓ parathion. As shown in Fig. 23, the tail moment was 15.0±2.27 in 2.0 Gy irradiated cells, and 5.3±1.13 in 1 mg/ℓ parathion treated cells while it significantly increased upto 21.3±2.17 in the lymphocytes irradiated with 2.0 Gy of gamma-ray after pretreatment of 1 mg/ℓ parathion ($p<0.01$). This implies that even the recommended concentration of parathion can induce high DNA damage when interact synergistically with the other factor like ionizing radiation. From the fig. 24 we can easily notice the synergistic effect of pesticide with gamma-radiation. The result indicates that the synergistic interaction should be taken into consideration for biological or environmental risk assessment.

The tail moment in unirradiated lymphocytes normally ranges 2~3. The value of the present study was in the normal range. The lower limit of detection for ionizing radiation was differently reported. Singh *et al.* (1995) reported it as 0.001 Gy for gamma-radiation [92]. Plappert (1995) reported 0.01 Gy of the lower limit of X-ray detection [91]. As we can know from the lower limit of radiation detection, the comet assay is a reliable method for assessing radiation damage in the lymphocytes and for analyzing the level of synergistic interaction between two different factors.

4. Abstract

Agricultural pesticides may cause certain biological risks since they are widely used to eradicate pests. Agricultural disasters may arise even from the possibility of their synergistic interaction with other harmful enviromnetal factors. The effect of pesticide on radiation-induced DNA damage in human blood lymphocytes was evaluated by the single cell gel electrophoresis (SCGE) assay. The lymphocytes, with or without pretreatment of the pesticide, were exposed to 0~2.0 Gy of ^{60}Co gamma ray. Significantly increased tail moment, which was a marker of DNA strand breaks in SCGE assay, showed an excellent dose-response relationship. The present study confirms that the

pesticide has the cytotoxic effect on lymphocytes and that it shows the synergistic interaction with radiation on DNA damage as well. The results may have a role of providing biological information necessary for the prevention of agricultural disaster.

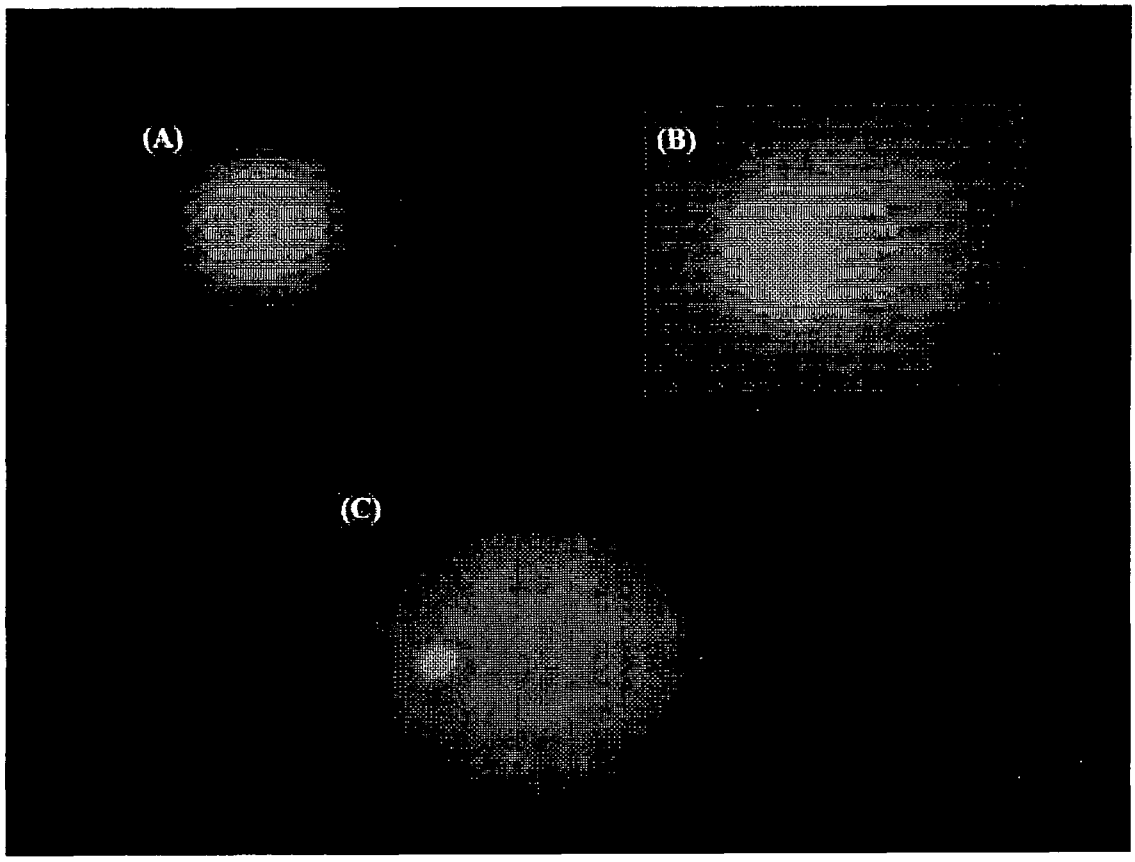


Fig. 20. Appearance of typical DNA comets. The unirradiated comet is shown in (A), the irradiated comet in (B), and the apoptotic comet in (C).

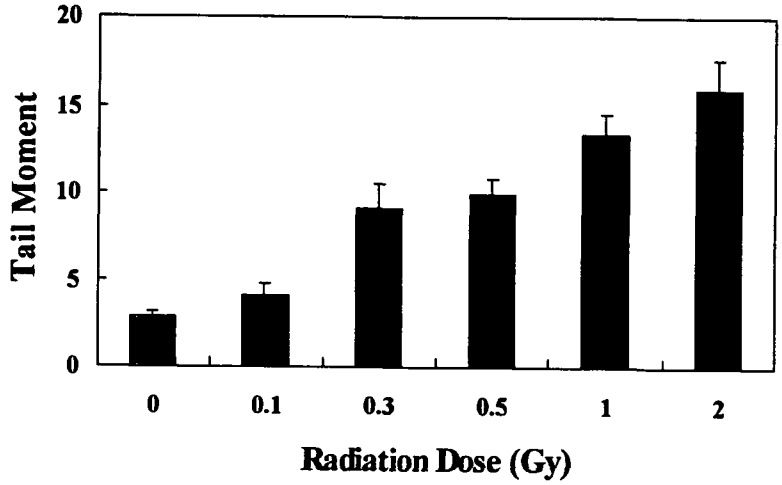


Fig. 21. Dose-response relationship of DNA damage in human lymphocytes exposed to γ-ray doses from 0 to 2.0 Gy. Error bars represent the standard error of the mean among 50 cells (25 cells per each slide).

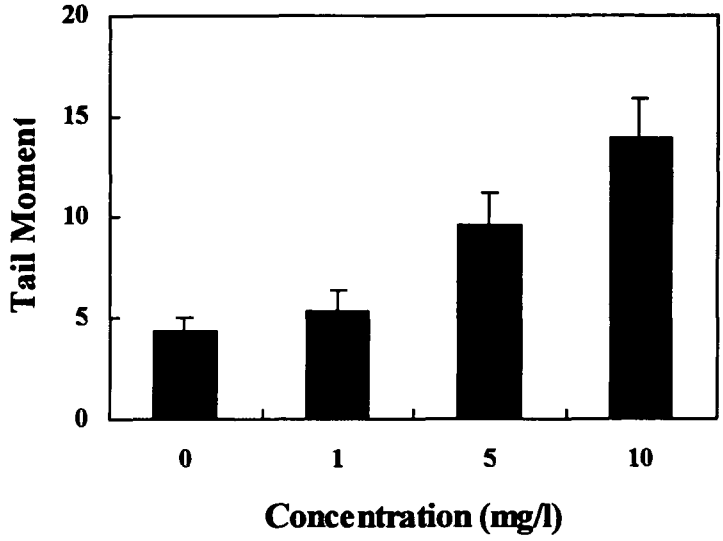


Fig. 22. DNA damage in human lymphocytes treated with various concentrations of parathion. Error bars represent the standard error of the mean among 50 cells (25 cells per each slide).

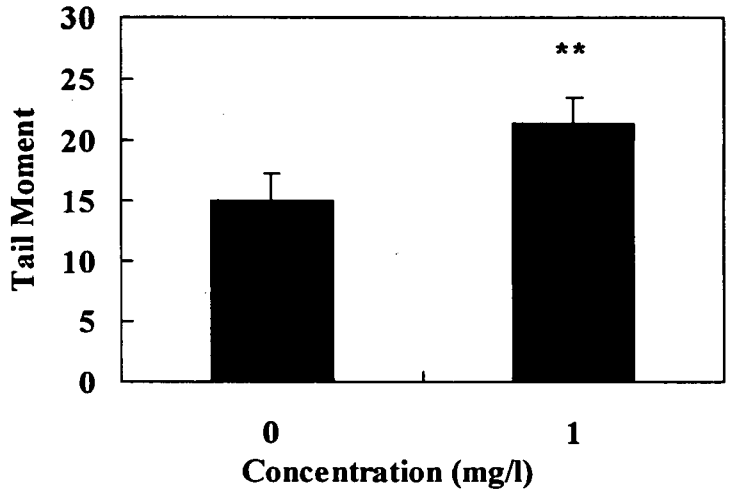


Fig. 23. DNA damage in human lymphocytes exposed to 2 Gy of γ-ray after pretreatment with 1 mg ℓ^{-1} of parathion. Error bars represent the standard error of the mean among 50 cells (25 cells per each slide) (**, $p<0.01$).

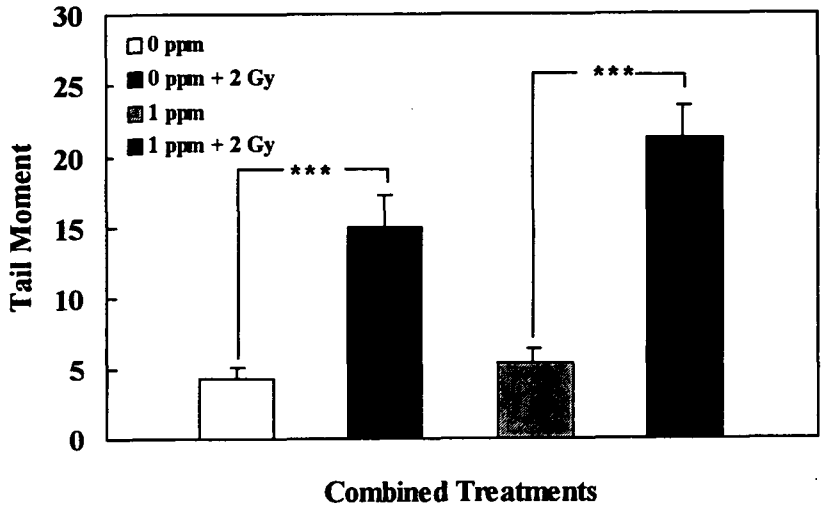


Fig. 24. Synergistic interaction of parathion with radiation on DNA damage in human lymphocytes. Error bars represent the standard error of the mean among 50 cells (25 cells per each slide) (***, $p<0.001$).

제5절 사람 림프구 DNA에 미치는 방사선과 B 및 Gd 화합물의 영향

(Section V. Radiomodifying effect of boron and gadolinium compounds in human peripheral blood lymphocytes)

1. Introduction

Comet assay, known as a single cell gel electrophoresis (SCGE), was introduced by Ostling and Johanson (1984) at first and named after the appearance of Comet [18]. This assay is considered to be a sensitive methodology for monitoring of genotoxic effects [93,94]. Above all, the alkaline single-cell gel electrophoresis assay has been used as a powerful and sensitive technique for detecting different types of DNA damages, ranging from DNA single-strand breaks to alkali-labile lesions, in individual eukaryotic cells [20,28,89]. The comets, consisting of heads and tails, reflect the types of DNA damages and the degree of DNA strand breaks.

The clinical application of treating brain tumors with boron neutron capture therapy (BNCT) is very encouraging. Also, ^{157}Gd is one of the nuclides that hold interesting properties of being a neutron capture therapy agent [102]. For neutron capture therapy (NCT), neutron beams often have a considerable portion of gamma rays and fast neutrons having high energies in the range of several MeV [100,103]. Gamma ray as one of the beam contaminants can cause considerable damage to normal tissues even if such tissues do not contain high 10 B concentrations. The present study was designed to study the modifying effect of boron and gadolinium compounds on the biological efficacy of gamma-ray in human lymphocytes using the comet assay to support the fundamental understanding of neutron capture therapy. Besides the neutron capture reactions, the treatment of the two chemicals can also give rise to radiomodifying effects in various biological systems irradiated with sparsely ionizing radiation. Such a modification of radioresponse in biological systems is an interesting phenomenon from the radiobiological point of view. In the present

study, with an application of the comet assay we examined the effect of boron and gadolinium compounds on the lymphocyte DNA damages induced by gamma-ray, one of the therapeutic neutron beam contaminants.

2. Materials and Methods

2-1. Isolation of lymphocytes

Human peripheral blood samples were obtained from a healthy male donor (28 years old). Lymphocytes were isolated by mixing 100 $\mu\ell$ of heparinized blood and 200 $\mu\ell$ of RPMI 1640 (Sigma) medium with 10% foetal bovine serum (FBS) and kept on ice. Two hundreds microliters of Histopaque 1077 (Pharmacia) was underlaid 300 $\mu\ell$ RPMI 1640 diluted blood. The blood cells were centrifuged at 400 X g for 4 min at 4℃. The obtained ficoll layer was washed in 1 ml cold phosphate buffered saline (PBS, pH 7.4). Viability of cells after isolation, determined using the trypan blue exclusion was ≥98%.

2-2. Chemical treatment and Irradiation

Isolated lymphocytes were treated with various concentrations (0~250 nM) of boron (Borax; $Na_2B_4O_7 \cdot 10H_2O$ supplied by Hayashi Pure Chemical Co., Osaka) and gadolinium (Gd-DTPA; as Magnevist supplied by Schering, Berlin) compounds for 10 min at 4℃, and then irradiated at 4℃ with various doses (0, 1, 2 and 4 Gy) of gamma ray from ^{60}Co source (dose rate: 20.1 cGy/min., source strength: 150 TBq, Panoramic Irradiator, Atomic Energy of Canada Ltd.) at Korea Atomic Energy Research Institute (KAERI). Under the same condition of 60Co source, the normal isolated lymphocytes without chemical pretreatment were irradiated with the same range of doses.

2-3. Comet assay

The comet assay was performed as described by Singh *et al.* (1988) with minor modifications [20]. All steps were carried out on ice to avoid repairing of damaged cell DNA and under dim light to prevent the occurrence of additional DNA damage. Two hundred microliters of 1% normal melting point

agarose (NMA) diluted in distilled water was added at 50℃ to fully frosted microscope slides, and kept for 10 min at room temperature to dry. The coverslips were removed. As a second layer, 100 ㎕ of 0.75% low melting point agarose (LMA) was added together with $1-2\times10^5$ suspended cells and covered with a coverslip. The slides were kept cold for 10 min at 4℃. After removal of the coverslips, 100 ㎕ of 0.75% LMA was added as a third layer and then the slides were again covered with coverslips, kept for 10 min at 4℃ and removed the coverslips. The slides were immersed in a jar containing cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100 and 10% DMSO were added fresh). The slides were kept at 4℃ for at least 1 hr. After the lysis, the slides were placed on a horizontal electrophoresis chamber. The unit was filled with a freshly made alkaline electrophoresis buffer (300 mM NaOH and 1 mM EDTA, pH 13.0) to a level of 0.25 cm above the slides. The cells were exposed to alkali for 20 min to allow for DNA unwinding and expression of alkali-labile sites. For the DNA electrophoresis an electric current of 25 V (0.75 V/cm) and 300 mA was applied for 20 min. After electrophoresis, the slides were placed horizontally and some drip of neutralization buffer (0.4 M Tris, pH 7.4) was added to remove the excess alkali. Finally, 50㎕ ethidium bromide (20 ㎍/ml) was added to each slide, covered with a coverslip, kept in a humidified box. DNA damage was identified using the image analyzing system Kinetics (Version 4.0). The images of 50 randomly selected cells (25 cells from each of two replicate slides) were analyzed from each group from a fluorescence microscope (Olympus BX50) equipped with an excitation filter of 515~560 nm and a barrier filter of 590 nm.

2-4. Statistical analysis

The significance of the difference was statistically evaluated by the non-parametric Mann-Whitney *U*-test (Instat, GraphPad software) in the effects between the experimental and the control groups.

3. Results

The results of this study showed the radiomodifying effects of boron and gadolinium compounds in human lymphocytes irradiated with gamma-ray using the comet assay.

3-1. Effect of gamma-radiation

The lymphocytes pretreated only with PBS were exposed to gamma-radiation doses from 0 to 4 Gy. The tail moment value in the comet assay reflects the level of DNA damages. As can be seen in Fig. 25 and Fig. 26, the increases in the tail moments clearly indicate the dose-response relationship of cellular DNA damage with radiation dose.

3-2. Effect of boron compound (Borax)

The results of this study indicated that both boron and gadolinium compounds had genotoxic effects on human lymphocytes *in vitro* (Fig. 25 and Fig. 26). Borax is previously known as cytotoxic agent [98]. However we found that borax had genotoxic effects as well. The radiation-induced DNA damages in boron-pretreated cells are shown in Fig. 25. The tail moments were increased by the treatment of boron compound in non-irradiated cells. However, the pretreatment of the boron compound resulted in decreased tail moments after irradiation. The higher concentration (250 nM) of boron was more efficient in decreasing DNA damages than the lower concentration.

3-3. Effect of gadolinium compound (Gd-DTPA)

The lymphocytes pretreated with 250 nM Gd-DTPA were found inappropriate for the comet assay because of its cytotoxicity. Thus the effects of 50 nM Gd-DTPA on human lymphocytes is shown in Fig. 26. The treatment of Gd-DTPA caused a significant increase in DNA damages not only in the non-irradiated control but also in the irradiated groups. The dose-response relationship of DNA damages was linear in the dose range 0 to 2 Gy. DNA damages in the Gd-DTPA pretreated cells were increased by more than 50 % at a dose of 4 Gy.

3-4. Effect of the mixture of boron and gadolinium compounds

The damages in DNA of lymphocytes pretreated with a mixture (1:1) of boron and gadolinium compounds were also evaluated by means of comparing the difference between the experimental groups (Fig. 27). The mixtures were the 50 or 250 nM of boron compound mixed with the same volume of 50 nM of gadolinium compound. The mixture caused a definite increase in the tail moment values in all the groups. The increase in the tail moments was somehow related with the concentration of boron compound in the mixture in both non-irradiated and irradiated groups. Higher concentration of boron compound in the mixture made a role in a decrease of DNA damages. This result also confirmed the decrease in radiosensitivity of lymphocyte DNA due to the pretreatment of boron compound. In addition, both type of the mixtures had the radiomodifying effects on the lymphocytes, by which the decrease in DNA damages appeared in a boron concentration-dependent manner.

4. Discussion

Recently, the use of the comet assay, which has many advantages for genotoxicity studies, has been increasing [93,101]. It appears to be sensitive enough for the detection of DNA damages induced by a low dose of radiation [94,97]. It was also shown that DNA damages detected by SCGE assay are directly related to the chromosomal damage in following mitosis [85,95]. Ionizing radiation is known to induce a variety of cellular responses. It is also well known that ionizing radiation causes DNA damages, inducing predominantly single-strand breaks, double-strand breaks, alkali-labile sites, and oxidized purines and pyrimidines [96].

The objective of the present experiment was to study the modification effect of boron and gadolinium compounds on gamma-ray-induced DNA damages using the comet assay. The results indicate that the radiomodifying effect of sodium borate (Borax) is different from those of other boron compounds, such as sodium borocaptate-^{10}B (BSH) and boronophenylalanine-^{10}B (BPA) and so on [99]. Though this study revealed that the pretreatment of sodium borate caused a decrease in radiosensitivity of lymphocyte DNA to gamma ray, the detailed

mechanisms of radiomodification by boron compounds remain to be further elucidated. It is also of interest to note that, DAC-1, one of boron neutron capture agents did not affect the biological efficacy of gamma-ray [104].

Gd-DTPA is widely used to enhance the contrast of magnetic resonance imaging. And the element of gadolinium gathers a lot of attention since it has a high neutron capture cross section. The present study was encouraged by the possibility of the gadolinium compound as being an NCT agent. A high concentration of Gd-DTPA proved highly cytotoxic to the lymphocytes. The cells treated with 250 nM Gd-DTPA were not suitable for further evaluation. On the other hand, addition of 50 nM of the compound led to a significant increase in the DNA damage of lymphocytes in terms of tail moment (Fig. 26). The tail moment values of the lymphocytes treated with Gd-DTPA before γ -irradiation were higher than those of the cells irradiated with gamma-ray without any pretreatment. However, the increase in the tail moment values was not dose-dependent. The increase in DNA damage seen after the addition of Gd-DTPA may indicate the production of hydroxyl radicals, as increasing of sister chromatid exchange (SCE) in the presence of Gd-DTPA [105,106]. Fig. 27 shows the effect of the mixture of Gd-DTPA and borax on the damage in lymphocyte DNA.

Of the two concentrations of the boron compound, the higher *one* was more effective to reduce radiation-induced damage. The result indicates that the boron compound plays a major role in reducing the DNA damages due to gamma-ray irradiation in a cellular level. However, it is very difficult to explain such a radioprotective action of the boron compound. The possible mechanisms of radioprotective action may be summarized as follows: 1) radical scavenging action, 2) induction of increased repair ability, 3) changes in oxygen tension (competition with oxygen), 4) protection against direct action of radiation. It is considered that the radiomodification effect comes from either the free radical scavenging action of the boron compound or the direct action of sodium-containing compounds.

A great potential of NCT lies in the fact that physiological differences between healthy and tumor cells can be utilized to enhance the delivered dose to tumor cells selectively. This is achieved by administering, prior to the radiation

treatment, a boron or gadolinium compound that can selectively accumulate or be retained in the desired target cells. Subsequently, the organ containing the tumor is irradiated with neutrons. ^{10}B and ^{157}Gd, by virtue of their high neutron capture cross section, will give rise to two densely ionizing particles through the neutron capture reaction. For an effective BNCT, the ^{10}B must be selectively delivered to and distributed in the target tumor cells. The tumor region is then irradiated with low-energy epithermal neutrons because thermal neutrons lack the capability of penetration into tissues. The low-energy neutron beams currently used for BNCT are produced using nuclear reactors even if neutron beams contain high-energy contaminants. It is apparent from our data that the boron compound could reduce the DNA damage induced by gamma-radiation, one of beam contaminants of therapeutic beams from a nuclear reactor. To our knowledge, the present study is the first demonstration of the modifying effect of boron and gadolinium compounds on the biological efficacy of gamma-ray in human lymphocytes by means of the comet assay. Therefore, the present results may support the fundamental understanding of neutron capture therapy. Although the mechanisms by which those compounds affect radiation responses are not clearly defined, possible explanations that are amenable to further study do exist.

5. Abstract

The modification of radioresponse in human lymphocytes pretreated with boron or gadolinium compound was studied by assessing the DNA damage using the single cell gel electrophoresis (SCGE), the comet assay. The lymphocytes from the human peripheral blood were irradiated with 0, 1, 2 and 4 Gy of gamma rays from a 60Co isotopic source without or with pretreatment of boron or gadolinium compound for 10 minutes at 4℃. Following the processes such as slide preparation, cell lysing, unwinding and electrophoresis, neutralization, staining, and analytic steps, gel electrophoresis was performed. The results show that pretreatment with boron compound (50 nM or 250 nM of 10B) is effective in reducing the radiosensitivity of the lymphocyte DNA.. On

the other hand, pretreatment with gadolinium compound (50 nM) led to a dose-dependent increase in the radiosensitivity, which was the most prominent with a dose of 4 Gy. ($P<0.001$). Furthermore, when the lymphocytes were pretreated with a mixture (1:1) of boron (250 nM) and gadolinium (50 nM) compounds, the reduced radiosensitivity was also observed.

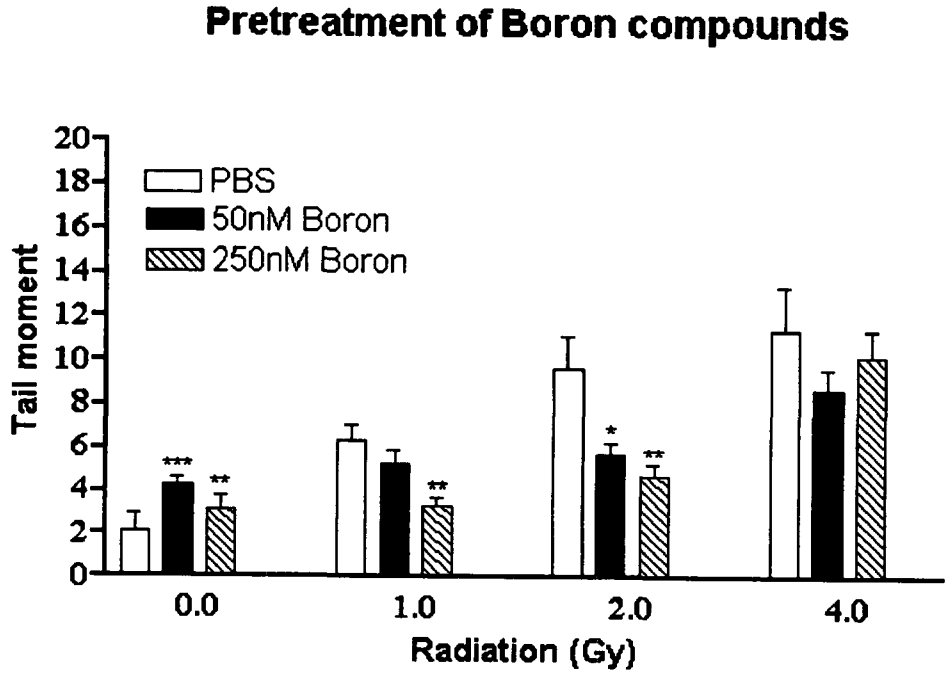


Fig. 25. Effect of boron compound (Borax) on radiation-induced DNA damage. Bars indicate the standard error of the mean with duplicated measurements in each. Values significantly different from that of the control are given as: *P<0.05, **P<0.01, and ***P<0.001.

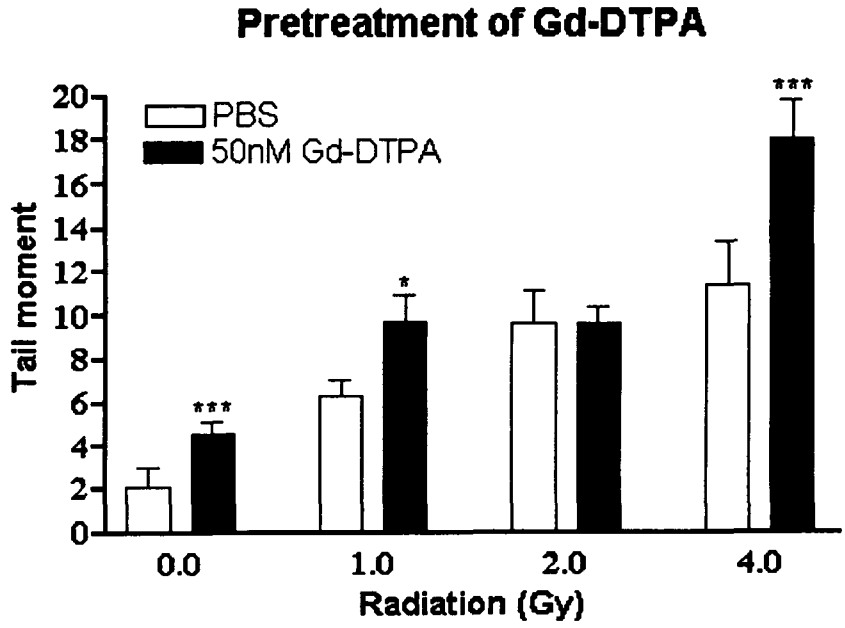


Fig. 26. Effect of gadolinium compound (Gd-DTPA) on radiation-induced DNA damage. Bars indicate the standard error of the mean with duplicated measurements in each. Values significantly different from that of the control are given as: *P<0.05, and ***P<0.001.

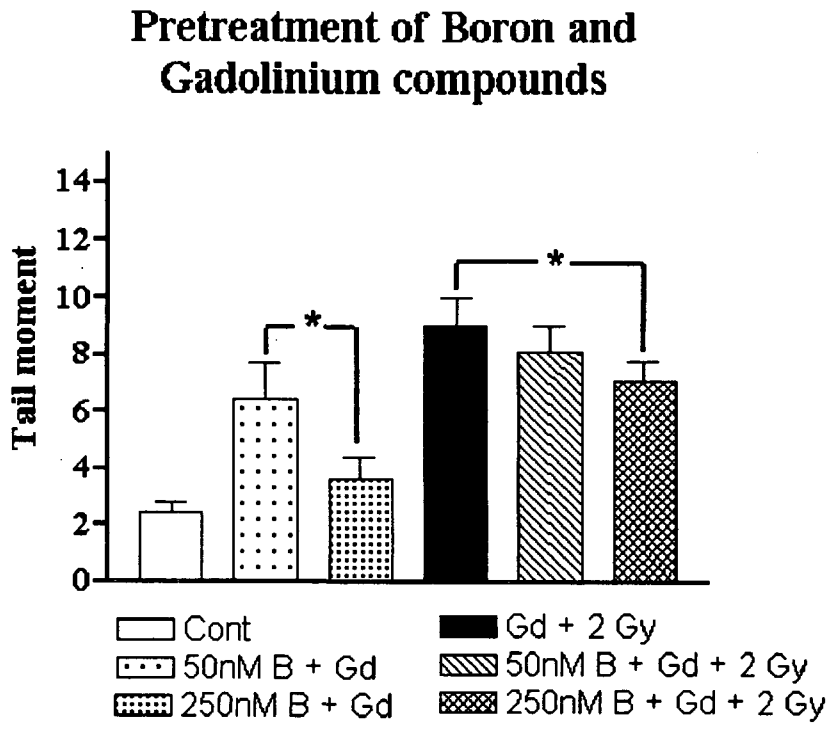


Fig. 27. Effect of the mixture of boron and gadolinium compounds on radiation-induced DNA damage. The concentration of gadolinium compound was limited to 50 nM. Bars indicate the standard error of the mean with duplicated measurements in each. Values significantly different between those of the experimental groups are given as: *P<0.05.

제6절 복합적 상승작용에 관한 수학적 해석

(Section VI. Mathematical description of synergistic interactions)

1. Introduction

The assessment of potential significance of synergistic interaction of combined action of two inactivating agents is an important problem. Despite the potential importance of interactions between different agents, there is little information regarding regularities and mechanisms of action of ultraviolet (UV) light and hyperthermia. Several lines of evidence show that UV light exhibits synergistic interaction between UV light of different energies [107], with ionizing radiation [108-110], various chemical agents [111,112] and hyperthermia [113-115]. It was shown [115] that, at any fixed UV light intensity, the synergistic effect occurred within the given temperature interval. It means that the optimal temperature providing the highest synergistic effect may be indicate inside of this interval for every fluence rate examined. It is of significance that the temperature range synergistically interacting with UV light has been removed towards lower temperature values with an decreasing fluence rate [115]. Similar results have been obtained for yeast cells exposed to ionizing radiation and hyperthermia [116]. Taken these data as a whole, one may conclude, that, in principle, synergistic interaction may take place between low intensities of UV or ionizing radiations and relatively low temperatures. In relation to essential value of this conclusion it is of actual task to understand the reason and mechanism of such a regularity. But at first it would be of interest to obtain an additional experimental demonstration of this conclusion.

Earlier a simple mathematical model of simultaneous combined action of ionizing radiation and hyperthermia has been proposed [117]. The model predicts the dependence of synergistic interaction of the ratio of lethal lesions produced by every agent used, as well as the maximal value of the synergistic effect, conditions at which the maximal interactive effect can be achieved, and the dependence of synergistic effect on dose rate. The model was tested for

thermoradiation action to various unicellular organisms including yeast cells. Thus, it would be also of interest to accommodate this model for description, optimization and prognosis the results obtained for simultaneous action of UV light and hyperthermia.

Therefore, the main purposes of this study were as follows: (a) to obtain new experimental data confirming the dependence of synergistic interaction of ultraviolet light and hyperthermia on fluence rate; (b) to suggest a new mathematical model for combined action of UV light and hyperthermia; (d) to test the validity of the model proposed.

2. Materials and methods

A wild-type diploid yeast of *S. cerevisiae* (strains T1 and XS800) were used in these experiments. Before irradiation, the cells were incubated for 3-5 days at 30℃ on a full nutrient agar layer. The cells were irradiated with germicidal lamps that emitted predominantly UV light of wavelength 254 nm (far-UV radiation) at fluence rates of at 0.15 and 1.5 W/m^2 for T1 strain either 0.25 and 1.5 W/m^2 for XS800 strain under ambient conditions at different temperatures (47.5~57.5°C), which were adjusted within ±0.2 °C. The source of far-UV light at 254 nm was a low pressure mercury vapour lamp with an integral filter that provided more than 95% of the emitted radiation at 254 nm. Variation of the intensity was achieved by means of calibrated metal wire nets. The fluence rates at 254 nm were measured using a calibrated General Electrical germicidal meter.

It should be noted that, in all our combined experiments, both modalities were applied simultaneously. This means that the durations of the UV light irradiation and heat exposure were identical, and might be determined as the ratio of the UV dose (J/m^2) to the fluence rate (W/m^2). The duration of heat incubation alone at different temperatures was varied to reduce cell survival to about 10% or less.

The following heating method was used. 0.1 ml of cell suspension at room temperature (about 1.5×10^6 cells) was placed into 1.4 ml of sterile water

preheated to a required temperature in a water bath. For the simultaneous thermal and UV light treatment, the time interval between introduction of the cells into the preheated water and the beginning of exposure was about 0.1 min, which was significantly less than the total treatment time. This means that the durations of UV light and heat exposure were identical. At the end of the treatment, the samples were rapidly cooled to room temperature. A known number of cells were then plated such that 150~200 colonies per dish would form after 5-7 days of incubation at 30°C. To avoid photoreactivation, UV exposure, dilution and other procedures were performed under red ambient light, while post-irradiation incubation was carried out under dark conditions.

3. Experimental results

Fig. 28 provides a typical example of the basic experimental data observed in this investigation. Here, survival curves were obtained for *S. cerevisiae* diploid yeast cells (strain T1) after heat treatment (50.0°C) alone (curve 1), and exposure to UV light (254 nm) at 1.5 W/m^2 and room temperature (curve 2). An experimental curve was also obtained after simultaneous action of both modalities (curve 4). Curve 3 represents a hypothetical curve that would be obtained if inactivation by the combined heat and UV light was completely additive. These types of survival curves described above and depicted in Fig. 28 were obtained for every temperature, fluence rate and strain investigated. They are not presented here and used to calculate the synergistic enhancement ratio.

To estimate quantitatively the effect of combined action of both modalities, we used the synergistic enhancement ratio $k = D2/D1$ (Fig. 28). In other words, k is defined as the ratio of the calculated UV light dose (assuming an additive effect of UV light and hyperthermia), $D2$, to the UV light dose, $D1$, observed from the experimental survival curve for the simultaneous action of UV light and hyperthermia at a fixed level of survival. In our case this parameter was calculated for 10% survival. Results of three to five experiments were used to calculate interexperiment standard errors.

It was shown in our previous publication [119] that, for simultaneous action

of ionizing radiation and hyperthermia on *S. cerevisiae*, the thermal enhancement ratio increases indefinitely with increasing exposure temperature, while the synergistic enhancement ratio first increases, then reaches a maximum, which is followed by a decrease. In other words, a specific temperature which maximizes the synergistic effect may be found. This is why, in this section, we have also used the synergistic enhancement ratio. Pooled results of 3~5 experiments were used to calculate the mean number of these parameters and their statistical errors.

This parameter is depicted in Fig. 29 (strain T1) and in Fig. 30 (strain XS800) as functions of the exposure temperature and fluence rate. It is evident from these data that, for the simultaneous action of heat and UV light (254 nm), both the temperature and fluence rate affect the synergy. As can be seen, for a given intensity, there is a specific temperature that maximizes the synergistic effect. This implies that the synergistic interaction between hyperthermia and UV light is observed only within a certain temperature range. For temperatures below this range, killing of cells was mainly determined by UV light exposure, while, for temperatures above this range, killing of cells was caused by heat. Any deviation of the temperature from the optimal value results in a decrease in synergism. It is clear from the data presented that the fluence rate is also a very important parameter. From these findings, it can be inferred that the temperature at which UV light is delivered should be decreased to obtain the maximum synergistic effect with decreasing fluence rate. Indeed, for both yeast strains studied (Figs. 29, 30) for fluence rate 1.5 W/m^2 the highest synergistic effect was obtained under 55℃. The reduction of UV light fluence rate down to 0.15 W/m^2 for T1 cells (Fig. 29) and to 0.25 W/m^2 for XS800 cells (Fig. 30) resulted in the necessity to reduce exposure temperature down to 50 and 52.5℃ respectively to retain the greatest synergistic enhancement ratio. These results seem to be related to the fact that, if the fluence rate decreases, then the lethal UV dose is delivered for a longer time, so that the duration of heat incubation increases and could explain the lower temperature that should be applied to the cells to provide the greatest synergistic interaction. All the above described regularities are intrinsic to both strains studied.

4. Analytical approach

Synergism is defined as the combined interaction of two agents that exceeds the additive sum of their individual effects. On this definition, it might be reasonable to assume that some additional lethal lesions are produced during combined action. Our analytical approach is based on the supposition that the additional lethal lesions are arisen from the interaction of sublesions induced by both agents. These sublesions are non-lethal when each agent is applied separately. We assume that one sublesion produced by UV light interacts with one sublesion from heat exposure to produce one additional lethal lesion. It would seem probable to suppose that the number of sublesions was directly proportional to the number of lethal lesions. Let $p1$ and $p2$ be the number of sublesions that occur for one lethal lesion induced by UV light and hyperthermia respectively. Let $N1$ and $N2$ be the mean numbers of lethal lesions in a cell produced by these agents. A number of additional lesions $N3$ arising from the interaction of UV light and hyperthermia sublesions may be written as

$$N3 = min\{p1N1;\ p2N2\} \quad \text{----------} \quad (1)$$

Here, $min\{p1N1;\ p2N2\}$ is a minimal value from two variable quantities: p1N1 and p2N2., which are the mean number of sublesions produced by UV light and hyperthermia respectively. In other words, this value is the mean number of additional lethal lesions per cell arising from the interaction between the UV light and the hyperthermia and accounting for the synergism. Thus, the model describes the yield of lethal lesions per cell as a function of UV light (N1), hyperthermia (N2), and interaction (min{p1N1; p2N2}) lethal lesions. Then the synergistic enhancement ratio k may be expressed as

$$k = (N1 + N2 + N3)/(N1 + N2) \quad \text{----------} \quad (2)$$

The corresponding number of lethal lesions can be calculated [118] as

$$N = - lnS \quad \text{----------} \quad (3)$$

where S is the surviving fraction.

If the observed biological effect is mainly induced by heat ($p1N1 < p2N2$) then taking into account Eqs. (1-3), the parameter $p1$ can be expressed as

$$p1 = (k1 - 1)(1 + N2/N1) \quad \text{----------} \quad (4)$$

where $k1$ is the value of synergistic enhancement ratio observed in experiments performed in this condition. On the contrary, if the observed biological effect is mainly induced by UV light, we have

$$p2 = (k2 - 1)(1 + N1/N2) \quad \text{----------} \quad (5)$$

where $k2$ is the experimental value of the synergistic enhancement ratio observed for the condition $p2N2 < p1N1$. We can easily show that under condition

$$p1N1 = p2N2 \quad \text{----------} \quad (6)$$

the greatest synergistic enhancement ratio will be attained, which value is given by

$$kmax = 1 + [p1p2/(p1 + p2)] \quad \text{----------} \quad (7)$$

Thus, based on Eqs. (4) and (5) and experimental data of $k1$ and $k2$, we can estimate the basic model parameters $p1$ and $p2$ and then predict the value of the synergistic enhancement ratio for any $N1$ and $N2$ [Eq. (2)], the

highest value of the synergistic enhancement ratio [Eq. (7)], and condition [Eq. (6)] under which it can be achieved.

5. Comparison of experimental results with model predictions

The purpose of this Section is to assess the value of the model in describing experimental results obtained. Using the foregoing equations and the results described, we calculated the dependence of synergistic enhancement ratio k on the ratio $N2/N1$ for T1 (Fig. 31) and XS800 (Fig. 32) strains. The solid lines in these Figures are the prediction from our model [Eq. (2)], open circles denote experimental values of k obtained for lower fluence rates of UV light while closed ones for 1.5 W/m^2. The errors in k values were calculated from interexperimental variation. Predicted values of k were estimated with $p1$ = 1.5 and $p2$ = 0.77 for T1 strain and $p1$ = 0.92 and $p2$ = 1.62 for XS800 strain. These values were evaluated by means of Equations (4) and (5), and the value of $k1$ and $k2$ which have been derived from real experiments. The important aspect of these data is that there appears to be a sharp dependence of synergistic effect on the ratio $N2/N1$. The maximal values of the synergistic enhancement ratios predicted by Eq. (7) ($kmax$ = 1.51 and 1.59 for T1 and XS800 strains respectively) are expected in accord with Eq. (6) at $N2/N1$ = 1.95 (strain T1, Fig. 31) and $N2/N1$ = 0.57 (strain XS800, Fig. 32). The synergistic effect appears to decline with a deviation of the ratio $N2/N1$ from the optimal value. One can also see that experimental results are comparable to the predicted values of the synergistic effect. The degree of synergistic interaction was found to be dependent on the ratio of lethal damages ($N2/N1$) induced by both agents applied.

Another important aspect of the synergistic interaction of simultaneous application of UV light and hyperthermia is the dependence of synergy effect on UV light fluence rate which was obtained in our earlier work [115] and has been confirmed in this study. It would be of interest to verify the ability of the analytical approach proposed to describe and interpret the dependence of synergistic effect on UV light fluence rate.

Two significant consequences are ensued from Eq. (6). First of all, it follows the dependence of synergistic effect on intensity of agents applied. Indeed, Eq. (6) shows that the greatest synergistic effect will be achieved after equal numbers of sublesions induced by both UV light (*p1N1*) and hyperthermia (*p2N2*). The lower fluence rate is, the larger will be the time needed to achieve *N1*. The same is true for the number of sublesions produced by UV light (*p1N1*). As soon as both modalities were applied simultaneously, the number of heat lethal lesions (*N2*) and, consequently, the number of sublesions (*p2N2*) would be increased. Hence, to fulfil Eq. (6), *p2N2* must be decreased by reduction of the temperature at which the UV light illumination is delivered. Hence, the model qualitatively explains the dependence of synergy effect on UV light fluence rate, i.e. the necessity to reduce exposure temperature with fluence rate decreasing to obtain the maximal synergistic effect.

The second important consequence followed from Eq. (6) is concerned with the dependence of the synergistic enhancement ratio on the ratio of lethal effects *N2/N1* produced by every agent. It follows from Eqs. (6) and (7) that the position of the highest synergism on *N2/N1* axis and its value has not to be dependent on intensity of modalities used for combined action. Really, if the values of *p1* and *p2* stayed unchanged with fluence rate (that was observed for our case), then in accordance with Eq. (6) the ratio of *N2/N1*, providing the highest synergistic effect, should also stay unchanged. It means that alteration of both agents intensities should be compassed in such a manner to keep this ratio constant and equal to the ratio of *p1/p2*. In such a case, in accord with Eqs. (1) and (2), the relationship between the synergistic enhancement ratio *k* and the ratio *N2/N1* also shouldnt be dependent on the UV light fluence rate, that it was observed in our experiments (Figs. 31, 32). It implies that the model considered is able to describe and predict quantitatively the synergistic interaction of simultaneous action of UV light and hyperthermia on yeast cell inactivation.

6. Concluding remarks

New additional experimental data related with the dependence of synergistic interaction of simultaneous action of UV light (wavelength 254 nm) and hyperthermia on UV light intensity are presented in this section for yeast cells, the simplest model of eukaryotic cells. The most important data was that the synergistic interaction between both modalities was observed only within a definite range of high temperature. For other temperatures the effect was only additive. Hence, there is a specific temperature that maximizes the synergy. Any change in the number of lethal damages induced by UV light or heat resulting in a decreasing or an increasing the ratio of $N2/N1$ from optimal value was shown to cause the reduction of the synergistic interaction. The dependence of synergistic effect of UV light fluence rate was also demonstrated.

The other interesting new results of this work concern the mathematical model which has been proposed to explain the experimental data of synergistic interaction of UV light and hyperthermia for yeast cells. The model is based on the supposition that synergism takes place due to the additional lethal lesions arising from the interaction of non-lethal sublesions induced by both agents. These sublesions are considered noneffective after each agent taken alone. The model predicts the dependence of synergistic interaction of the ratio of lethal lesions produced by every agent applied, the greatest value of the synergistic effect as well as the conditions under which it can be achieved and the dependence of synergistic effect on UV light fluence rate. These predictions of the model have been tested for simultaneous combined action of UV light and heat (45~57.5℃) for two strains of wild-type diploid yeast cells of *Saccharomyces cerevisiae.* The model is not concerned with the molecular nature of sublesions, and the mechanisms of their interaction remain to be elucidated. In spite of the approximation used in this simplified model, it is evident from the data presented that a good agreement appears to exist between theoretical and experimental results.

It is worth to note that the identical model was used to describe the dependence of synergistic effect on the ratio of $N2/N1$ for various unicellular organisms irradiated with ionizing radiation at high temperatures [117] as well as for yeast cells undergoing to combined action of ultrasound and hyperthermia [119]. Taking this data and the results of this work as a whole, we may

conclude that different biological systems exposed simultaneously by various physical factors can be analyzed by the same model. It suggests a general validity of the model for the description, prognosis and optimization of synergistic interaction of two harmful agents.

7. Abstract

A new mathematical model for synergistic interaction of lesions produced by ultraviolet (UV) light and high temperature has been proposed. The model suggests that synergism is expected from the additional lethal lesion arising from the interaction of sublesions induced by both agents. These sublesions are considered noneffective after each agent taken alone. The model predicts the dependence of synergistic interaction of the ratio of lethal lesions produced by every agent applied, the greatest value of the synergistic effect as well as the conditions under which it can be achieved, and the dependence of synergistic effect on UV light fluence rate. These predictions of the model have been tested for simultaneous combined action of UV light (wavelength 254 nm) and heat (45~57.5℃) on two strains of wild-type diploid yeast cells of *Saccharomyces cerevisiae*. The theory appears to be appropriate and the conclusions valid.

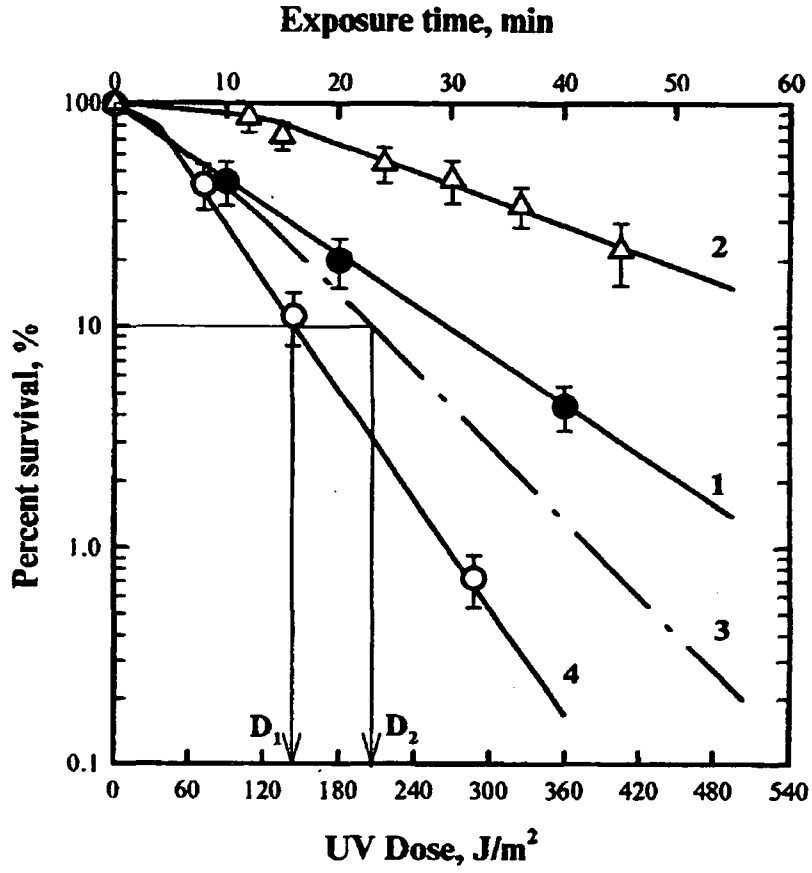


Fig. 28. Survival curves of *S. cerevisiae* diploid yeast cells (strain T1): 1, heat treatment (50.0 °C) alone; 2, UV light (254 nm) at 1.5 W/m2 and room temperature (20 °C); 3, calculated curve for independent action of UV light (1.5 W/m^2) and high temperature (50.0 °C); 4, experimental curve after simultaneous action of these modalities. Error bars represent the standard error of the mean.

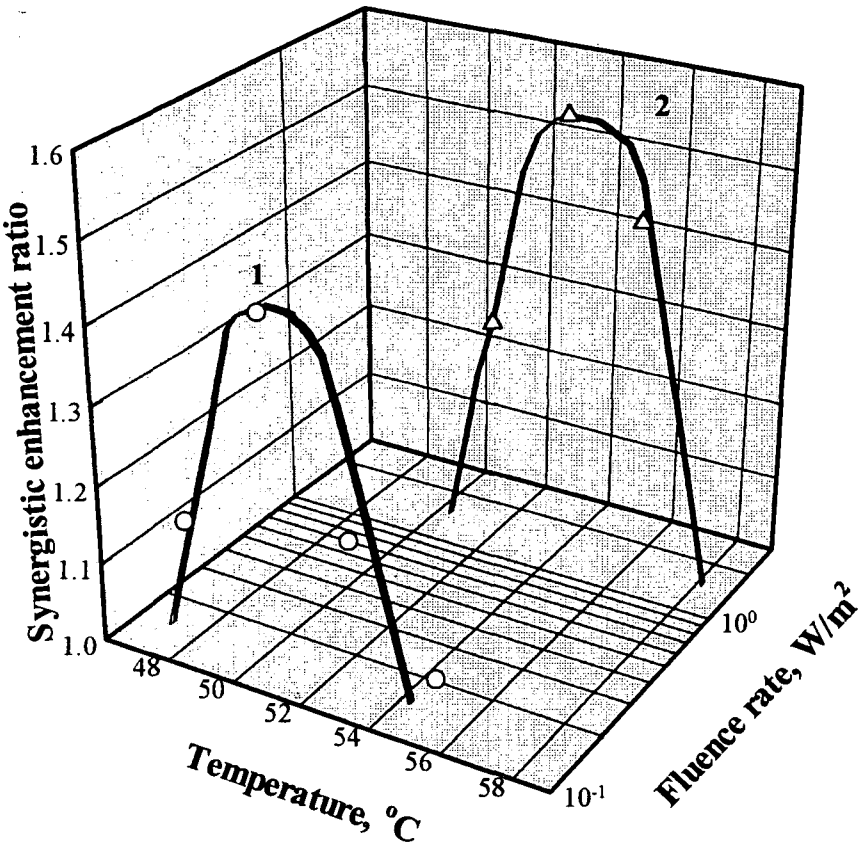


Fig. 29. The dependence of the synergistic enhancement ratio (k) upon the exposure temperature and fluence rate (1, 0.15 W/m^2 ; 2, 1.5 W/m^2) for *S. cerevisiae* diploid yeast cells (strain T1).

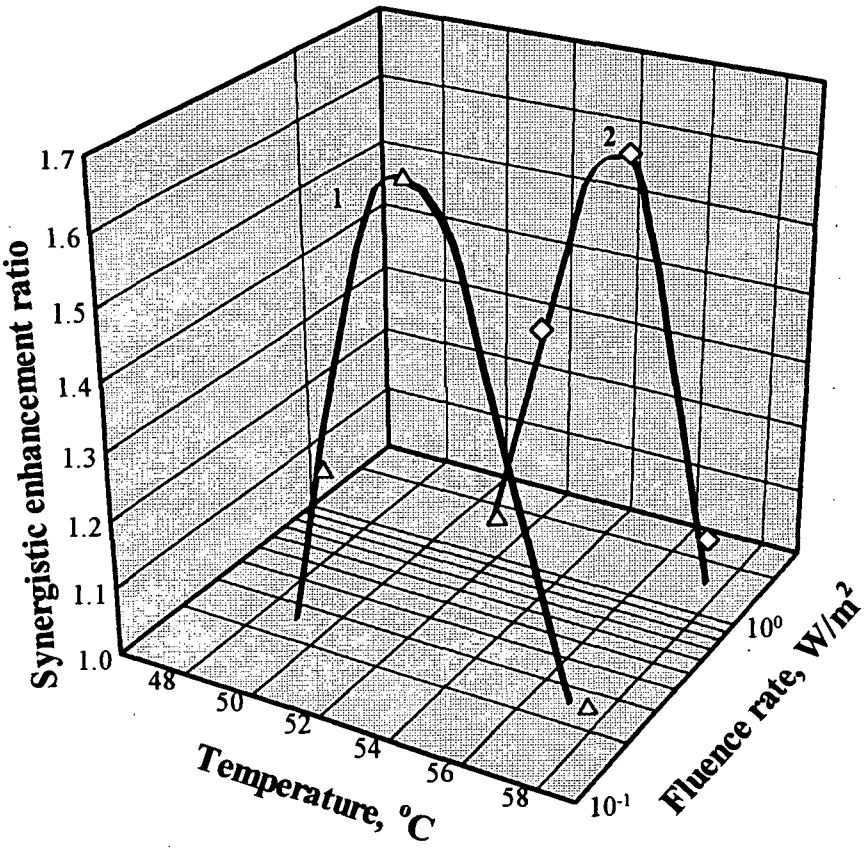


Fig. 30. The dependence of the synergistic enhancement ratio (k) upon the exposure temperature and fluence rate (1, 0.25 W/m^2 ; 2, 1.5 W/m^2) for *S. cerevisiae* diploid yeast cells (strain XS800).

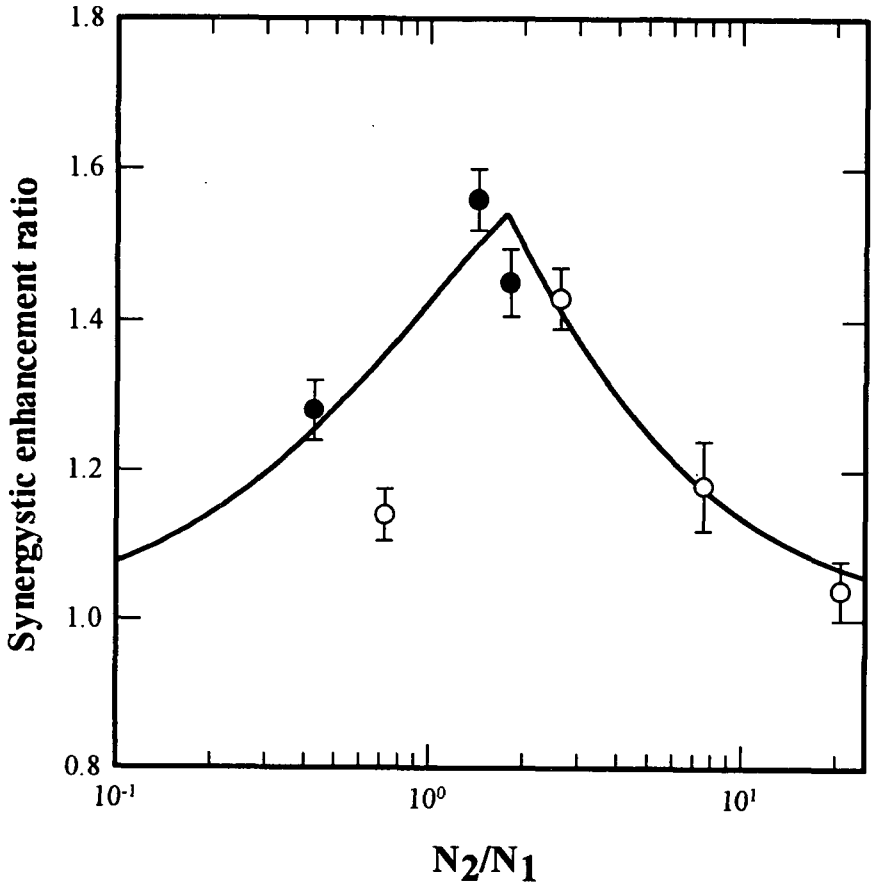


Fig. 31. The dependence of the synergistic enhancement ratio (k) as a function of ratio *N2/N1* for *S. cerevisiae* diploid yeast cells (strain T1) exposed by UV light at various intensities (open circle, 0.15 W/m^2; closed circles, 1.5 W/m^2) and temperature. Solid line theoretically predicted dependence; circles experimental data. Error bars show inter-experimental errors.

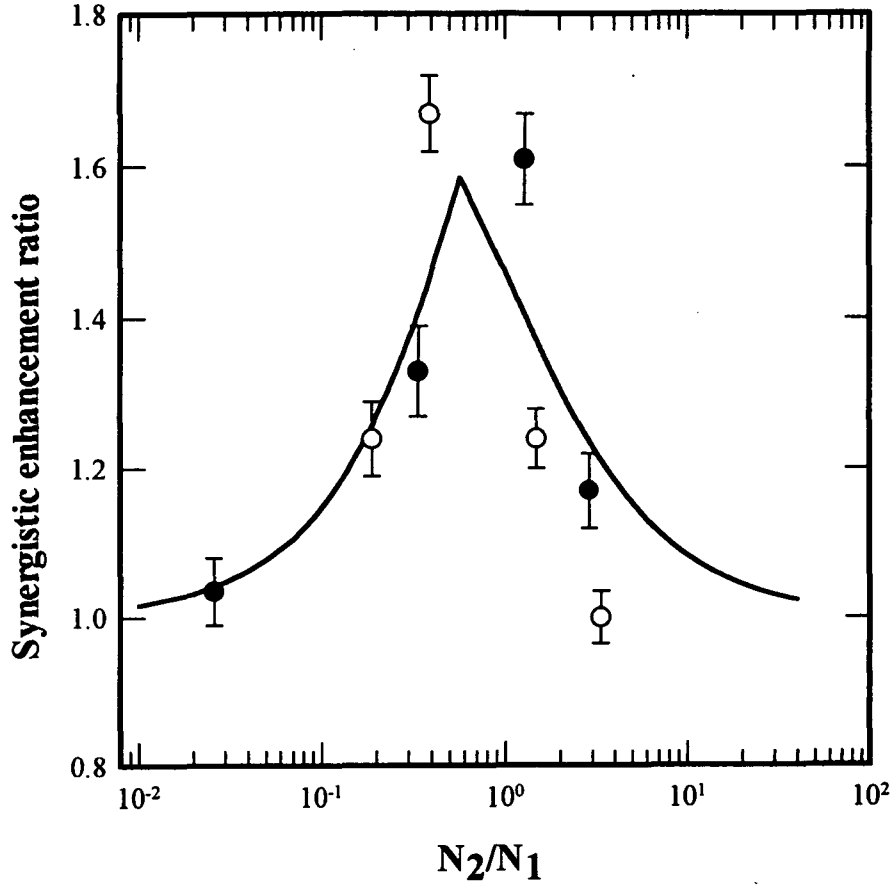


Fig. 32. The dependence of the synergistic enhancement ratio (k) as a function of ratio *N2/N1* for *S. cerevisiae* diploid yeast cells (strain XS800) exposed by UV light at various intensities (open circle, 0.25 W/m^2; closed circles, 1.5 W/m^2) and temperature. Solid line theoretically predicted dependence; circles experimental data. Error bars show inter-experimental errors.

제7절 상승작용에 관한 일반 규칙성 해석

(Section VII. General regularities of synergistic interactions)

1. Introduction

It is well known fact that interactions can take place between hyperthermia and other numerous physical and chemical agents found in modern life. A number of biological objects can be inactivated more strongly by various physical and chemical agents applied together with hyperthermia compared with normal conditions. Some of these combinations lead to a synergistic interaction when the final observed effects are greater than the addition of the effects from separate exposures to the individual inactivating agents. Heat is known to be extensively utilized to enhance cell inactivation induced by ionizing radiation [128,132,133,140,141], ultraviolet light [113,114,133,134], ultrasound [119,126,127] and the majority of chemical agents [123,129,132,143].

The problem of the synergistic interaction of hyperthermia with various physical and chemical agents is very important both from theoretical and practical points of view. Theoretically, it may shed light on the mechanism of synergy. In practice, an improvement in combined therapy of cancer or a variety of sterilization methods may be expected. To elucidate the mechanism of synergy, it is of interest to reveal common patterns of synergistic effects displayed independently on the agents applied, the end point registered and biological test systems. Using the results of many publications [123,128,132,135,140,141], it is easily recognized that the degree of synergistic effect appears to be influenced by many factors such as cell line, thermotolerance, recovery and repair, phase of cell cycle, temperature, exposure time, dose, intensity of the physical factors, concentration of the chemical agents, and treatment sequence. The greatest synergistic interaction was observed for the simultaneous treatment of hyperthermia with another factor used in combination. The intervals between the exposures may affect the degree of synergy, it being known that increasing the time between successive

treatment of hyperthermia and another inactivating agent results in decreasing their synergistic interaction.

Despite the potential importance of synergistic interaction, information regarding other new common regularities of synergistic interaction of hyperthermia with various physical and chemical inactivating agents was largely missing. To reveal some new general rules describing the response of various unicellular organisms to the combined action of hyperthermia with another inactivating agent, in this section, synergistic interaction of the simultaneous action of hyperthermia with ionizing radiation, ultraviolet light, ultrasound and some chemical agents has been analysed using the experimental data presented here for bacterial and yeast cells. To check the universality of the rules obtained, the results published by others for viruses, bacterial spores and cultured mammalian cells were involved.

2. Materials and methods

2-1. Strains and cultivation conditions

The following yeast strains were used in the experiments: *Saccharomyces cerevisiae* (two diploid strains, XS800 and T4), *Endomyces magnusii* (diploid). Yeast cells were incubated before irradiation for 3~5 days at 30℃ on a complete nutrient agar layer to a stationary phase. The cells were then washed with distilled water and resuspended to make a stock solution. The repair-proficient bacterial strain *Escherichia coli B/r* was also used in the present investigation. Before treatment, bacterial cells were grown in YEP broth (yeast extract - 1%, glucose - 1%, peptone - 1%, NaCl -1%) at 37℃ during 18 h of incubation. Final suspensions prepared in this manner contained approximately 10^8 cells per mℓ for bacterial and 10^6 cells per mℓ for yeast cells.

2-2. Irradiation with ionizing radiation

Aliquots with 10^6 yeast cells/mℓ in a glass tube were exposed to graded doses of γ-radiation. The ^{60}Co source was a Gammacell 220 (Atomic Energy of Canada Ltd.). The γ-ray dose-rate, estimated by ferrous sulphate dosimetry

and by a Siemens ionization chamber, was 10 Gy/min. The electron beam from a 25 MeV pulsed linear accelerator was also used in these experiments. The pulse duration at half peak output was 2-7 s and the pulse repetition rate was at 50 Hz, the average dose rates were 5, 10, 25 and 250 Gy/min as determined by ferrous sulphate dosimetry. Dosimetric control of the accelerator was performed immediately before the irradiation procedure.

2-3. UV light exposure

The cells were irradiated with germicidal lamps that emitted predominantly UV light of wavelength 254 nm (far-UV radiation) at fluence rates of 0.033, 0.15, 0.25, 1.5 W/m^2 at different temperatures (47.5~57.5 °C). The source of far-UV light at 254 nm was a low pressure mercury vapour lamp with an integral filter that provided more than 95% of the emitted radiation at 254 nm. Variation of the intensity was achieved by means of calibrated metal wire nets. The fluence rates at 254 nm were measured using a calibrated General Electrical germicidal meter. To avoid photoreactivation, UV exposure, dilution and other procedures were performed under red ambient light, while post-irradiation incubation was carried out under dark conditions.

2-4. Sonication

A continuous mode of sonication was accomplished by a 20 kHz ultrasonic unit (Fisher sonic dismembrator Model 300). The diameter of the ultrasonic transducer was 15 mm. The ultrasonic dose rates were 0.05 and 0.2 W/cm^2. This ultrasonic intensity was measured by the calorimetric method. To estimate the effect induced by ultrasound alone, the following method was used: 0.1 ㎖ of cell suspension (10^8 cells/㎖) at room temperature was put into 9.9 ㎖ of sterile water which was placed into a metal vessel constructed together with the transducer. To determine the effect induced by ultrasound alone, the absorbed ultrasound heat was completely removed by cooling with water.

2-5. Heat treatment applied alone or combined with other agents

Hyperthermia was given in a water bath where a desired temperature ±0.1 °C was maintained by a constant temperature circulator. The exposed cell

suspension was adjusted to pH 7.4. For the simultaneous action of hyperthermia and other physical or chemical agents, the time interval between the introduction of the cells into the preheated water and the beginning of exposure was about 0.1~0.3 min, which was significantly less than the total treatment time. At the end of the treatment, the samples were rapidly cooled to room temperature. This meant that the duration of physical agent treatment and heat exposure were identical. A known number of cells were then plated such that 150~200 colonies per dish would form by the surviving yeast cells after 5~7 days of incubation at 30 °C and 50~100 colonies by bacterial cells after 24 hrs of incubation at 30 °C. All experimental series were repeated 3~5X. The linear regression calculation based on the multi-target single-hit mathematical model was used to fit the survival curves. The details were described elsewhere [115,116,119,131].

3. Results

3-1. The thermal enhancement ratio and the synergistic enhancement ratio

Fig. 33 exhibits some examples of the basic experimental data used in this investigation. Here, survival curves were obtained for diploid yeast cells of different species and bacterial cells after heat treatment alone (curve 1) or after exposure with another inactivating agent applied alone at room temperature (curve 2). Curve 3 represents the theoretical survival curves that would be expected by the simultaneous combined action of both modalities if their inactivation action was completely additive. Curve 4 shows the real survival curves obtained experimentally after the simultaneous action of heat and another inactivating agent. In all cases, the experimental curves go below the survival curves predicted for the case of both agents produce their effect without interaction. This means that a synergistic interaction takes place. These four types of survival curves depicted in Fig. 33 have been obtained in this section for every temperature, intensity of physical factors, concentration of chemical agents and cell kind investigated. They are not presented here and were used to evaluate the effectiveness of the interaction.

To estimate quantitatively the sensitization action of hyperthermia, one can

employ (for example, [139]) the thermal enhancement ratio (TER) defined as the ratio t3/t1 (Fig. 33), i.e. TER is defined by the ratio of dose (or exposure duration) required to give a specified level of damage induced by the applied inactivating agent without heat to the dose required to give the same level of damage produced by the same agent used together with heat. This ratio indicates an increase of cell sensitivity by high temperature. However, it does not reflect the kind of interaction (whether it was antagonistic, independent or synergistic). In some cases, for practical and theoretical reasons, it is of great importance to provide information concerning the interaction. Hence, it would be of interest to estimate quantitatively the synergy on the dependence of environmental conditions including the temperature under which the treatment occurred. In this section, to calculate the synergistic effect we used the synergistic enhancement ratio (k) suggested earlier [133,134] and defined as the ratio of the exposure time expected for an additive effect of both modalities to that observed from the experimental survival curve for the simultaneous action of hyperthermia and another inactivating factor. This parameter should be estimated for a fixed level of survival, for example for 10% survival, k = t2/t1 (Fig. 33). For purely exponential survival curves, k does not depend on the surviving level.

Both these parameters are plotted in Fig. 34 against the exposure temperature for the simultaneous action of hyperthermia with various inactivating agent and cell systems. The remarkable feature of Fig. 34 is that the thermal enhancement ratio (curve 1) increases permanently with increasing exposure temperature, while the synergistic enhancement ratio (curve 2) increases at first, then reaches a maximum, which is followed by a decrease. This pattern was observed independently of the agent (ionizing radiation, UV light, ultrasound, and zinc sulfate) and the cell line (yeast or bacterial cells) used in this investigation. Noteworthy is the fact that such a dependence of the synergistic effect on the temperature under which the exposure occurred was also obtained for the combined action of ionizing radiation and heat on bacteriophage [142], bacterial spores *Bacillus subtilis* var. *niger* [124,137], as well as for cultured mammalian cells subjected to the simultaneous treatment of hyperthermia with some chemical inactivating agents [130]. This implies that,

irrespective of the biological object investigated, the synergistic interaction between hyperthermia and other inactivating agents is observed only within a certain temperature range. For temperatures below this range, cell killing is only additive and was predominantly induced by the physical factor or the chemical agent combined with heat. On the contrary, for temperatures above this range, cell killing was caused mainly by heat and is also only additive. Hence, one can conclude that for a given intensity of physical factors or concentration of chemical agents there would be a specific temperature that maximizes the synergistic interaction. Any deviation of the acting temperature from the optimal value results in a decrease of synergism. Therefore, it can be supposed that for any fixed temperature there should be a specific intensity of the agent applied that maximizes the synergistic effect. It would then be of interest to examine the relationship between the acting temperature and the intensity of the physical factors or the concentration of the chemical agents, the combined action of which provides a definite synergy or its highest value.

3-2. Dose rate and synergistic interaction of hyperthermia with ionizing radiation

A detailed study of the dependence of synergism of a simultaneous thermoradiation exposure on the ionizing radiation dose rate was performed using the simplest model of eukaryotic cells - diploid yeast cells of *Saccharomyces cerevisiae* of the wild type (XS800 strain) exposed at the stationary growth stage simultaneously to ionizing radiation (25 MeV electron) and hyperthermia. Some results of these studies were published earlier [116,136,144]. The final results of these studies are summarized in Fig. 35 where the experimentally obtained dependencies of the synergistic enhancement ratio on temperature at which the cell irradiation occurred are depicted for all the used dose rates. The presented data suggest the following conclusions. First, for each of the studied dose rates there is a certain range of temperatures synergistically enhancing the effect of ionizing radiation as well as a temperature at which the effect of synergism is maximal. Second, the effect of synergism depends both on the temperature at which the irradiation occurs and on the dose rate of ionizing radiation. For example, with a dose rate of 250 Gy/min (curve 4) the highest

synergistic enhancement ratio is observed at 54℃. A decrease in the dose rate to 25 (curve 3), 10 (curve 2), and 5 (curve 1) Gy/min leads to the fact that the temperatures at which synergism will be maximal become lower and equal 52.5, 50.0, 48.0℃. Thus, with decreasing dose rate the temperatures at which irradiation occurs should be lowered in order to achieve the greatest synergistic interaction. In relation with the cardinal importance of this conclusion it seems of interest to analyze the data on the relationship between the synergism of the other harmful physical and chemical environmental factors and their intensities.

3-3. Fluence rate and synergistic interaction of hyperthermia with UV light

A wide set of survival curves for two diploid strains (XS800 and T1) of *Saccharomyces cerevisiae* yeast cells of the wild type exposed at the stationary growth stage simultaneously to 254 nm UV light and hyperthermia. Some results of these studies were published earlier for different fluence rates and temperatures [115,136,145]. The final results of these studies are summarized in Fig. 36 which demonstrates the dependence of the synergistic enhancement ratio on the temperature at which irradiation occurred and on the intensity of UV radiation. It follows from these data that synergism is determined both by the temperature and fluence rate of UV light. Similar to the simultaneous exposure to ionizing radiation and hyperthermia, for every constant fluence rate of UV radiation there is a certain range of temperatures synergistically enhancing the effect of this factor as well as a temperature at which the synergistic interaction is the highest. For temperatures below this range, the interaction of both agents is only additive and cell killing was mainly determined by UV light exposure. For temperatures above this range, cell killing is additive again and is predominantly caused by heat. It can also be inferred from these data that with a decrease in the UV light fluence rate the temperature of the exposed cell suspension should be lowered in order to provide the highest synergistic effect. It can be supposed on this basis that, for any fixed temperature, there should be a specific UV light fluence rate that maximizes the synergistic effect, and any deviation of the UV light intensity from the optimal value would result in a decrease in synergy.

3-4. Ultrasound intensity and synergistic interaction of hyperthermia with ultrasound

Diploid *Saccharomyces cerevisiae* yeast cells (strain XS800) were subjected to the simultaneous action of 20 kHz ultrasound and hyperthermia. Yeast cells were sonicated at intensities of 0.05 and 0.20 W/cm^2 at temperatures ranging from 44℃ to 54.5℃. A part of the results has already been published [119]. New results on the relationship between synergism, exposure temperature and ultrasound intensity are presented in Fig. 37. It is obvious that for both intensities there is an optimal temperature at which the synergistic interaction of the agents is maximal. In particular, at the ultrasound intensity of 0.20 W/cm^2 a maximal synergistic enhancement ratio was achieved at 52.5℃ whereas to provide the highest synergism at the ultrasound intensity of 0.05 W/cm^2 the temperature should be reduced down to 47℃. This means that as the ultrasound intensity decreased, the temperature at which the ultrasound treatment of the cells occurred should be diminished to provide the highest synergistic interaction. Thus we may infer that the conclusions made for the above-described combination of physical factors are also true for the simultaneous action of ultrasound and hyperthermia.

3-5. Concentration of chemical agents and their synergistic interaction with hyperthermia

Since chemical reactions in general are accelerated by increasing temperature, one would expect that the rate of cell injury by chemical agents would also be accelerated at elevated temperatures. Temperature dependence in the cytotoxicity of the chemical agents as well as the synergistic interaction of the combined action of hyperthermia with a number of chemical environmental pollutions or therapeutic agents have been reported [129,130,143]. It would be of interest to analyze the dependence of synergism on the intensity of some chemical agents. The treatment duration may be considered as an analog of the chemical agent dose while the concentration of preparation may serve as a measure of its intensity.

Johnson and Pavelec (1973) obtained the relationship between temperature and the cytotoxicity of tris(1-aziridinyl)-phosphine sulfide (thio-TEPA) in vitro

with the inactivation of clone-forming hamster cells [129]. Cells were exposed to thio-TEPA in 2 concentrations, 5 and 10 g/mℓ at various temperatures. The effect of various concentrations of *cis*-diamminedichroloplatinum (II) (*cis*-DDP) and hyperthermia on cultured Chinese hamster ovary (CHO) cells was investigated by Urano *et al.* (1990, [143]). In both these reports, a precise and useful relationship between temperature and cytotoxic effect was defined, and in addition, the thermodynamic characteristics of the main chemical reactions involved in the cytotoxicity of the agents have been published. However, the authors of the cited works did not estimate the dependence of synergism on the concentration (intensity) of the drugs. Using their data, including extrapolation which was made for higher temperatures [129], we have calculated the relationship between the concentration of drugs, treatment temperatures and the synergistic enhancement ratio. The results of the calculations are given in Fig. 38A for thio-TEPA and in Fig. 38B for *cis*-DDP. It can be seen that with a decrease in concentration of both drugs the exposure temperature should also be decreased to display the highest synergistic interaction.

4. Discussion

In this section, synergistic interaction of the simultaneous action of hyperthermia with ionizing radiation, ultraviolet light, ultrasound and some chemical agents has been analysed using the experimental data obtained by authors with bacterial and yeast cells and published by others for cultured mammalian cells. Some universal rules in the responses of various cellular systems to the combined action of hyperthermia with another inactivating agent were revealed. The existence of a definite temperature range inside which the synergistic interaction of both modalities is observed was demonstrated. The remarkable feature of this interaction is that at first the synergistic effect increases with increasing exposure temperature, then reaches a maximum, which is followed by a decrease. For temperatures below this temperature range, the synergistic effect was not observed and cell killing was mainly determined by the damages induced by the physical or chemical factor employed in combination

with hyperthermia. For temperatures above this temperature range, the synergistic effect was also not observed, but cell killing was chiefly caused by hyperthermia.

The temperature range strengthening the effect of ionizing radiation has been varied with different cell systems and shifted toward lower temperatures for temperature-sensitive cell lines. As it has been already pointed out [117,135], this range was about 95~105℃ for thermoresistant bacterial spores *Bacillus subtilis*, 50~60℃ for bacterial cells, 45~55℃ for yeast cells, and 40~45℃ for cultured mammalian cells whose sensitivity to hyperthermia is the greatest. Inside the temperature range, synergistically increasing the effect of the inactivating agents, a specific temperature which maximizes the interactive effect may be found. In other words, there is a definite ratio of damages produced by agents employed in combination that would ensure the greatest synergistic effect. Any deviation of this ratio from the optimal one resulted in a decrease in synergism.

The data presented in this section clearly indicate that the intensity of the physical factor or the concentration of the chemical agent may serve as a determinant of synergy. It was demonstrated for yeast cells that, with a decreasing dose rate of ionizing radiation to obtain the maximum synergistic effect, the temperature at which the radiation is delivered should be diminished. To justify the acceptability of this conclusion to other biological objects we have analyzed the experimental data published by other authors. Inactivation of attenuated poliovirus [125] and T4 bacteriophage [142] were dependent on both dose rate (1.32 and 5.1 Gy/min) and exposure temperature. It has been shown for *Bacillus subtilis* spores that for a given temperature (95 or 105°C) there is a specific dose rate which maximizes the rate of inactivation [137,138]. For this cell system, a temperature- and dose rate-dependent model was suggested for the kinetics of cellular response to simultaneous treatment with ionizing radiation and temperature [122]. For mammalian cells the temperatures increasing the effect of ionizing radiation shifted toward lower temperatures with a decrease of dose rate [120,121]. Our calculations showed [136] that the value of synergism was the highest for bacterial spores (k_{max} = 2.2) but was intermediate for bacteriophage (k = 1.3) and cultured mammalian cells (k = 2.2). This was due to

the fact that for last cell systems the highest synergistic effect was not obtained for all dose rates used in the experiments. However, the main conclusion of the present work was also valid for these objects. To preserve the same level of synergy, the exposure temperature should be lowered with diminishing of dose rate of ionizing radiation. Consequently, the data for yeast cells presented here are consistent with those described for cells of various origin and thus demonstrate that with decreasing dose rate the temperatures at which irradiation occurs should be lowered in order to provide the greatest or some a definite level of synergy.

For diploid yeast cells, the synergistic interaction of the simultaneous action of 254 nm ultraviolet light or 20 kHz ultrasound with hyperthermia was also studied. It was shown that, at any fixed UV light or ultrasound intensity, the synergistic effect occurred within a certain temperature interval. For other temperatures, the effect was only additive. The optimal temperature to achieve the greatest synergistic effect may be shown for every intensity of these physical factors. The correlation between the optimal temperature that maximized the synergy and UV light or ultrasound intensity was estimated: this temperature shifted towards lower temperature values with a decreasing intensity of the acting physical factor. These data are in complete agreement with the discussed set of data demonstrating the importance of dose rate for the synergistic interaction of ionizing radiation and hyperthermia.

Using the experimental data published by others for the simultaneous action of hyperthermia and various concentration of thio-TEPA [129] or *cis*-DDP [143] on cultured mammalian cells, it was demonstrated that with a decrease in concentration of both drugs the exposure temperature should also be decreased to achieve the highest synergistic interaction. Admitting the concentration of the chemical agent may be considered as an analog of the intensity of the inactivating factor, we may conclude that these data agree with the whole complex of the above discussed results evidencing the importance of the intensity of the acting agents for their synergistic interaction.

Taking all these data obtained for the simultaneous treatment of hyperthermia with ionizing radiation, ultraviolet light, ultrasound and some chemical drugs on various cell systems as a whole, one can conclude that the

intensity of the physical factor or the concentration of the chemical agent may be considered as a determinant of synergy. It was shown that the lesser intensity of one of the agent applied, the lesser temperature under which the treatment occurred should be used to obtain the highest value of synergism. These results seem to be related to the fact that, if the intensity decreases, then the effective lethal dose is delivered over a long time, so that the duration of heat incubation increases, which could explain the lower temperature that should be applied to the cells. In other words, for a short duration of interaction, relatively higher intensities of both agents should be used to provide the greatest synergistic interaction. In contrast, for a long duration, relatively smaller intensities must be used. Besides being of interest for interpretation of the mechanism of synergistic interaction, these results may be important in diverse practical applications. Although all these data were obtained at temperatures far beyond the physiological range, it is not excluded, in principle, that for even physiological temperatures of homeothermal animals and man there could be exist optimal intensities of existing natural or man-made background of ionizing radiation, UV light, ultrasound or concentrations of chemical pollutants providing the highest synergistic interaction and thereby increasing their biological effects.

5. Abstract

Synergistic interaction of the simultaneous action of hyperthermia with ionizing radiation, ultraviolet light, ultrasound and some chemical agents has been analysed using experimental data obtained by authors with bacterial and yeast cells and published by others for viruses, bacterial spores and cultured mammalian cells. Some general non-trivial rules in responses of these cellular systems to the combined action of hyperthermia with one of the other inactivating agents were revealed. For every constant intensity or concentration, there was a specific temperature that maximizes the synergistic interaction of hyperthermia with another agent employed. Any deviation of temperature from the optimal one resulted in a reduction of the synergy. The intensity of the physical factors or the concentration of chemical agent strongly influenced the

synergy: the lesser intensity of the agent applied, the lesser temperature under which the treatment occurred should be used to obtain the highest or some definite value of synergism.

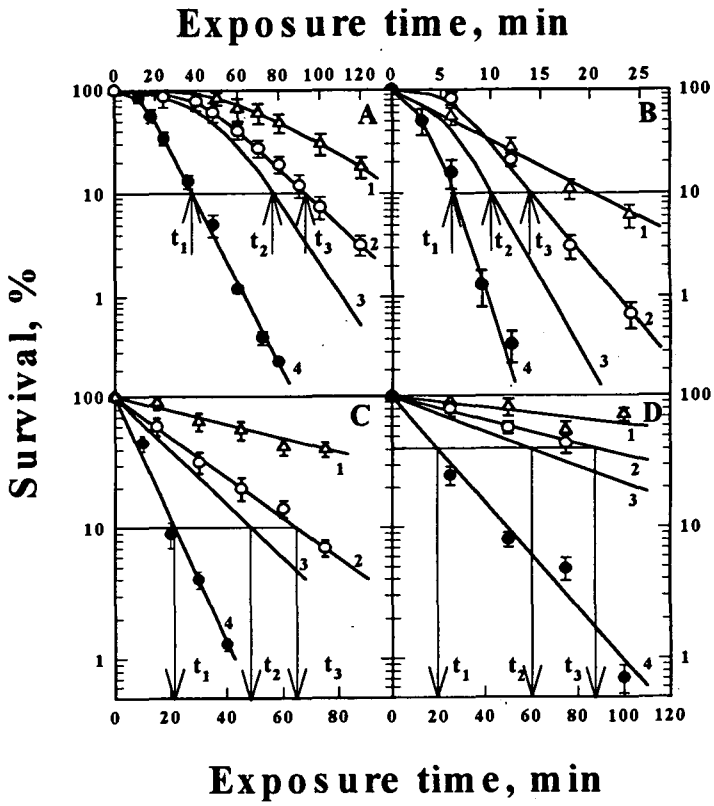


Fig. 33. Survival curves of diploid yeast cells (A - *Endomyces magnusii*, B, C *Saccharomyces cerevisiae*, strain XS800) and bacterial *Escherichia coli B/r* cells exposed to various agents. Curve 1 - heat treatment alone (A - 40℃, B - 52, 5℃, C - 48℃, D - 40℃); curve 2 another inactivating agent applied alone at room temperature (A - γ-ray, 10 Gy/min, B - 254 nm UV light, 0,25 W/m^2, C - 20 kHz ultrasound, 0,05 W/cm^2, D - 0,01 M zinc sulfate); curve 3 - calculated effect for the independent action of the agents used; curve 4 - experimental surviving curves after the simultaneous action of the agents used. Error bars show inter-experimental standard errors.

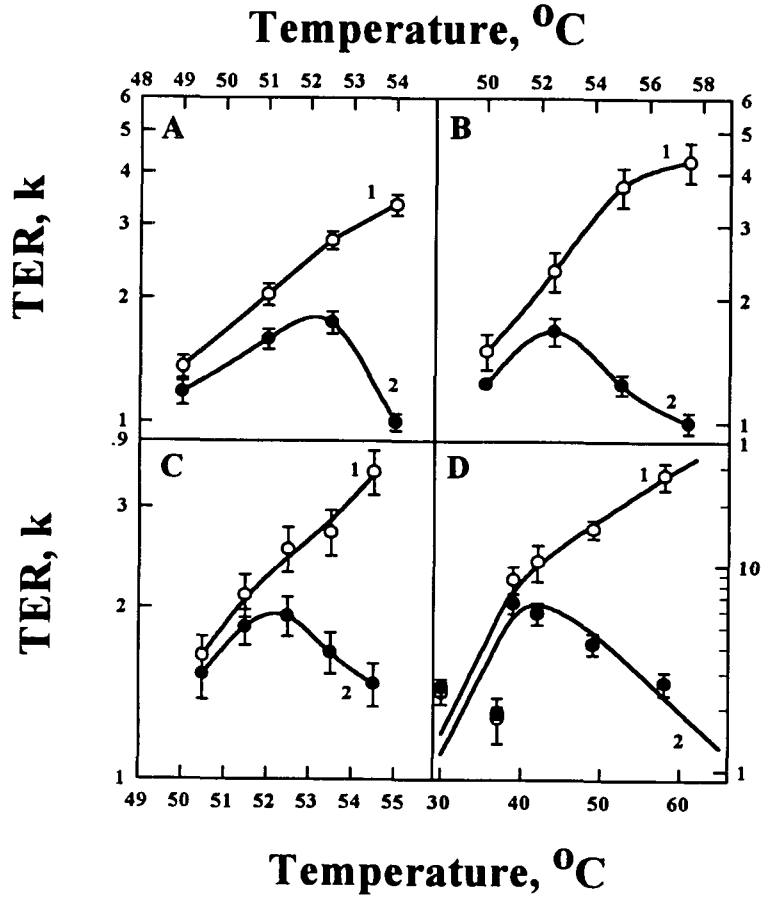


Fig. 34. The dependence of the thermal enhancement ratio TER (curve 1) and synergistic enhancement ratio *k* (curve 2) upon exposure temperature after the simultaneous action of high temperature and another inactivating agent (A - 25 MeV electron radiation, 25 Gy/min, B - 254 nm UV light, 0,25 W/m^2, C - 20 kHz ultrasound, 0,05 W/cm^2, D - 0,01 M zinc sulfate) on *Saccharomyces cerevisiae* diploid yeast cells, strain XS800 (A, B, C) and *Escherichia coli* bacterial cells, strain *B/r* (D). Error bars show inter-experimental standard errors.

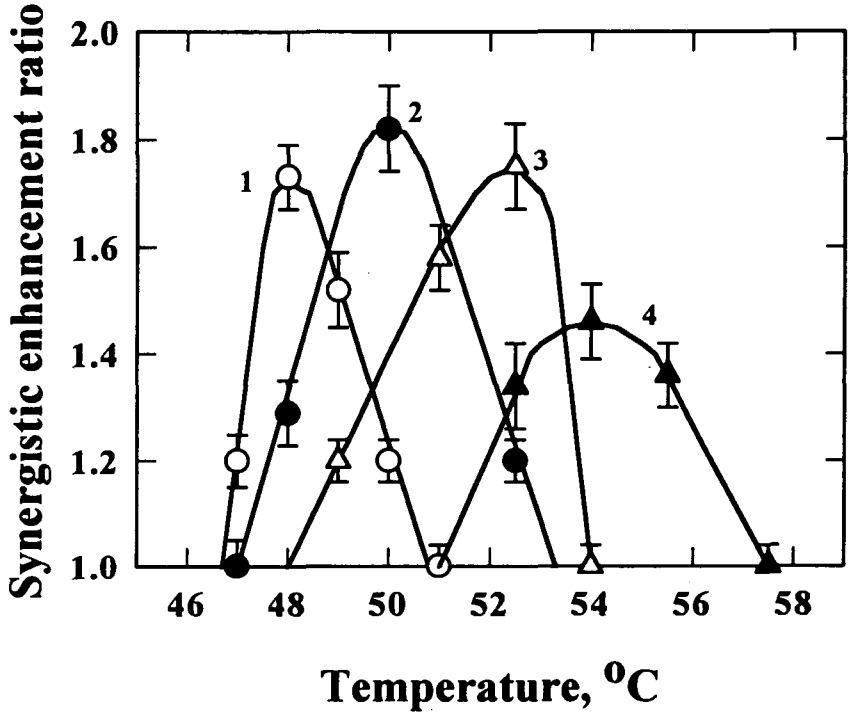


Fig. 35. The dependence of the synergistic enhancement ratio (k) upon exposure temperature and the dose rate of 25 MeV electron radiation (1 - 5, 2 - 10, 3 - 25, 4 - 250 Gy/min) for *Saccharomyces cerevisiae* diploid yeast cells (strain XS800). Error bars show inter-experimental standard errors.

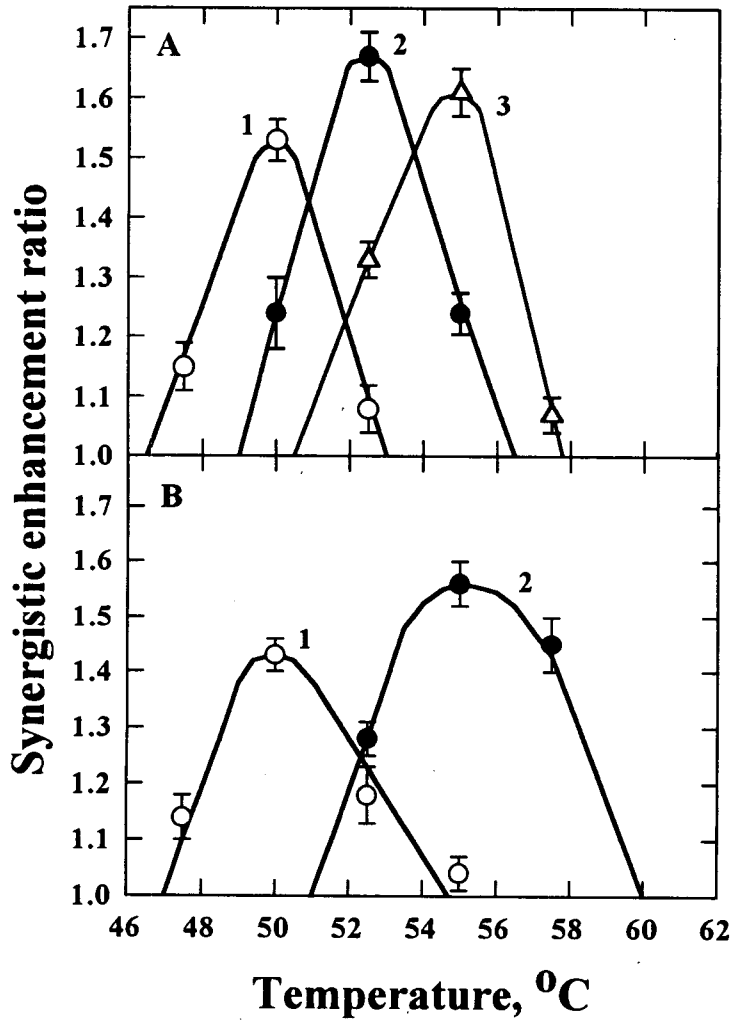


Fig. 36. The dependence of the synergistic enhancement ratio (k) upon exposure temperature and the fluence rate of ultraviolet light (254 nm). A: curve 1 - 0.033, 2 - 0.25, 3 - 1.5 W/m^2; B: curve 1 - 0.15, 2 - 1.5 W/m^2 for *Saccharomyces cerevisiae* diploid yeast cells, strains XS800 (A) and T1 (B). Error bars show inter-experimental standard errors.

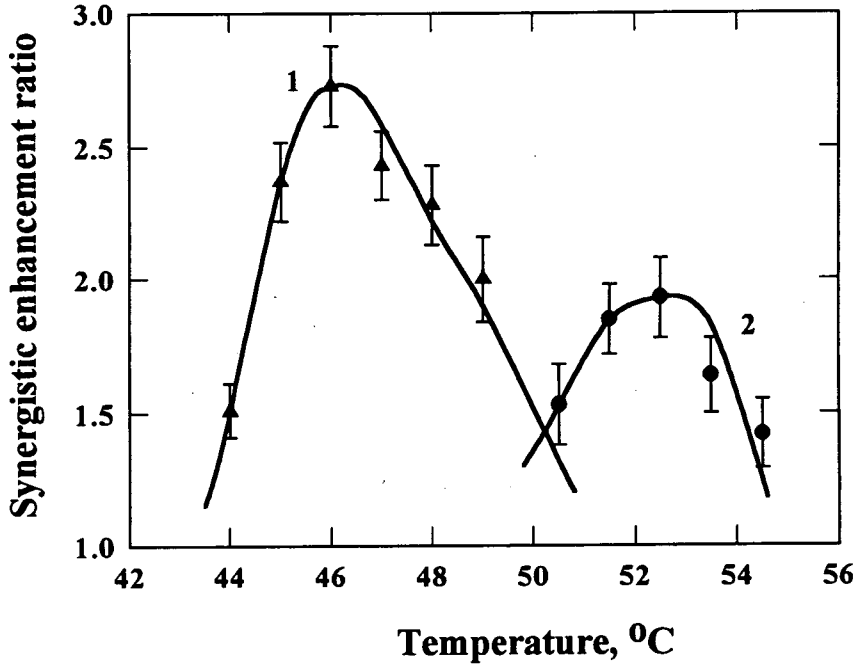


Fig. 37. The dependence of the synergistic enhancement ratio (k) upon exposure temperature and ultrasonic (20 kHz) intensity (1 - 0.05, 2 - 0.2 W/cm^2) for *Saccharomyces cerevisiae* diploid yeast cells (strain XS800). Error bars show inter-experimental standard errors.

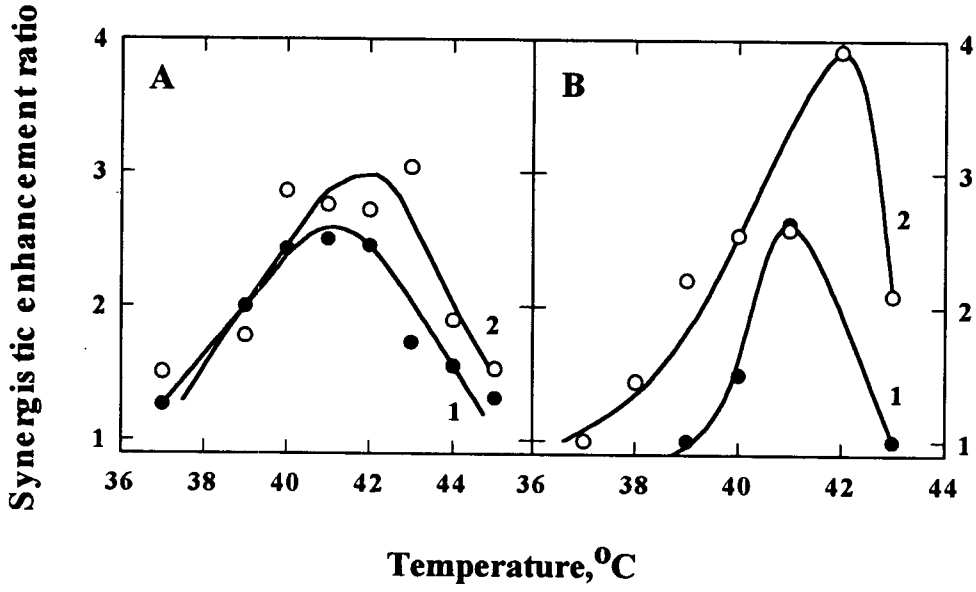


Fig. 38. The dependence of the synergistic enhancement ratio (k) upon exposure temperature and concentration of thio-TEPA (A: curve 1 5 g/mℓ, curve 2 10 g/mℓ) and *cis*-DDP (B: curve 1 3 M, curve 2 6 M) for Chinese hamster cells.

제8절 방사선 및 화학물질의 상승작용에 있어서 결정인자 분석

(Section VIII. Determinant of synergistic interaction between radiation, heat and chemicals in cell killing)

ABSTRACT

Experimental data obtained for simultaneous action of ionizing radiation with different physical or chemical agents on various cellular systems evidence that the lesser the intensity of physical factor or the concentration of chemical agents, the smaller the temperature that has to be used to provide the highest or a definite level of synergistic interaction. On this basis, it is inferred that the synergism may take place at small intensities of harmful environmental factors existing in the biosphere. Hence, the assessment of health or environmental risks should take into account the synergistic interaction between harmful agents.

1. Introduction

Combined exposures are an essential feature of modern life. It is well known that almost all physical and a wide array of chemical harmful agents, both natural and man made, are capable of interacting with each other in a synergistic manner when the final biological effect exceeds the sum of individual effects produced by interacting agents. Therefore any risk assessment must consider the question whether combined effects will influence the health outcome. Of all possible situations of combined actions, long term exposure of living objects to low levels of the agents widely presented in the nature is especially important. However, the assessment of potential significance of synergistic interaction between adverse environmental factors acting together at the level of intensity and concentration found in biosphere is still an intriguing and unresolved problem. Real experiments with low intensities found in

environmental and occupational settings is prone to large uncertainties. A feasible approach to this problem is to analyze the dependence of the efficiency of synergistic interaction on the intensity of agents used. Hypothetically, some possibilities could be realized. The case when synergism is decreasing with intensities of deleterious agents applied is unimportant. The same is true for the situation when a decrease in the intensity of one factor should be accompanied by an increase in the intensity of another to retain their synergistic interaction at the same level. The only possibility would be of great importance: if the lesser intensity of one of the agents is applied, then the smaller intensity of another agent should be employed for the display of the highest or a definite level of synergy. In such a case, it would be expected that even at low intensity environmental agents may, in principle, interact with each other in a synergistic manner and thereby to enhance their harmful action. In this section, we present conclusive evidence confirming the last opportunity for various cellular systems and various a

2. Materials and methods

Zygosaccharomyces bailii haploid and *Saccharomyces cerevisiae* diploid (strain XS800) and haploid (strain S288C) yeast cells were used in the experiments. Yeast cells were incubated before irradiation for 3~5 days at 30℃ on a complete nutrient agar layer to a stationary phase. Aliquots with 106 yeast cells/㎖ were exposed to various physical agents applied alone or combined with hyperthermia. We used a ^{60}Co source Gammacell 220, Atomic Energy of Canada Ltd. The γ-ray dose-rate, estimated by a Siemens ionization chamber, was 10 Gy/min. The electron beam from a 25 MeV pulsed linear accelerator was also used in these experiments. The pulse duration at half peak output was 2.7 s and the pulse repetition rate was at 50 Hz, the average dose rates were 5, 10, 25 and 250 Gy/min as determined by ferrous sulphate dosimetry.

For ultraviolet (UV) irradiation, the cells were exposed with germicidal lamps that emitted predominantly UV light of wavelength 254 nm. Variation of the intensity was achieved by means of calibrated metal wire nets. The fluence

rates were measured using a calibrated General Electrical germicidal meter. To avoid photoreactivation, UV exposure, dilution and cell plating were performed under red ambient light, while post-irradiation incubation was carried out under dark conditions.

Ultrasound (20 kHz) was generated by a Fisher sonic dismembrator (Model 300). The ultrasonic dose rates were 0.05 and 0.2 W/cm^2 which was measured by the calorimetric method. To determine the effect induced by ultrasound alone, the absorbed ultrasound heat was completely removed by cooling with water.

Experiments on the simultaneous action of high temperature and other agents were performed as follows. The special dish and tube containing 1.4 (for UV light irradiation) or 9.9 (for ionizing radiation and ultrasound exposures) ml of sterile water was preheated to a specified temperature which was maintained with an accuracy of 0.2℃. Cell suspension was added immediately before irradiation or sonication (an 0.1-ml aliquot containing 1.5x107 cells for UV-light and 108 cells for ionizing radiation and ultrasound exposures). For the simultaneous action of hyperthermia and other physical or chemical agents, the time interval between the introduction of the cells into the preheated water and the beginning of exposure was about 0.1~0.3 min, which was significantly less than the total treatment time. At the end of the treatment, the samples were rapidly cooled to room temperature, and, hence, the exposure to high temperature and another physical agent lasted for the same period of time. Thereafter, cell suspension was diluted to a necessary concentration and plated onto the standard nutrient medium to determine the cell survival by the method of macrocolonies. All experimental series were repeated 3~5 times. The details were described elsewhere [115,116,133,134,146].

3. Results

It is now generally accepted that the highest synergistic interaction is observed under the simultaneous action of harmful agents. The increasing in the interval between exposure results in a diminution of synergy. That is why in this work we analyse only simultaneous application of agents. Cell killing is the

main end-point envisaged. Fig. 39 provides an example of the basic experimental data used in this investigation. To estimate quantitatively the sensitization action of hyperthermia, one can apply the thermal enhancement ratio [139] defined as the ratio D3/D1 or t3/t1 (Fig. 39). This ratio indicates an increase of cell radiosensitivity by high temperature. However, it does not reflect the kind of interaction (whether it was independent or synergistic). To calculate the synergistic effect we used the synergistic enhancement ratio (k), defined as the ratio of the calculated radiation dose (assuming an additive effect of radiation and hyperthermia) to that observed from the experimental survival curve for the simultaneous action of radiation (or other agents employed) and hyperthermia at a fixed level of survival. For example for 1% survival, k = D2/D1 = t2/t1 (Fig. 39). For exponential survival curves, this parameter is independent of the survival level for which it is calculated. For sigmoidal survival curves, the synergistic enhancement ratio was calculated for 10% survival. Both these parameters are plotted in Fig. 40 against the irradiation temperature for XS800 diploid (Fig. 40a) and S288C haploid (Fig. 40b) *Saccharomyces cerevisiae* yeast cells simultaneously exposed to ^{60}Co γ-ray (10 Gy/min) and high temperature. The noticeable feature of Fig. 40 is that the thermal enhancement ratio (curve 1) increases indefinitely with increasing exposure temperature, while the synergistic enhancement ratio (curve 2) at first increases, then reaches a maximum, which is followed by a decrease. This implies that the synergistic interaction between hyperthermia and ionizing radiation is observed only within a certain temperature range. Noteworthy is the fact that such a dependence of synergistic effect on temperature under which the exposure was occurred was also obtained upon the simultaneous combination of hyperthermia with UV light [90,115], ultrasound [134,146] and some chemical inactivating agents [130,147]. Hence, one can conclude that for a given intensity of physical factors or concentration of chemical agents there would be a specific temperature that maximizes the synergistic interaction. Any deviation of the acting temperature from optimal value results in a decrease of synergism. One more important conclusion can be made from the results presented in Fig. 40. One can see that the effectiveness of synergistic interaction was smaller for haploid cells than for diploid ones. It is in agreement with the well known fact that the mechanism of

synergy is related with cell ability to repair radiation damage [120,121] while repair of DNA double strand breaks requires two homologous DNA duplexes [148].

To evidence the importance of synergistic effects at low intensity of inactivating agents, we analysed the dependence of synergistic interaction on the intensity of physical factors or on the concentration of chemical agents applied in combination with hyperthermia. Using survival curves data published for simultaneous action of hyperthermia and ionizing radiation on bacteriophage [142], bacterial spores [137,138], yeast [161,133] and mammalian cultured cells [120,121], we were able to calculate the synergistic enhancement ratio for various cell systems and irradiation condition. It allowed us to establish the correlation between the dose rate and the exposure temperature, which both provide maximum or other arbitrary levels of synergistic interaction (Fig. 41). Open circles denote the results of our calculations based on experimental results published in the cited papers. The value of synergism was the highest for bacterial spores (k_{max} = 2.2), diploid yeast cells (k_{max} = 1.7) and was intermediate for bacteriophage (k = 1.3) and cultured mammalian cell (k = 2.2). This was due to the fact that for last cell systems the highest synergistic effect was not obtained for all dose rates used in the experiments. One can see that linear relationships are found between these values for various cellular objects. This means the general importance of the dose rate of ionizing radiation in the manifestation of synergistic interaction. It can be inferred that the temperature at which ionizing radiation is delivered should be diminished to obtain the maximum or a definite synergistic effect with dose rate decreasing and *vice versa*.

To check this regularity for other inactivating agents, the data on simultaneous effect of hyperthermia combined with UV light [90,115] or ultrasound [134,146] on diploid yeast cells, as well as with tris(1-aziridinil)-phosphine sulfide (thio-TEPA) [129] and *cis*-diamminedichloroplatinum (II) (*cis*-DDP) [143] on cultured mammalian cells were involved. The last two set of data include the relationship between exposure temperature, concentration, and rate of cell inactivation for chemical agents used in clinical chemotherapy. Hence, they have no direct attitude toward

environmental harmful agents and they used here to demonstrate that the concentration of chemical agents is also important for their synergistic interaction with heat. Using data published in the above cited works, we could obtain the relationships between the intensity of physical factors or the concentration of chemical agents with the exposure temperature which both provide the greatest synergy (Fig. 42). Here again, open circles denote the results of our calculations based on the survival curve data published earlier. The value of the synergistic enhancement ratio was about 1.6 for UV light, 2.5 for ultrasound, 2.5 for thio-TEPA and 3.2 for *cis*-DDP combined with hyperthermia. In all cases, at a smaller intensity of the physical factor or concentration of the chemical agents, it was required to reduce the acting temperature to preserve the highest synergistic effect.

4. Discussion

The experimental data presented in this section and obtained by authors with yeast cells and published by other authors with cultured mammalian cells after simultaneous action of heat and various physical and chemical agents revealed two remarkable features. First of all, for any constant intensity of physical agent or concentration of chemical compounds there is an optimal temperature at which their synergistic interaction shows the highest effectiveness. In other words, there exists a definite temperature range inside which the synergistic interaction takes place.

The second relevant feature, followed from the data presented in this section is the evidence of the importance of synergistic effects at low intensity of acting agents. Experimental results obtained for the simultaneous treatment of hyperthermia with ionizing radiation, ultraviolet light, ultrasound and some chemical drugs on various cell systems clearly indicate that the intensity of the physical factor or the concentration of chemical agent predestines the effectiveness of their synergistic interaction with heat. One can conclude therefore that time factor may be considered as a determinant of synergy. It was shown that the lesser intensity of one of the agent applied, the lesser

temperature under which the treatment occurred should be used to provide the highest or a specified level of synergism. These results seem to be related to the fact that, if the intensity decreases, then the effective lethal dose is delivered over a long time, so that the duration of heat incubation increases, which could explain the lower temperature that should be applied to the cells.

Taking all these data as a whole, one can conclude that for a long duration of interaction, which are important for problems of health physics, small intensities of deleterious environmental factors may synergistically interact with each other either with environmental heat or physiological temperatures of homoiothermal animals and man. Hence, the assessment of health or environmental risks from numerous natural and man-made agents at the level of intensities or concentrations found in environmental and occupational settings should take into account the synergistic interaction between harmful agents.

5. Conclusions

It can be therefore concluded that (i) the exposure rate of environmental agents is an important relevant determinant of synergy, and that (ii) there perhaps may be exist a common rule among the objects tested and agents employed in this work: the lesser intensity of one of the agent applied, the smaller temperature has to be used to provide the highest or a definite level of synergistic interaction. It can be inferred that, in principle, the synergistic effect may take place between small intensities of harmful environmental factors existing in the biosphere and environmental heat and/or body temperature of homeothermal animals and man. This inference can have important bearing on the possible outcome of combined exposure, the risk assessment and disaster-prevention after action of numerous deleterious agents existing in comtemporary life.

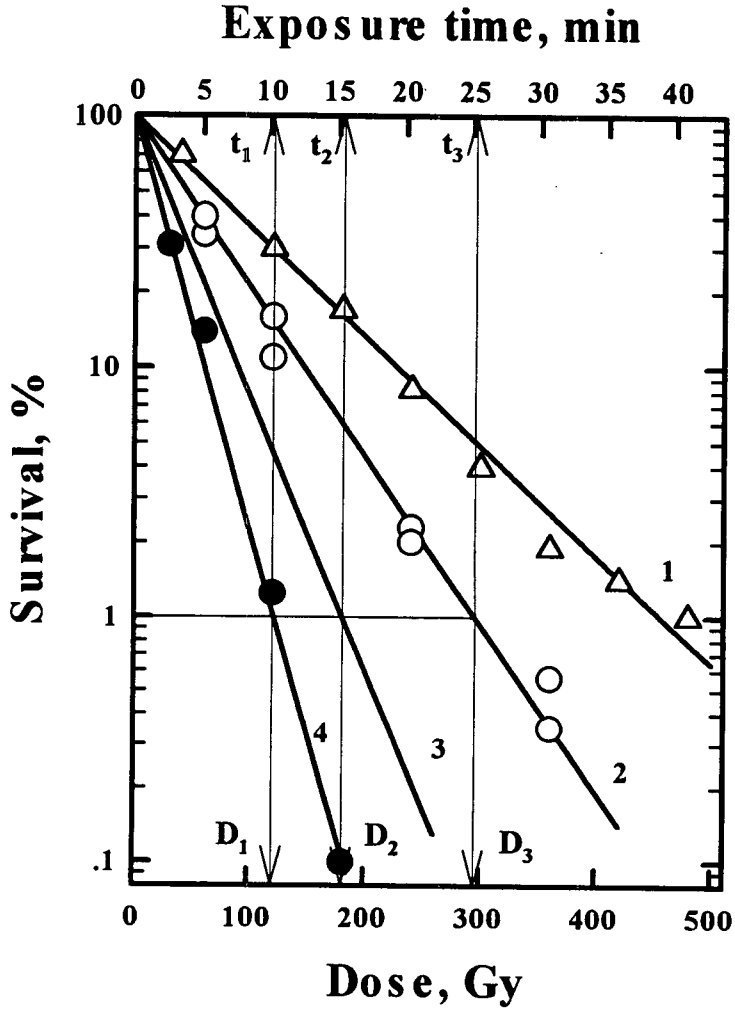


Fig. 39. Survival curves of Zygosaccharomyces bailii haploid yeast cell. Curve 1 - heat treatment (45℃) alone; curve 2 - γ-radiation at about 10 Gy/min and room temperature; curve 3 - calculated curve for independent action of ionizing radiation and heat; curve 4 - experimental curve after simultaneous thermoradiation action.

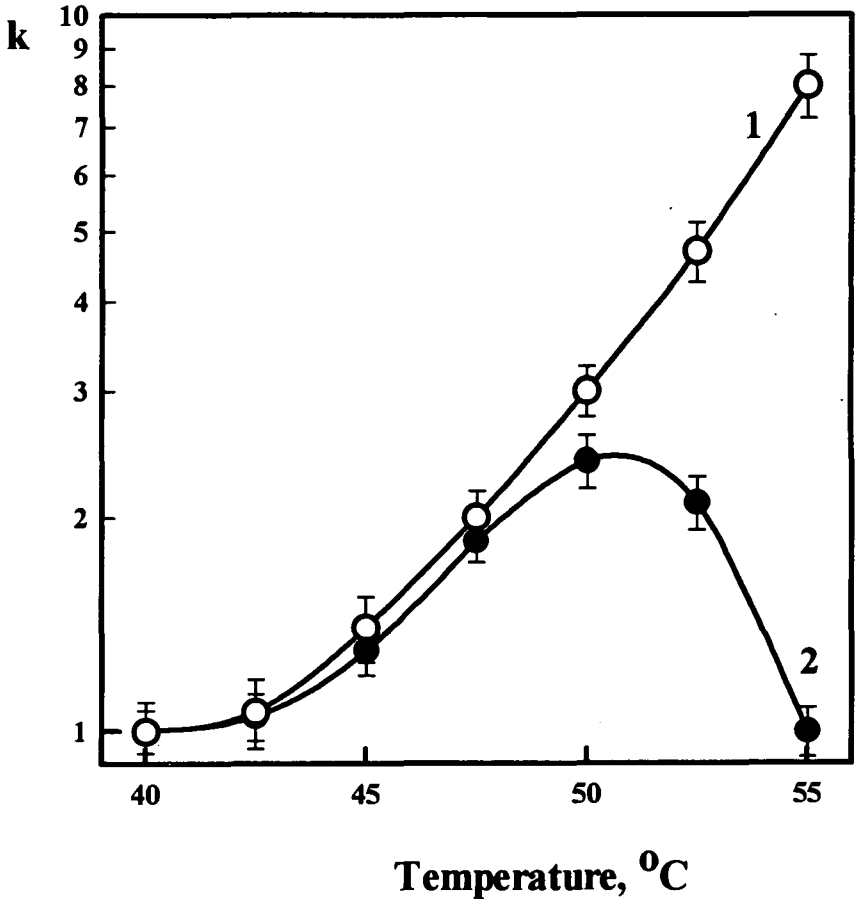


Fig. 40. Thermal enhancement ratio (TER) (curve 1) and synergistic enhancement ratio (k) (curve 2) of *Saccharomyces cerevisiae* diploid (a, strain XS800) and haploid (b, strain S288C) as a function of temperature during exposure of γ-ray at 10 Gy/min.

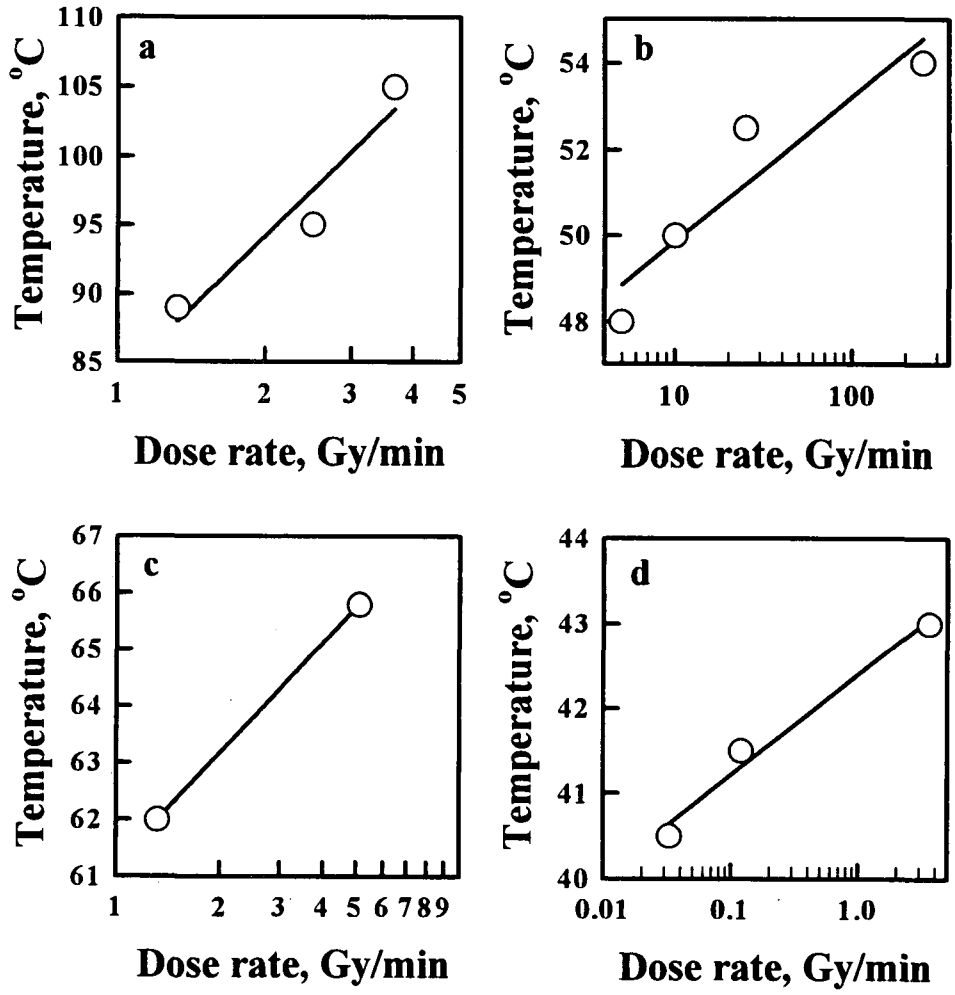


Fig. 41. Correlation of dose rate and exposure temperature providing the same synergistic interaction under simultaneous thermoradiation action: a - bacterial spores (*Bacillus subtilis*); b - diploid yeast cells (*Saccharomyces cerevisiae*, XS800); c - bacteriophage (T4); d - cultured mammalian cells (Chinese hamster cells). The original survival curve data were partly obtained by authors and the publications. The calculated values of synergism were about 2.2 for bacterial spores, 1.7 for yeast cells, 1.3 for bacteriophage, 2.2 for cultured mammalian cells. The total doses were about 5000 Gy for bacterial spores, 1000 Gy for yeast cells, 500 for bacteriophage, and 10 Gy for cultured mammalian cells.

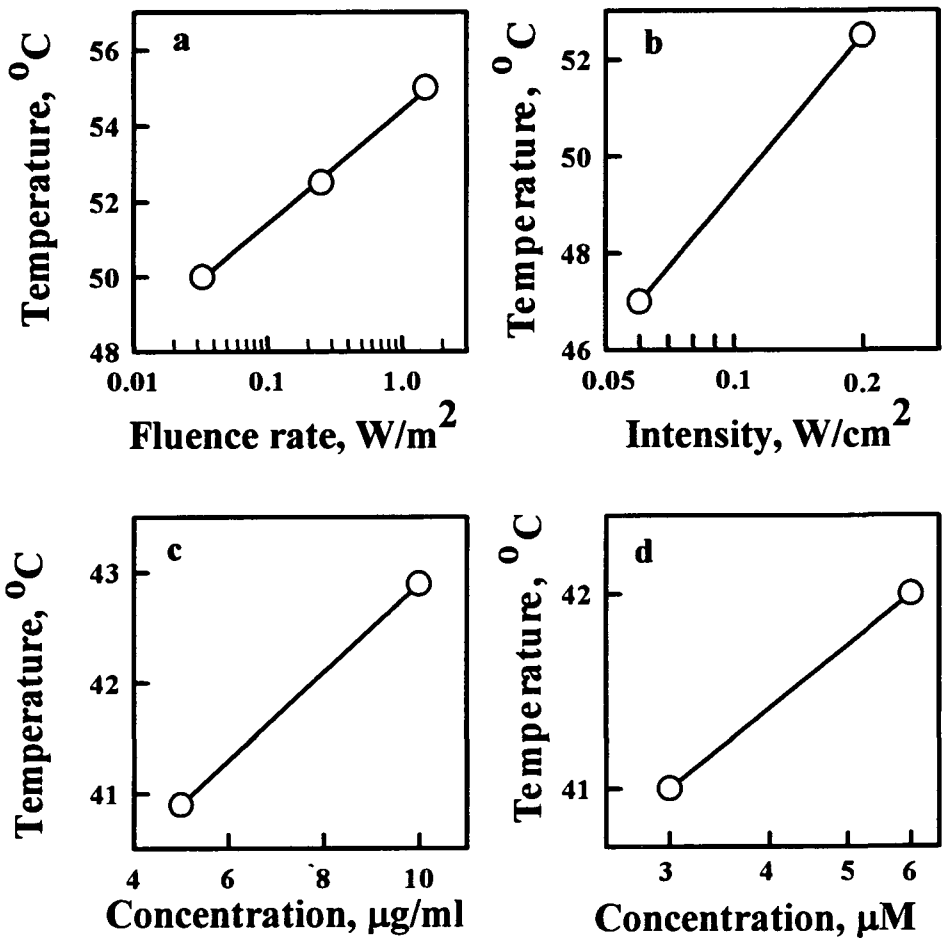


Fig. 42. Correlation of exposure temperature with UV light fluence rate (a), ultrasound intensity (b), and concentration of *tris*(1-aziridinil)-phosphine sulfide (thio-TEPA) (c) and *cis*-diamminedichloroplatinum (II) (*cis*-DDP) (d) providing the highest synergistic interaction under their simultaneous action on yeast cells (a, b) and cultured mammalian cells (c, d). The original survival curve data were partly obtained by authors and were taken from the publications. The calculated values of synergism were about 1.6 for UV light, 2.5 for ultrasound, 2.5 for thio-TEPA, and 3.2 for *cis*-DDP combined with hyperthermia.

여 백

제 4 장 연구개발목표 달성도 및 대외기여도

총 3년에 걸친 연구기간 중 일차년도 (1998. 9~1999. 8)에 설정한 연구목표 '방사선과 화학물질의 상호작용에 의한 TSH 체세포 돌연변이 반응성 규명'을 위해 붕소화합물의 전처리가 ^{252}Cf 중성자에 의하여 유발된 TSH 체세포 돌연변이를 변화시키는 정도를 상대생물효과비 (RBE)의 개념을 적용해서 정량적으로 평가하였다. 또한 감마선 및 살충제가 TSH 돌연변이에 미치는 영향을 비교·분석하여 계량화하였다. 일차년도 연구기간중 두 편의 논문을 국내학술지에 게재하였으며 3편의 논문발표를 수행하는 등 설정된 연구목표를 달성하였다.

연구개발의 이차년도 (1999. 9~2000. 8)는 '방사선 및 화학물질 상호작용에 의한 림프구내 DNA손상 반응성 규명'을 연차목표로 설정하였다. 연차별 목표를 달성하기 위해서 의학적으로 이용되고 있으며 많은 관심을 받고 있는 붕소화합물 전처리가 사람림프구의 방사선민감도를 변화시키는 정도를 정량화하는 실험을 수행하는 한편 가돌리늄 화합물에 의한 radiomodification 효과 또한 실험적으로 평가하였다. 또한 농업적으로 널리 사용되는 살충제 (일차년도에 TSH assay를 적용하여 실험을 수행한 동일 농약)와 감마선이 사람 림프구 DNA 손상에 미치는 상승작용의 영향을 구체적으로 분석하고 그 결과를 연구실적물 (SCI 학술지 논문 4편, 국내학술지 2편, 학술발표 5건)로서 제시함으로써 연구목표를 완수하였다.

연구개발의 삼차년도 (2000. 9~2001. 8)에는 '방사선과 화학물질 상호작용에 의한 생체효과 해석기술 수립'을 목표로 방사선과 기타요인에 의한 synergism 관련 논문 2편 게재, synergistic interaction 해석을 위한 수학모델 설정 및 관련 논문 2편 게재를 정량적 목표량으로 설정하였으며 이를 모두 달성하였다.

연차별 연구목표를 모두 달성함으로서 당초 제시된 연구개발의 최종목표인 '방사선과 화학물질에 의한 복합적 생체영향 해석기법의 확립'을 이룩하였다. 연구기간중 국내외 학술지에 게재했거나 국내외 관련 학술회의에서 발표된 실적물은 다음과 같다.

연 구 성 과	내 용			
	명 칭	저널·학회명	게재일	비 고
논문 게재	Relative biological efficiency of californium-252 neutrons in the induction of gene and lethal mutations in Trad-SH normal and enriched with boron-10 cells	*Mutation Research - Fundamentals and Molecular Basis of Muagenesis* 474: 57-70	2001. 4	SCI
	Mitotic recombination and inactivation in *Saccharomyces cerevisiae* induced by UV (254 nm) radiation and hyperthermia depend on UV fluence rate	*Mutation Research - Fundamentals and Molecular Basis of Muagenesis* 478: 169-176	2001. 7	SCI
	Induction and repair of DNA damage as measured by the Comet assay and the yield of somatic mutations in gamma-irradiated tobacco seedlings	*Mutation Research - Genetic Toxicology and Environmtal Mutagenesis* 491: 17-23	2001. 4	SCI
	Effects of combined irradiation of neutrons and γ-rays on the pink mutation frequencies in *Tradescantia*	*Journal of the Korean Association for Radiation Protection* 25(2): 67-73	2000. 6	국내학술지
	Mathematical description of synergistic interaction of UV-light and hyperthermia for yeast cells	*Journal of Photochemistry and Photobiology B: Biology* 55(1):74-79	2000. 5	SCI
	Dose-response relationship of micronucleus frequency in pollen mother cells of *Tradescantia*	*Journal of the Korean Association for Radiation Protection* 24(4):187-192	1999. 12	국내학술지

연 구 성 과	내 용			
	명 칭	저널·학회명	게재일	비 고
논문 게재	Considerations on the radiomodifying effect of boron and gadolinium compounds in human peripheral blood lymphocytes as assessed by the comet assay	*Toxicology Letters* (submitted) ref: jpk 00-68	(2000. 6)	SCI
	Exposure rate as a determinant of synergistic interaction of heat combined with ionizing or ultraviolet radiations in cell killing	*Journal of Radiation Research* (submitted) ref: JRR#01-10	(2001. 7)	SCI
	Survival and recovery of yeast cells after simultaneous treatment of ionizing radiation and heat	*International Journal of Radiation Biology* (submitted) IJRB/2001/000109	(2001. 7)	SCI
	Synergistic Interaction of Radiation with Pesticide on DNA Damage in Human Lymphocytes as Biological Information for Prevention of Environmental Disaster	*Kor. J. Environ. Biol.* 19: 19-24	2001. 3	국내 학술지
	Effect of NaCl and gamma ray on pink mutations in the stamen hair cells of *Tradescantia* 4430	*Kor. J. Environ. Agr.* 18: 41-47	1999. 6	국내 학술지
	Protective effect of pesticide on radiation-induced cell damage in *Tradescantia* 4430 stamen hairs	*Kor. J. Environ. Biol.* 17: 21-26	1999. 3	국내 학술지

연 구 성 과	내 용			
논문 발표	명 칭	저널·학회명	발표일	비 고
	Radioresponse in hyman lymphocytes pretreated with B and Gd compounds as assessed by the comet assay	*International Comet Assay Workshop*	2001. 07. 22-24	Ulm (독일)
	Influence of environmental stresses on radiation-induced pink mutations in *Tradescantia* stamen hairs	*Second International Symposium on Ionizing Radiation*	1999. 05. 10-14	Hiroshima (일본)
	붕소화합물이 처리된 자주달개비 체세포 돌연변이 유발에 있어서 중성자의 상대생물효과비 변화	한국원자력학회 2000춘계학술발표회 (한전원자력연수원, 고리)	2000. 05. 26-27	
	유해 환경요인의 복합적 상조작용에 관한 이론적 고찰	한국환경생물학회 2000춘계학술대회 (경북자연환경연구원, 구미)	2000. 05. 19-20	
	선질이 다른 두가지 방사선 조사의 선후 방식에 따른 자주달개비 체세포 분홍돌연변이율의 변화	대한방사선방어학회 2000년도 춘계학술발표회 및 심포지움	2000. 04. 21	논문집 p.43-48
	자주달개비 화분모세포 미세핵 생성의 방사선량-반응 관계	한국원자력학회 '99 추계학술발표회 (서울대학교, 서울)	1999. 10. 29-30	초록집 p.337
	Radiomodifying effect of boron and gadolinium compounds in human peripheral lymphocytes evaluated by the comet assay	대한방사선방어학회 '99추계학술발표회 (경북대학교, 대구)	1999. 10. 22-23	논문집 p.41-45
	자주달개비 체세포 돌연변이에 대한 방사선과 농약의 상조작용	한국환경생물학회 '98추계학술 대회	1998. 10. 16	상명대학교, 서울
	살충제와 감마선에 의한 자주달개비 수술털의 분홍돌연변이 빈도 변화	대한방사선방어학회 '98추계 학술발표회	1998. 11. 6	논문집 p.11-14,
	염분에 의한 방사선 유발 세포돌연변이 변화	한국환경생물학회 '99춘계학술대회	1999. 5. 14	대구대학교, 대구
기술이전 실적 등	명 칭	수요기관	이전기간	비 고

제 5 장 연구개발결과의 활용계획

KAERI-INP 공동연구를 통하여 방사선생물학, 핵의학, 인간 모니터링, 환경생물학 분야 등에 폭넓게 응용 가능한 제반 연구기법을 확립하였다. 특히 기관간 연계실험을 수행함으로써 두 기관이 보유한 연구장점을 최대로 활용할 수 있었을 뿐 아니라 한국원자력연구소 보유기술의 수준을 검증할 수 있었다. 본 공동연구를 통해 획득된 모든 연구 결과들은 SCI (science citation index)에 등재된 유명학술지에 공동 논문으로 게재될 것이며 이를 통해 한국·폴란드 간의 과학기술 분야 관계개선에 기여토록 할 것이다. TSH 돌연변이 분석기술, SCGE 기법 및 상승작용 해석기술 등은 개선을 거쳐 한국·폴란드 간의 호혜적 협력관계 구축 및 발전을 위한 기술근거로 계속해서 활용될 것이다.

○ 각종 방사선 및 화학물질에 대한 실험을 거쳐 확립된 TSH assay 연구기법은 이온화 방사선 및 비이온화 방사선의 생물학적 위해성 평가, 환경방사선 감시, 오염 토양 및 수질 평가 등에 활용될 것이며 이를 통하여 환경 위해 요인 (environmental risk factor)에 대한 보다 과학적인 생물학적 안전성 분석자료를 제공할 예정이다.

○ 본 연구를 통해 확립된 SCGE assay 기법은 방사선생물학 및 핵의학 분야의 응용연구에 핵심기술로 활용될 수 있다. 특히 방사선의 생물학적 효율을 변화시킬 수 있는 물질 (radiomodifier)들의 영향을 연구하고 방사선방어제 또는 방사선민감제 등을 개발하기 위한 평가수단으로도 활용성이 크다. 이 기술은 약간의 개선 및 변형과정을 거치면 동식물 세포 모두에 적용이 가능하기 때문에 환경감시, human monitoring 등에 적용될 수 있다.

○ 기관간 연계공동실험을 통하여 확립된 실험기법중 특히 이원적 연계실험의 설계 및 시료 생물체의 기관간 이송과 관련된 장거리, 장시간 운송처치 기술은 향후 생물체를 이용한 다국간 공동실험 (multi-national joint experiment)을 위한 기반기술로서 활용될 수 있다.

○ 본 연구의 수행 결과를 국제적으로 인지도가 높은 학술지 논문으로 게재함으로써 우리나라의 위상을 제고하는데 기여토록 할 것이며, 다년간의 공동연구 수행 경험은 한국·폴란드 간의 상호 협력과 이해의 폭을 넓히는 역할에서의 일익을 담당하게 될 것이며 특히 과학기술분야의 호혜적 동반자관계 발전을 위한 선험사례로 활용될 것이다.

여 백

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서 지 정 보 양 식			
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연구책임자 및 부서명	김진규 (동위원소·방사선응용연구팀)
연구자 및 부서명	송희섭, 김재성, 천기정, 이영근, 이병헌 (동위원소·방사선응용연구팀) ; 신해식 (학연생), 이경희 (연구생) ; 블라디슬라프 페틴 (러시아 MRRC) 안토니나 체불스카-바실레브스카 (폴란드 INP)

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초록(300단어 내외)

한국원자력연구소와 폴란드핵물리연구소간에 체결된 공동연구협약에 따라 양 기관 병립적 공동연구를 수행하였으며 주요내용에 대해서는 공동실험을 실시하였다. 핵심연구기법으로는 TSH assay, comet assay 및 synergism assay가 이용되었으며 다음과 같은 연구내용을 다루었다. ① 방사선 및 화학물질의 영향 분석을 위한 TSH 생물지표 응용, ② 중성자와 붕소화합물이 TSH 돌연변이에 미치는 영향, ③ 방사선에 의해 유발되는 TSH 돌연변이에 대한 살충제의 효과, ④ 감마선 및 살충제가 사람 림프구 손상에 미치는 영향, ⑤ 사람 림프구 DNA에 미치는 방사선과 B 및 Gd 화합물의 영향, ⑥ 복합적 상승작용에 관한 수학적 해석, ⑦ 상승작용에 관한 일반 규칙성 해석, ⑧ 방사선 및 화학물질의 상승작용에 있어서 결정인자 분석.

본 연구를 통하여 확립된 각종 연구개발 기술 및 공동연구의 결과는 향후 한국과 폴란드간의 호혜적 과학기술분야의 호혜적이며 상호보완적인 협력관계 발전을 위한 기술기반으로 활용될 것이다.

주제명키워드 (10단어내외)

방사선, 화학물질, 상승작용, 림프구, TSH 분석, 단세포겔전기영동

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Researcher and Dept.	H.S. Song, J.S. Kim, K.J. Chun, Y.K. Lee B.H. Lee (Radiation · RI Application Res. Team); H.S. Shin, K.H. Lee (Res. Assistant); Vladislav G. Petin (MRRC, Russia) Antonina Cebulska-Wasilewska (INP, Poland)

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Abstract(about 300 words)

KAERI nd INP (Poland) have been carried out parallel study and joint experiments on the major topics according to MOU about their cooperative project. Major experimental techniques were TSH assay, comet assay, and synergism assay. The research consisted of the following workscopes. ① Application of TSH bioindicator for studying the biological efficiency of radiation, ② Relative biological efficiency of californium-252 neutrons in the induction of gene and lethal mutations in TSH cells normal and enriched with boron compound, ③ Effect of pesticide on radiation-induced mutations in TSH cells, ④ Interaction of radiation with pesticide on DNA damage in human peripheral blood lymphocytes, ⑤ Radiomodifying effect of boron and gadolinium compounds in human peripheral blood lymphocytes, ⑥ Mathematical description of synergistic interactions, ⑦ General regularities of synergistic interactions, and ⑧ Determinant of synergistic interaction between radiation, heat and chemicals in cell killing.

Both institutes have established wide variety of research techniques applicable to various radiation research through the cooperation. The results of research can make the role of fundamental basis for the better relationship between Korea and Poland.

Subject Keywords (about 10 words)

Radiation, chemicals, synergistic interaction, lymphocyte, TSH assay, SCGE