Korean J. Environ. Biol. 20 (4) : 277~286 (2002)

〈Review Paper〉

Theoretical Conception of Synergistic Interactions

Jin Kyu Kim* and Vladislav G. Petin[1]

Korea Atomic Energy Research Institute, Daejeon 305-353, Korea
[1]Medical Radiological Research Center, 249036 Obninsk, Kaluga Region, Russia

Abstract – An increase in the overall biological effect under the combined action of ionizing radiation with another inactivating agent can be explained in two ways. One is the supposition that synergism may attribute to a reduced cellular capacity of damage repair after the combined action. The other is the hypothesis that synergism may be related to an additional lethal or potentially lethal damage that arises from the interaction of sublesions induced by both agents. These sublesions are considered to be ineffective when each agent is applied separately. Based on this hypothesis, a simple mathematical model was established. The model can predict the greatest value of the synergistic effect, and the dependence of synergy on the intensity of agents applied, as well. This paper deals with the model validation and the peculiarity of simultaneous action of various factors with radiation on biological systems such as bacteriophage, bacterial spores, yeast and mammalian cells. The common rules of the synergism are as follows. (1) For any constant rate of exposure, the synergy can be observed only within a certain temperature range. The temperature range which synergistically increases the effects of radiation is shifted to the lower temperature for thermosensitive objects. Inside this range, there is a specific temperature that maximizes the synergistic effect. (2) A decrease in the exposure rate results in a decrease of this specific temperature to achieve the greatest synergy and vice versa. For a constant temperature at which the irradiation occurs, synergy can be observed within a certain dose rate range. Inside this range an optimal intensity of the physical agent may be indicated, which maximizes the synergy. As the exposure temperature reduces, the optimal intensity decreases and vice versa. (3) The recovery rate after combined action is decelerated due to an increased number of irreversible damages. The probability of recovery is independent of the exposure temperature for yeast cells irradiated with ionizing or UV radiation. Chemical inhibitors of cell recovery act through the formation of irreversible damage but not via damaging the recovery process itself.

Key words : synergism, mathematical model, radiation, hyperthermia, sublesions

INTRODUCTION

Ecosystems and living organisms have never been exposed to merely one harmful agent. Many physical, chemical, biological and social factors may simultaneously exert their influence to organisms and the environment. In many examples of interactions between ionizing radiation and other toxic physical or chemical agents, the biological responses range from strong antagonism to strong synergism. The most effective simultaneous exposure to ionizing radiation and another factor may result in four possible modes of interaction: (i) no interaction, the effects observed are based on the most

---

* Corresponding author: Jin Kyu Kim, Tel. 042-868-2057, Fax. 042-868-2091, E-mail: jkkim@kaeri.re.kr

toxic agent; (ii) additivity, the effects produced by separate application of each agent are simply summed up; this mode can be subdivided on the additive and independent interaction; (iii) antagonism, the effect is less than expected additivity; (iv) synergism or supra-additivity, the effect is greater than expected additivity.

The combined exposure to two harmful agents could result in a higher effect than would be expected from the addition of the separate exposures to individual agents (UNSCEAR 1982; Streffer and Müller 1984; UNSCEAR 2000). Hence, there is a possibility that, at least at high exposures, the combined effect of ionizing radiation with other environmental factors can result in a greater overall risk. The problem is not so clear for low intensity and there is no possibility of testing all conceivable combinations of agents. Moreover, there are contradictions in literatures devoted to synergy problems relative to interaction effectiveness in the dependence of dose of applied agents and their intensity which results in various opinions about the importance of the synergistic interaction at low intensity of harmful agents found in biosphere (Streffer and Müller 1984; UNSCEAR 2000; Dethlefsen and Dewey 1982; Kuzin 1990).

A great number of experimental data obtained in this area stresses the need for a mathematical approach in efforts to optimize and predict the interaction of environmental agents. Recently, a simple mathematical model was proposed to describe the synergistic effect of simultaneous action of ionizing radiation and high temperature (Petin and Komarov, 1997). The basic postulates underlying the model were not related to a particular cytotoxic agent, so the model may have an application to describe the synergistic interaction of other agents. Some examples have been already published. Our purpose is to discuss the simplest model to account for currently available experimental findings. This work describes a part of continuing research activity directed at optimizing the combined action of two harmful agents by achieving some new insight into the synergistic mechanism. To gain deeper insight into the mode of interaction, we have discussed the following tasks in this paper. (1) To reveal common peculiarities of synergistic interaction display. (2) To study post-irradiation cell recovery after combined actions. (3) To suggest a concept of synergistic interaction for the interpretation of reveled regularities and to formulate a common mathematical model to describe, optimize and predict the synergistic effects. (4) To prove the condition under which the highest or any equieffective values of the synergistic enhancement ratio can be achieved. (5) To compare the model predictions with experimental results. (6) To demonstrate that, for any fixed intensity of one agent, the synergistic effect *might be increased, decreased or remain without change* with alteration of the intensity of another agent. The results obtained are discussed from the viewpoint of potential significance of synergistic interaction of deleterious agents delivered at intensities occurring in the human environment.

EXPERIMENTAL ANALYSIS OF SYNERGISTIC INTERACTION

A lot of experimental data have been obtained for the action of various agents combined with hyperthermia (Petin and Zhurakovskaya 1995, 1997; Petin *et al.* 1998; Kim *et al.* 2001; Petin and Kim 2002; Kim *et al.* 2002). For these cases, it was shown that synergistic enhancement ratio increased, reached the highest value and then dropped with increase in the ambient temperature. This dependence suggests that the equieffective synergy *may be realized at various temperatures.*

Fig. 1 provides an example of the basic experimental data used in this investigation. Four types of survival curves were obtained for every condition of thermoradiation action: a heat treatment alone (curve 1), ionizing radiation (or another physical agent or chemical compound) without heating (curve 2), composite simultaneous heat and radiation exposure (curve 4). Curve 3 represents a theoretically expected survival curve that would be obtained if inactivation by composite heat and radiation were completely independent. To estimate quantitatively the sensitization action of hyperthermia, one can apply the thermal enhancement ratio (Stewart and Denecamp 1978), defined as D_3/D_1 (Fig. 1). This ratio indicates an increase in cell radiosensitivity by high temperature. However, it does not reflect the kind of interaction. To evaluate the synergistic effect, the synergistic enhancement ratio, defined as D_2/D_1 (Fig. 1) has been used in various studies (Petin and Zhurakovskaya

1995, 1997; Petin *et al.* 1998; Kim *et al.* 2001; Petin and Kim 2002, Kim *et al.* 2002). It is curious that the thermal enhancement ratio increases indefinitely with increasing exposure temperature, while the synergistic enhancement ratio at first increases, then reaches a maximum, which is followed by a decrease. This implies that a synergistic interaction between hyperthermia and ionizing radiation is observed only within a certain temperature range. Noteworthy is the fact that such a dependence of the synergistic effect on temperature under which the exposure occurred was obtained for diploid yeast cells upon the simultaneous combination of hyperthermia with ionizing radiation, UV light, ultrasound, and some chemical inactivating agents. Hence, it can be understood that for a given intensity of physical factors or concentration of chemical agents there will be a specific temperature that maximizes the synergistic interaction. Any deviation of temperature from the optimal value results in a decrease of synergism. Another example of similar synergy pattern is reported by Kim *et al.* (2002).

Here again, the dependence of the synergistic enhancement ratio upon the exposure temperature is depicted for relatively thermosensitive (*Endomyces magnusii*), mildly thermoresistant (*Zygosaccharomyces bailli*) and thermoresistant (*Saccharomyces ellipsoideus*) diploid yeast strains. It is noteworthy that the temperature range strengthening the effect of ionizing radiation has been shifted toward the lower temperatures for temperature-sensitive cell lines.

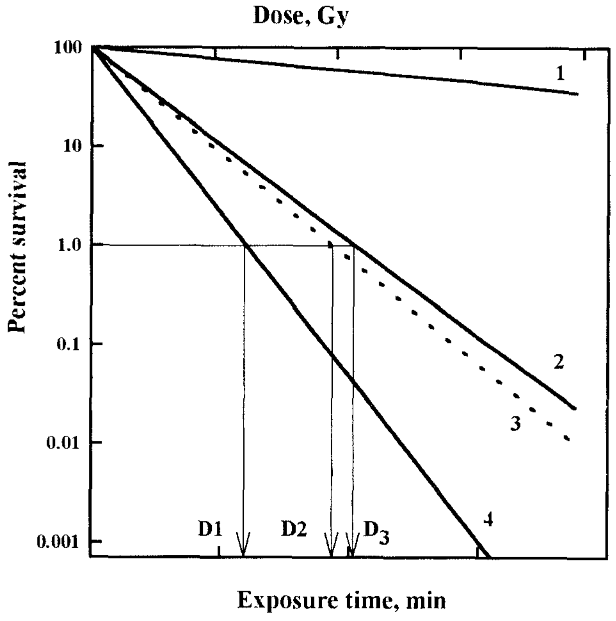

Fig. 1. Example of the basic experimental data. A. Survival curves of haploid yeast cell: curve 1-heat treatment (45°C) alone; curve 2-ionizing radiation (^{60}Co) at about 10 Gy/min and room temperature; curve 3-calculated curve for independent action of ionizing radiation and heat; curve 4-experimental curve after simultaneous thermoradiation action.

Thus, it is clear that the synergistic interaction between hyperthermia and other inactivated agents is realized only within a certain temperature range independently of the object analyzed. For temperatures below this temperature range, the synergistic effect was not observed and cell killing was mainly determined by the damages induced by ionizing radiation. For temperatures above this temperature range, the synergistic effect was also not observed but cell killing was chiefly caused by hyperthermia. It follows that for a given intensity of physical factors or concentration of chemical agents there will be a specific temperature that maximizes the synergistic interaction. Any deviation of the exposure temperature from optimal value will result in a decrease of the synergistic interaction. These results, besides being an important key for searching the synergy, can be considered as an indication of the possibility to optimize and achieve a desirable level of synergy.

On the basis of the results presented the following conclusion should also be valid: for any constant ambient temperature (or concentration of chemical agent) there should be an optimal intensity of ionizing or UV light radiation resulting in the greatest synergy. Some selected experimental results confirming this prediction are presented in Fig. 2.

The data on the simultaneous thermoradiation inactivation of *Bacillus subtilis* spores (Reynolds and Garst 1970; Reynolds and Brannen 1973) were used to estimate the dependence of the synergistic enhancement ratio on the dose rate at the exposure temperature of 95°C. The results are depicted in Fig. 2A. It can be seen that for a constant temperature, at which the irradiation occurs, synergy can be obtained within a certain dose rate range. Inside this range an optimal dose rate of ionizing radiation may be indicated, which maximizes the synergy. Very similar results were obtained for inactivation of diploid yeast cells exposed to electron beam from a 25

MeV pulsed linear accelerator at 51°C (Fig. 2B).

Fig. 2C demonstrates the relationship between the synergistic enhancement ratio and the dose rate of ionizing radiation for a combined effect of lead nitrate and chronic irradiation of *Arabidopsis thaliana* seeds. This relationship was calculated using the experimental results obtained by others (Dineva *et al.* 1993). The seeds were selected in wild populations grown for five years in places with different levels of radioactive pollution inside the 30 km zone around the Chernobyl nuclear power station and then treated with lead nitrate (3.39 g l^{-1}).

The frequency of mutant embryos and the proportion of lethal embryos were estimated. For this case, the synergistic enhancement ratio was defined as the ratio between the increment of the mutant or lethal embryos after the combined action and the sum of these increments after separate action of each agent. As it can be seen from Fig. 2C, the synergistic effect has a pronounced maximum at a certain dose rate of ionizing radiation.

Fig. 2D shows the relationship between the synergistic enhancement ratio and power flux density after simultaneous action of microwave exposure and high environment temperatures (30°C, curve 1 and 38°C, curve 2) on rabbit heating (Kolganova and Zhavoronkov 2001). Here again, the synergistic effect has a pronounced maximum at a certain intensity of microwave radiation. The only experimental point obtained for rabbits exposed at 100 mW cm^{-2} and 38°C, shows that the synergistic enhancement ratio was increased for this power flux density. It turned out that further investigation of the synergy for lower intensities of microwaves delivered at 38°C was fruitless because animals did not sustain this high environmental temperature for a long time.

Nevertheless, this data show that the relationship between the synergy and the power flux density may be shifted to higher intensities with an increase in ambient

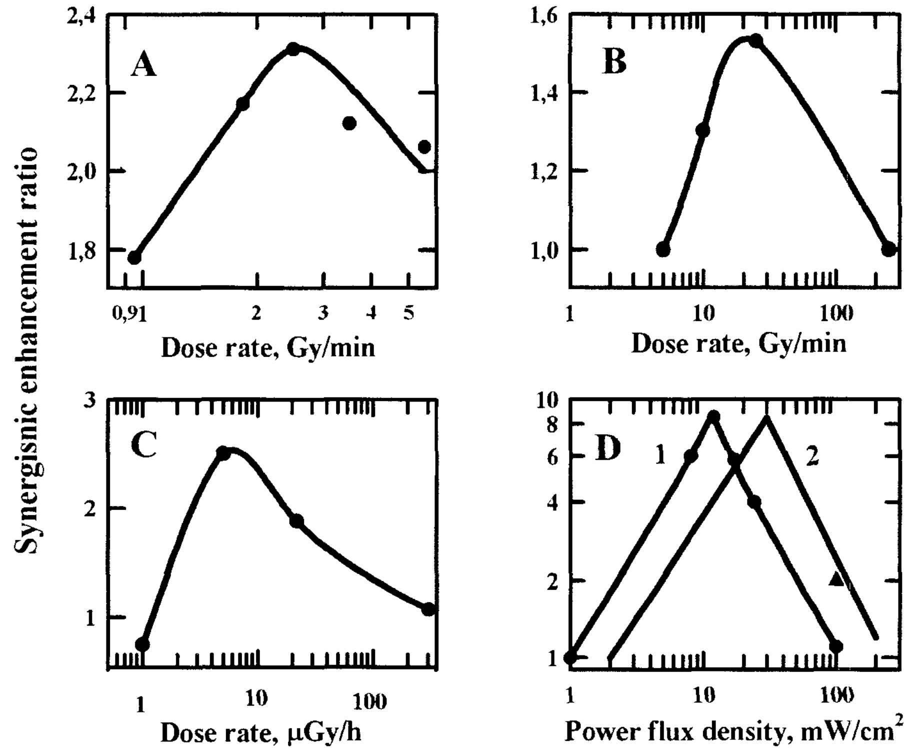

Fig. 2. The dependencies of the synergistic enhancement ratio upon the intensity of physical factors. The role of dose rate for inactivation of bacterial spores *Bacillus subtilis* (A), diploid *Saccharomyces cerevisiae* yeast cells (B) exposed to ionizing radiation at 95 and 51°C, respectively. C-the relationship between the synergistic enhancement ratio and the dose rate of ionizing radiation for a combined effect of lead nitrate and chronic irradiation of *Arabidopsis thaliana* seeds. D-the relationship between the synergistic enhancement ratio and power flux density after simultaneous action of microwave exposure and high environment temperatures (30°C, curve 1 and 38°C, curve 2) on rabbit heating.

temperature. Then it can be expected that as the exposure temperature is reduced, the optimal dose rate decreases and *vice versa*. The universality of this important conclusion is supported by the author's extensive data with diploid yeast cells exposed by heat combined with ionizing radiation, 254 nm UV light and ultrasound.

The synergistic interaction may increase, decrease or stay unchanged with the decrease in the intensity of any physical factor combined with heat. Nevertheless, the equieffective values of the synergistic interaction and the whole temperature range, within which the synergy can occur, are shifted to a lower temperature. To demonstrate a potential significance of synergistic interaction at low intensity of agents applied, the correlation between the intensity of physical factor or the concentration of chemical compound and the exposure temperature, which both provide equieffective levels of synergistic interaction (Kim *et al.* 2001). To estimate this correlation, original data were taken from a number of publications for simultaneous thermoradiation action on bacterial spores (Reynolds and Garst 1970; Reynolds and Brannen 1973), phage (Trujillo and Dugan 1972), mammalian cells (Ben-Hur *et al.* 1974; Ben-Hur 1976), and rabbit heating (Kolganova and Zhavoronkov 2001). Original data for mammalian cells exposed to heat combined with cis-DDP or TAPS were taken from the papers (Urano *et al.* 1990) (Johnson and Pavelec 1973), respectively. Linear relationships are found between these values for various biological objects. In all cases, at a smaller intensity of the physical factor or the concentration of the chemical agents, one has to reduce the acting temperature to preserve the highest or any arbitrary synergistic effect. These data, in principle, indicate a potential significance of synergistic interaction at low intensity of adverse factors encountered in the natural environment.

CELL RECOVERY AFTER COMBINED ACTIONS

The inhibition of cell recovery is commonly considered as a reason of synergy of the combined action of ionizing radiation with various agents (UNSCEAR 1982; Streffer and Müller 1984; UNSCEAR 2000; Dethlefsen and Dewey 1982). However, the inhibition may be proceeded *via* either the damage of the mechanism of the recovery itself or *via* the formation of irreversible damage which can not be repaired at all. Therefore, it would be of interest to estimate quantitatively the probability of recovery per time unit and the fraction of irreversible damage for various combinations.

It is known (Kapultsevich 1978; Korogodin 1993) that the decrease in the effective dose $D_{eff(t)}$ with the recovery time t was fitted to an equation of the form:

$$D_{eff(t)} = D_l [K + (1 - K) e^{-\beta t}] \quad (1)$$

where D_l is the initial radiation dose; K is an irreversible component of radiation damage; e is the basis of the natural logarithm, and β is the recovery constant characterizing the probability of recovery per time unit. The estimation of the recovery parameters (K and β) after different combined treatments, at which a considerable synergistic interaction occurred make it possible to determine whether the mechanism of synergistic interaction was related to the impairment of the recovery capacity *per se* or to the production of irreversible damages. For various biological objects and different conditions of the combined action, the irreversible component increased with either the exposure temperature or the concentration of chemicals while the probability of recovery stayed unchanged. It can be concluded on this basis that the recovery process itself is not damaged and the inhibition of recovery is entirely due to the enhanced yield of irreversibly damaged cells.

MATHEMATICAL MODEL OF SYNERGISTIC INTERACTION

The results of the previous section, pointing out the negligible role of the recovery inhibition itself in the mechanism of synergistic interaction, strongly invoke the need to elaborate a new theoretical conception of the synergy which, being useful for environmental radiation protection, take into account the regularities revealed. It might be reasonable to assume that some additional lethal lesions produced during combined action are responsible for the synergistic interaction. The supposition is that the additional lethal lesions are arisen from the interaction of sublesions induced by both agents and these sublesions are non-lethal when each agent is applied separately. It is assumed that one sublesion produced for instance by ionizing radiation interacts with one sublesion from another environmental agent (for specificity sake, let it be heat) 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 p_1 and p_2 be the number of sublesions that occur for one lethal lesion induced by ionizing radiation and hyperthermia, respectively. Let N_1 and N_2 be the mean numbers of lethal lesions in a cell produced by these agents. A number of additional lesions N_3 arising from the interaction of ionizing radiation and hyperthermia sublesions may be written as

$$N_3 = \min\{p_1N_1;\ p_2N_2\} \qquad (2)$$

Here, $\min\{p_1N_1;\ p_2N_2\}$ is a minimal value from two variable quantities: p_1N_1 and p_2N_2, which are the mean number of sublesions produced by ionizing radiation and hyperthermia, respectively. Thus, the model describes the mean yield of lethal lesions per cell as a function of ionizing radiation (N_1), hyperthermia (N_2), and interaction ($\min\{p_1N_1;\ p_2N_2\}$) lethal lesions. Then the synergistic enhancement ratio k may be expressed as

$$k = (N_1+N_2+N_3)/(N_1+N_2) \qquad (3)$$

Taking into account Eqn. 2, the last expression can be rewritten as

$$k = 1+\min\{p_1;\ p_2N_2/N_1\}/(1+N_2/N_1) \qquad (4)$$

It is evident from here that the highest synergistic interaction will be determined by the least value from the two functions: $f_1 = 1+p_1/(1+N_2/N_1)$ and $f_2 = 1+(p_2N_2/N_1)/(1+N_2/N_1)$. Fig. 3A shows the dependence of both this functions on the ratio of N_2/N_1, calculated for arbitrary chosen p_1 and p_2 ($p_1 = 6$, $p_2 = 4$). The bold line at this figure depicts the dependence of the synergistic enhancement ratio on the ratio N_2/N_1, i.e. the ratio of the effects produced by each agent used in combination. Since f_1 decreases while f_2 increases with N_2/N_1, the greatest synergistic effect will be obtained when $f_1 = f_2$, i.e.

$$p_1/(1+N_2/N_1) = (p_2\,N_2/N_1)/(1+N_2/N_1) \qquad (5)$$

From here, the condition of the highest synergistic interaction can be obtained:

$$p_1N_1 = p_2N_2 \qquad (6)$$

It means that the highest synergistic interaction occurred when both agents produce the equal number of sublesions. Taking into account Eqns. 3 and 5, the value of the greatest synergistic enhancement ratio is given by

$$k_{max} = 1+[p_1p_2/(p_1+p_2)] \qquad (7)$$

Some examples of theoretically predicted dependency of the synergistic enhancement ratio on the N_2/N_1 ratio

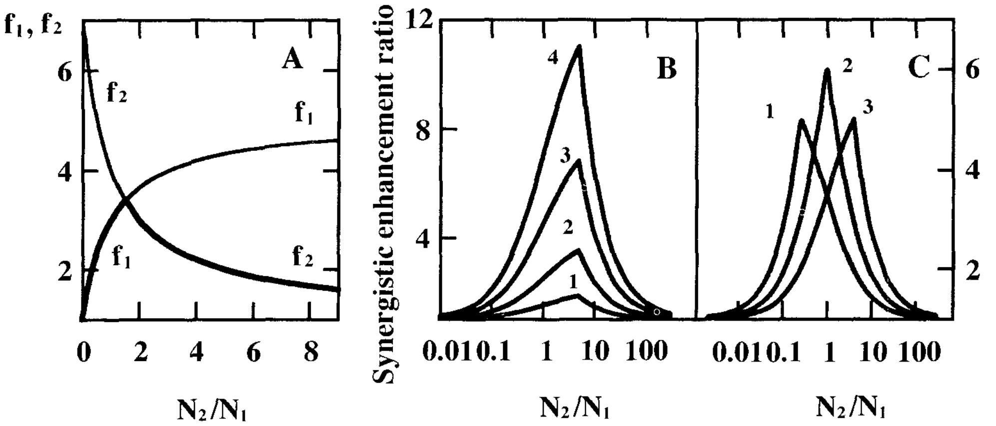

Fig. 3. The calculated dependencies of functions f_1 and f_2 on the N_2/N_1 ratio for the following values of the basic parameter: $p_1 = 6$ and $p_2 = 4$ (A) and theoretically expected dependencies of the synergistic enhancement ratio on the N_2/N_1 ratio for the following values of the basic parameter: B – $p_1 = 5$, $p_2 = 1$ (curve 1), $p_1 = 15$, $p_2 = 3$ (curve 2), $p_1 = 35$, $p_2 = 7$ (curve 3), $p_1 = 60$, $p_2 = 12$ (curve 4); C – $p_1 = 5$, $p_2 = 20$ (curve 1), $p_1 = 10$, $p_2 = 10$ (curve 2), $p_1 = 20$, $p_2 = 5$ (curve 3).

for various values of the basic model parameters p_1 and p_2 are depicted in Figs. 3B and 3C. If the observed biological effect is mainly induced by heat ($p_1N_1 < p_2N_2$) then taking into account Eqn. 4, the parameter p_1 can be expressed as

$$p_1 = (k_1 - 1)(1 + N_2/N_1) \qquad (8)$$

where k_1 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 ionizing radiation, we have

$$p_2 = (k_2 - 1)(1 + N_1/N_2) \qquad (9)$$

where k_2 is the experimental value of the synergistic enhancement ratio observed for the condition $p_2N_2 < p_1N_1$. The corresponding number of lethal lesions can be calculated (Haynes 1966) as

$$N = -\ln S \qquad (10)$$

where S is the surviving fraction.

It is easily to demonstrate that the model under consideration can predict two N_2/N_1 ratio, at which equieffective values of the synergistic enhancement ratio (ki) can be observed. For the case $p_1N_1 < p_2N_2$, we have

$$N_2/N_1 = (p_1 - ki + 1)/(ki - 1) \qquad (11)$$

while for the case $p_2N_2 < p_1N_1$

$$N_2/N_1 = (ki - 1)/(p_2 - ki + 1) \qquad (12)$$

Some examples of calculations based on Eqns. 11 and 12 can be found elsewhere (Petin *et al.* 2000).

COMPARISON OF THE MODEL PREDICTIONS AND EXPERIMENTAL DATA

Several examples of this model application for optimization and prediction of the synergy have already been published (Petin and Komarov 1997; Petin *et al.* 1999; Petin *et al.* 2000). The main value of the mathematical approach presented is the possibility to predict the equieffective synergy including the highest synergism and the N_2/N_1 ratio at which it can be achieved. Tests have been done on the applicability of the model for quantitative description, prediction and optimization of the synergistic interaction observed for various biological objects and test systems.

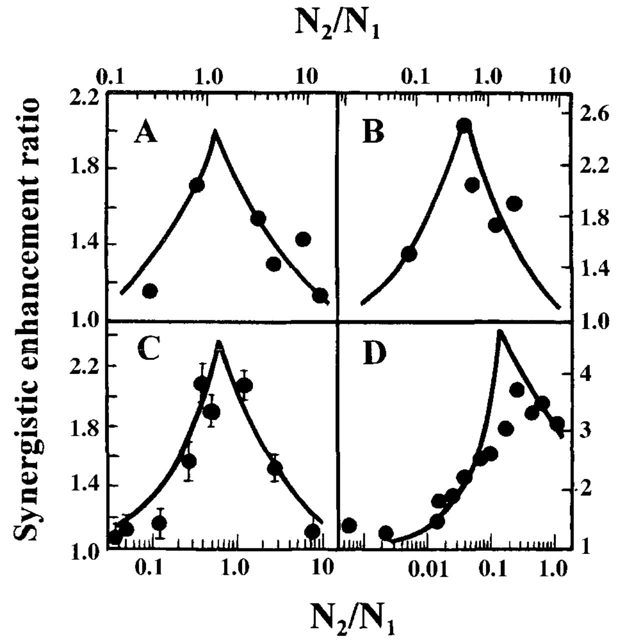

Fig. 4. Experimentally obtained (circles) and theoretically predicted (solid lines) dependencies of the synergistic enhancement ratio k on the N_2/N_1 ratio for simultaneous thermoradiation inactivation of T4 bacteriophage (A), *Bacillus subtilis* spores (B), diploid *Saccharomyces cerevisiae* yeast cells (C) and cultured mammalian cells.

Fig. 4 presents the experimentally obtained (circles) and theoretically predicted (solid lines) relationships between the synergistic enhancement ratio and the N_2/N_1 ratio for inactivation of T_4 bacteriophage (A), *Bacillus subtilis* spores (B), diploid *Saccharomyces cerevisiae* yeast cells (C) and cultured mammalian cells (D). The procedures for calculating these relationships have been described in detail in the previous section. Initial experimental data used for these calculations were taken from earlier papers on simultaneous thermoradiation inactivation of bacteriophage (Trujillo and Dugan 1972), bacterial spores (Raynold and Garst 1970), yeast (Petin and Komarov 1997) and mammalian cells (Ben-Hur *et al.* 1974; Ben-Hur 1976). The errors in the synergistic enhancement ratio values (k) were calculated, if it was possible, from interexperimental variation. Predicted values of k were estimated by Eqn. 3 using the basic parameters p_1 and p_2 of the model which have been derived (Eqns. 7 and 8) from real experiments.

CONCLUSIONS

The main common regularities of the synergistic interaction obtained in this investigation may be summarized as follows. (1) For any constant rate of exposure, the synergy can be observed only within a certain temperature range. (2) The temperature range which synergistically increases the effects of radiations is shifted to the lower temperature for thermosensitive objects. (3) Inside this range, there is a specific temperature that maximizes the synergistic effect. (4) A decrease in the exposure rate results in a decrease of this specific temperature to achieve the greatest synergy and vice versa. (5) For a constant temperature at which the irradiation occurs, synergy can be observed within a certain dose rate range. (6) Inside this range an optimal intensity of the physical agent may be indicated, which maximizes the synergy. (7) As the exposure temperature reduces, the optimal intensity decreases and vice versa. (8) The recovery rate after combined action is decelerated due to an increased number of irreversible damage. (9) The probability of recovery is independent of the exposure temperature for yeast cells irradiated with ionizing or UV radiation. (10) Chemical inhibitors of cell recovery act through the formation of irreversible damage but not *via* damaging the recovery process itself.

The remarkable result of this paper concerns the mathematical model that has been proposed to explain the experimental data of synergistic interaction of hyperthermia with other inactivating agents. 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 when each agent is applied saparately. The idea of sublesions is widely used in radiobiology (Leenhouts and Chadwick 1978; Murthy *et al.* 1979; Zaider and Rossi 1980). In the model, the synergistic effect is given by min $\{p_1N_1; p_2N_2\}$ (Eqn. 2). This means that one sublesion caused by irradiation or chemicals interacts with one sublesion produced by heat. This process is assumed to proceed until the sublesions of the less frequent type is used up. To estimate the basic parameters p_1 and p_2 we have used the experimental values of the synergistic enhancement ratio k_1 and k_2 (Eqns. 8 and 9). It means that the model takes into consideration only the actual interaction determining the synergistic effect. The model predicts the dependence of synergistic interaction on the ratio N_2/N_1 of lethal lesions produced by every agent applied (Eqn. 4), the greatest value of the synergistic effect (Eqn. 7) as well as the conditions under which it can be achieved (Eqn. 6). The model is not concerned with the molecular nature of sublesions, and the mechanism of their interaction remains to be elucidated. In spite of the approximation used in this simplified model, it is evident from the data presented that a good agreement exists between theoretical and experimental results. Moreover, the model discussed here can be used to predict conditions under which the greatest synergy can be observed and its value. The degree of synergistic interaction was found to be dependent on the ratio of lethal damage (N_2/N_1) induced by the two agents applied. The synergistic interaction is not observed at any N_2/N_1 ratios. The effectiveness of synergistic interaction appears to decline with a deviation of the ratio N_2/N_1 from optimal value.

The most interesting results, obtained from the model, is the conclusion that for a lower intensity of physical agents or a lower concentration of chemicals a lower temperature must be used to provide the greatest synergy. Actually, any decrease in the intensity of physical agents would result in a increase of the duration of thermoradiation action to achieve the same absorbed dose. Therefore, the number of thermal sublesions will also be increased resulting in the disruption of the condition at which the highest synergy will be observed (Eqn. 6). Hence, to preserve an optimal N_2/N_1 ratio with any decrease in the dose rate (or the intensity of other agents) the exposure temperature should be decreased. For a long duration of interaction, which are important for problems of radiation protection, low intensities of deleterious environmental factors may, in principle, synergistically interact with each other or with environmental heat.

ACKNOWLEDGEMENT

This work has been partly supported by the Russian

Fund of Fundamental Researches and Administration of Kaluga Region, the National R&D Program and the Korea-Russia Scientists Exchange Program by the Ministry of Science and Technology of Korea.

REFERENCES

Ben-Hur E, MM Elkind and BV Bronk. 1974. Thermally enhanced radioresponse of cultured Chinese hamster cells: inhibition of repair of sublethal damage and enhancement of lethal damage. Radiat. Res. 58:38-51.

Ben-Hur E. 1976. Mechanisms of the synergistic interaction between hyperthermia and radiation in cultured mammalian cells. J. Radiat. Res. 17:92-98.

Dethlefsen LA and WC Dewey (Eds.). 1982. Cancer Therapy by Hyperthermia,Drugs and Radiation. National Cancer Institute Monograph. 61.

Dineva SB, VI Abramov and VA Shevchenko. 1993. The genetic effects of treatment of *Arabidopsis thaliana* seeds by the sodium lead of chronic irradiatied population. Genetics. 29: 1914-1920 [in Russian].

Haynes RH. 1966. The interpretation of microbial inactivation and recovery Phenomena. Radiat. Res. 6:(Suppl.) 1-29.

Johnson HA and M Pavelec. 1973. Thermal enhancement of thio-TEPA cytotoxicity. J. Natl. Cancer Inst. 50:903-908.

Kapultsevich Yu G. 1978. Quantitative Regularities of Cell Radiation Damage. Moscow: Energoatomizdat. [in Russian].

Kim JK, HS Shin, VG Petin and YY Lee. 2002. Experimental analysis on regularities of synergistic interaction of temperature with physico-chemical environmental factors. Korean J. Environ. Biol. 20:165-172.

Kim JK, VG Petin and GP Zhurakovskaya. 2001. Exposure rate as a determinant of synergistic interaction of heat combined with ionizing or ultraviolet radiations in cell killing. J. Radiat. Res. 42:361-365.

Kolganova OI and LP Zhavoronkov. 2001. Thermocompensative rabbit response to the microwave exposure at various environmental temperatures. Radiation Biology. Radioecology. 41:712-717 [in Russian].

Korogodin VI. 1993. The study of post-irradiation recovery of yeast: the premoleular period. Mutat. Res. 289:17-26.

Kuzin AM (Ed.). 1990. Synergism in Radiobiology. Pushchino. [in Russian].

Leenhouts HP and KH Chadwick. 1978. An analysis of synergistic sensitization. Br. J. Cancer. 37(3):198-201.

Murthy MSS, VV Deorukhakar and BS Rao. 1979. Hyperthermic inactivation of diploid yeast and interaction of damage caused by hyperthermia and ionizing radiation. Int. J. Radiat. Biol. 35:333-341.

Petin VG and GP Zhurakovskaya. 1995. The peculiarities of the interaction of radiation and hyperthermia in *Saccharomyces cerevisiae* irradiated with various dose rates. Yeast. 11:549-554.

Petin VG, GP Zhurakovskaya and LN Komarova. 1997. Fluence rate as a determinant of synergistic interaction under simultaneous action of UV light and mild heat in *Saccharomyces cerevisiae*. J. Photochem. Photobiol. B: Biology. 38:123-128.

Petin VG and VP Komarov. 1997. Mathematical description of synergistic interaction of hyperthermia and ionizing radiation. Mathem. Biosci. 146:115-130.

Petin VG, GP Zhurakovskaya, LN Komarova and SV Ryabova, 1998. Synergism of environmental factors as a function of their intensity. Russian J. Ecology. 29:383-389.

Petin VG, GP Zhurakovskaya and LN Komarova. 1999. Mathematical description of combined action of ultrasound and hyperthermia on yeast cells. Ultrasonics. 37: 79-83.

Petin VG, JK Kim, GP Zhurakovskaya and AV Rassokhina. 2000. Mathematical description of synergistic interaction of UV-light and hyperthermia for yeast cells. J. Photochem. Photobiol. B: Biology. 55:74-79.

Petin VG, JK Kim, GP Zhurakovskaya, IP Dergacheva, 2002. Some general regularities of synergistic Interaction of hyperthermia with various physical and chemical inactivating agents. Int. J. Hyperthermia. 18:40-49.

Reynolds MC and DM Garst. 1970. Optimizing thermal and radiation effects for bacterial inactivation. Space Life Sci. 2:394-399.

Reynolds MC and JP Brannen. 1973. Thermal enhancement of radiosterilization. In: Radiation Preservation of Food. Vienna: International Atomic Energy Agency. 165-176.

Stewart FA and J Denekamp. 1978. Combined X-rays and heating: is there a Therapeutic gain? In: Cancer Therapy by Hyperthermia and Radiation, (Ed. by C. Streffer) Baltimore-Munich: Urban & Schwarzenberg. 249-250.

Streffer C and WU Müller. 1984. Radiation risk from combined exposures to ionizing radiations and chemicals. Adv. in Radiat. Bio. 11:173-210.

Trujillo R and VL Dugan. 1972. Synergistic inactivation of viruses by heat and ionizing radiations. Biophys. J. 12: 92-113.

UNSCEAR. 1982. Ionizing Radiation: Sources and Biological Effects. New York: United Nations Publications.

UNSCEAR. 2000. Combined Effects of Radiation and Other Agents. New York: United Nations Publication.

Urano M, J Kahn, H Majima and LE Gerweck. 1990. The cytotoxic effect of cisdiamminedichloro platinum (II) on culture Chinese hamster ovary cells at elevated temperatures: Arrhenius plot analysis. Int. J. Hyperthermia. 6:581-590.

Zaider M and HH Rossi. 1980. The synergistic effects of different radiations. Radiat Res. 83:732-739.

(Received 14 October 2002, accepted 1 November 2002)