Effects of Thermal Neutrons on Living Cells
II. Combined Effects of Thermal Neutrons and Several Agents which Interact with DNA

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(Received December 6, 1976; Revised version received January 18, 1977)

In order to understand the further mechanisms of lethal actions of thermal neutrons, the combined effects of thermal neutrons and several chemical agents which interact with DNA, mainly bleomycin and actinomycin D, have been studied. Marked sensitization was observed with bleomycin even in its lower concentration, while in the case of actinomycin D, slight or no sensitization was found in its lower concentration, though considerable sensitization was observed in its high concentration (100 µg/ml). The sensitizing effects were stronger when the drug was administered before the irradiation than the case when the sequence was reversed. The marked sensitization found in the high dose of actinomycin D is considered to be the consequence of the single strand breaks in DNA chain, just as in the case of sensitization due to bleomycin.

INTRODUCTION

Because of the peculiar interaction of thermal neutrons with a matter, quite different approach as compared to that in the case of ultraviolet light or X-ray irradiation is required for an analytical work concerning the biological actions of this particles. In the previous paper,¹ we proposed that the lethal action of thermal neutrons on the amoebae must arise by the nuclear reaction, namely, $^{31}$P(n,γ)$^{32}$P on DNA chain. In order to understand the further mechanisms of lethal actions of thermal neutrons the combined effects of thermal neutrons and several chemical agents which interact with DNA have been studied. The agents mainly used were actinomycin D (AMD), known to bind preferentially to DNA,²³ and bleomycin (BLM) to cause single or double chain scission in DNA.⁴⁻⁵ The thymidine analogue 5-bromodeoxyuridine (BUdR) and the guanine analogue 8-azaguanine which have been shown to be radiosensitizer in mammalin cells⁷ were also used. The antibiotic, puromycin, which stops the growth of the polypeptide chain in the ribosome⁸ was used as a comparison.

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MATERIALS AND METHODS

Amoeba: The experimental organism, mononuclear M-type of Amoeba proteus, used in this study was cultured according to the method of Prescott and James. Five days after the feeding of Tetrahymena, the amoebae were put into agar medium which contain suitable concentration of the drugs, and then they were subjected to irradiation.

Irradiation: Thermal neutron irradiation was carried out in the heavy water facility of KUR (Kyoto University Reactor) for 5 hours. Thermal neutron flux was approximately $3.2 \cdot 10^9$ n.cm$^{-2}$.sec$^{-1}$. For $\gamma$-irradiation, 1.10$^4$Ci of $^{60}$Co-$\gamma$-ray source was used. Dose rate was $3.2 \cdot 10^6$ rad/hr. Both irradiations were carried out at room temperature.

Experimental procedures: Before irradiation, amoebae were treated with suitable concentration of BLM, AMD or puromycin for 1 hour unless otherwise stated. BUdR or 8-azaguanine treatment was continued for 24 hours prior to irradiation. Irradiation was carried out on the agar medium as described above. After irradiation, amoebae were transferred into normal medium (Prescott-James solution) and 20–30 individuals of them were put into hole slide (size of hole: 20 mm in diameter and 3 mm in depth). Three duplicates were prepared from the same sample. Number of amoebae in each hole slide was counted daily until 15th day under stereomicroscope. Tenth day-survivals in each duplicate were pooled and mean survival fraction was calculated. Under these conditions, non-irradiated amoebae could survive at least more than 20 days. Of course the cells could not induce cell division unless foods were given.

RESULTS

1. Effects of the antibiotics on the survivals of amoebae

Lethal effects of the both antibiotics, BLM and AMD, which have been shown to interact with DNA were examined. Figure 1 shows the lethality of amoebae at 10th day after 1 hour-treatment with the drugs as a function of the drug concentration. As shown, actually no effect was observed at the concentration below 1 $\mu$g/ml. It is noted that the lethal effects of both drugs increased with drug concentration beyond this critical point.

2. Effects of the both antibiotics on lethality due to $\gamma$-rays or thermal neutrons

Effects of the both antibiotics, BLM and AMD, at various concentration on the
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Fig. 2. Effect of various concentration of BLM (Fig. 2-A) and AMD (Fig. 2-B) on the survival of thermal neutron (upper) or 60Co-γ-ray (lower) irradiated amoebae.

Table 1
Comparison of D37 in each treatment

<table>
<thead>
<tr>
<th></th>
<th>Thermal neutrons (10^{11} n. cm^{-2})</th>
<th>60Co-γ-rays (10^{6} rad)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.1</td>
<td>2.5</td>
</tr>
<tr>
<td>BLM D_{37} ratio to control</td>
<td>AMD D_{37} ratio to control</td>
<td>BLM D_{37} ratio to control</td>
</tr>
<tr>
<td>1 μg/ml</td>
<td>4.18 (0.45)</td>
<td>9.11 (1.09)</td>
</tr>
<tr>
<td>10 μg/ml</td>
<td>3.09 (0.34)</td>
<td>5.25 (0.57)</td>
</tr>
<tr>
<td>100 μg/ml</td>
<td>1.82 (0.20)</td>
<td>4.31 (0.47)</td>
</tr>
</tbody>
</table>

lethality due to γ-rays or thermal neutrons were examined (Fig. 2). From the figure, D_{37} in each treatment was obtained, and they were summarized in Table 1. As shown in Fig. 2-A and Table 1, remarkable sensitizing effects were observed with BLM against the both radiations even at a lower concentration of the drug. For example, D_{37} was decreased from 9.1·10^{13} n. cm^{-2} to 4.18·10^{13} n. cm^{-2} by pretreatment with 1 μg/
ml of BLM. While in the case of AMD, slight or no sensitizing effect was observed at lower concentration, though considerable sensitization was found at its higher concentration (100 µg/ml) especially in the case of γ-irradiation (Fig. 2-B). In the next experiment, the sensitizing effects of both drugs in different administration state, namely, pre- or post-irradiation treatment were examined. In the case of BLM, sensitization was observed in the both administration against the both types of radiations. The sensitizing effect was stronger in this case, in pre-irradiation treatment (Fig. 3). The tendency was especially clear in the case of thermal neutron irradiation. In the case of AMD treatment, however, we could not observe any sensitization at this low concentration (1 µg/ml), in the both treatments against both radiations.

3. Interaction of several drugs with both types of radiations

Interaction of several antibiotics or analogues to the both types of radiations was examined (Table 2). As shown in the table, only BLM among all had sensitizing actions against both radiations irrespective of its administration state. The other antibiotics or analogues, at the concentration used, failed to increase or decrease the radiation effects.

DISCUSSION

In order to understand the mechanisms of lethal actions of thermal neutrons, the combined effects of thermal neutrons and several chemical agents which interact with DNA were studied. The degree of sensitization depends on the kind of drugs, concentrations and administration state. Only BLM had a sensitizing effect even at a lower concentration in which lethality due to the drug alone was actually negligible. The sensitizing effect was stronger when the drug was administered before the irradiation. Thus some injury in the cell due to BLM seems to have an effect to
sensitize radiation damage. From the fact that other drugs, except BLM and AMD, had no effect to sensitize radiation effects, it is suggested that the sensitization may occur at the level of DNA chain in which drug-induced scissions were preliminarily formed. Because it is generally accepted that the double chain scission of DNA is the cause of cell death, some injury, described above, by BLM the concentration of which could not kill any cells should be the single chain scission. It may be reasonable to assume that $^{31}\text{P}(n, \gamma)^{32}\text{P}$ reaction which occurs on the DNA chain having BLM-induced single chain scissions elsewhere along the chain, brings about double chain scission easily and thus makes the cell die more effectively. This situation is quite similar to that in the case of (γ-n) effect in which the cell-killing action of thermal neutrons was remarkably enhanced by pre-γ-irradiation.\textsuperscript{19} By the same reason as that, it is reasonable that the lethal action was larger in the case that BLM-treated cells were irradiated with radiations than the case when the sequence was reversed. The exact role of BLM in cellular effects has not yet been completely defined. However, it has been shown that BLM will bind to nuclear DNA, produces strand break in DNA, and thus has a maximal cell-killing efficiency in the M and G\textsubscript{2} stages of the cell cycle.\textsuperscript{16,17} On the other hand, AMD resolves the DNA complex after drug doses in the high survival range, and leads to single strand breaks after the low survival range.\textsuperscript{15} The marked radiosensitizing effects found at the high doses of AMD are considered to be the consequence of the single strand breaks, just as in the case of sensitization due to BLM.

ACKNOWLEDGMENT

The authors are grateful to Dr. Takamori (Radiation Center of Osaka Prefecture) for his kind advice and discussion. They thank to Mr. Horihata (Nihon Kayaku K.K.) for kindly donating bleomycin samples. This work was supported in part by a Scientific Grant from the Ministry of Education, Culture and Science of Japan.
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REFERENCES


