Communications to the editors

ENHANCEMENT OF THE LETHAL EFFECT OF ALKYLATION TO *ESCHERICHIA COLI* B/r BY SHOWDOMYCIN

Sir:

A similarity exists between the action of ionizing radiation and radiomimetics on living cells.[1,2] These points have been discussed in relation to lethal damage to the DNA molecule caused by ionizing radiation and radiomimetics, and the protection and recovery from such damage. The sensitization phenomenon, however, has investigated to any extent only for ionizing radiation.[3,4,5] We have attempted, therefore, to investigate the effect of two types of alkylating agents, 2,2'-dichlorodiethylamine (nor-HN 2) and methyl methanesulfonate (MMS), on *Escherichia coli* B/r. Although showdomycin is an antibiotic isolated from the culture media of a microorganism[6], its structural and radiosensitizing property[7] would indicate that it is a novel thiol-binding agent, and a one to one addition reaction with mercaptoethanol has actually been observed (S. WATANABE & K. TANAKA: personal communication); furthermore, such thiol-binding property of this compound has been also indicated in other biological systems[12,13]: *E. coli* B/r (CSH), grown overnight in an aerated nutrient broth culture, and M/15 sodium phosphate buffer (pH 6.8) was used throughout this experiment. Other details for the assay procedure will be found in our previous reports.[14,15] Nor-HN 2 (hydrochloride) was synthesized according to the method of WARD[8], and MMS was purchased from Kanto Chemical Co., Ltd. (Tokyo); both were stored at −20°C until use.

To assay the lethal effect of alkylating agent and showdomycin, approximatly 3×10^9 cells in 1 ml phosphate buffer were added to 9 ml of phosphate buffer solution containing an amount of alkylating agent appropriate to give the required final concentration. The mixture was then incubated at 37°C, and after 30 minutes 100 μl of showdomycin in buffer solution was added and incubation was continued for another 30 minutes.

Table 1. Loss of vialibity caused by the treatment of *E. coli* B/r with showdomycin in combination with alkylating agents.

	Concentration of alkylating agents (mg/ml)	Concentration of showdomycin (M)	Viability (%)
Nor-HN2	0	10^{-5}	90
		10^{-4}	68
	0.3	0	58
		10^{-5}	15
		10^{-4}	5
	1.0	0	15
		10^{-5}	0.8
		10^{-4}	0.06
MMS	0.05	0	8.0
		10^{-5}	2.0
		10^{-4}	0.9
	0.1	0	1.8
		10^{-5}	0.32
		10^{-4}	0.11

Nor-HN2: 2,2'-Dichlorodiethylamine
MMS: Methyl methanesulfonate

At the end of the incubation period, the cell suspension was cooled in ice to minimize further reaction. The suspension was then sufficiently diluted with ice cold phosphate buffer and viable cells were counted after 40 hours at 37°C on nutrient agar plates.

In Table 1 are summarized the effects of showdomycin on the lethal effect of these two alkylating agents. Although showdomycin alone did not show any serious toxicity to the cell, the lethal effects of alkylating agents are considerably enhanced by the addition of 10^{-4} and 10^{-5} M of showdomycin. This interesting phenomenon could be refered to as "radiomimetics sensitization", a term which has been often used for the enhancement of the lethal effect of ionizing radiation.

Before discussing the action of showdomycin it should be noted that the alkylating agents used affect the bacteria in different ways. Nor-HN 2, a mustard type difunctional alkylating agent, is believed to form

inter-strand bridges between the double helix of the DNA molecule[9,10] while MMS causes single-strand breaks in the DNA molecule[11]. It is interesting that showdomycin has enhanced the lethal effect of both alkylating agents in spite of their different mode of alkylation.

Two possibilities may be considered for the mode of action of showdomycin in this sensitization phenomenon; (1) the lowering of the concentration of cellular SH which reacts with and detoxicates the alkylating agents before they can attack the target molecules, (2) the inhibition of DNA repair machinery, the enzyme system in *E. coli* B/r which is now generally believed to effect repair to DNA damage caused by radiomimetics. The first hypothesis seems more reasonable because damage by alkylating agents has been well prevented by treatment with some sulfhydryl compounds in mammals and other living systems *in vitro*[1]. In this connection it would be also interesting to examine the effect of other kinds of thiol-binding agents. The validity of the second hypothesis would be examined by using mutant strains of *E. coli*, each lacking one or more of the repair enzymes; then, if showdomycin does inhibit one of these repair enzymes, the corresponding mutant strain would not be sensitized to alkylation by showdomycin.

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