Xymedon Restores T-cell Immune Response Inhibited by γ-Irradiation *in vivo*: Interrelations with Ca^{2+}-ATPase and DNA-Relaxing Activities

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 1, pp. 66-70, January, 1999
Original article submitted June 13, 1997

High radioprotective activity of xymedon was shown in mice. Radioprotective effects of this preparation were accompanied by restoration of the delayed-type hypersensitivity response and Ca^{2+}-ATPase activity in splenocytes which were inhibited by γ-irradiation (3 Gy). At concentrations of 10^{-3} and 10^{-6} M xymedon stimulated the activity of DNA topoisomerase-I of splenocyte nuclei. Here we discuss a mechanism of radioprotective effects of pyrimidine derivatives associated with the inhibition of apoptosis of lymphoid cells and the stimulation of proliferation and differentiation of lymphoid precursor cells.

Key Words: *xymedon; radioprotective effect; delayed-type hypersensitivity response; Ca^{2+}-ATPase; DNA topoisomerase-I*

Our previous studies demonstrated that the pyrimidine derivative xymedon displays radioprotective and immunotropic activities, stimulates DNA replication and reparation [9], and modulates the Ca^{2+}-ATPase activity (CAA) of immunocompetent cells [7].

It was assumed that xymedon-stimulated DNA synthesis in lymphocytes contributes to the radioprotective effects of this preparation [6]. Radioprotective effects of pyrimidine derivatives are realized through the restoration of colony-forming potencies of the bone marrow and spleen (proliferation of colony-forming precursor cells) [2]. Ca^{2+}-dependent and DNA-relaxing mechanisms of cell proliferation and differentiation control are probably involved in these processes.

Here we studied the effects of xymedon on the T-cell immune response and CAA of splenocytes in irradiated mice. We also investigated *in vitro* the influence of this preparation on DNA topoisomerase-I.

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MATERIALS AND METHODS

Radioprotective effects of xymedon were studied on 200 female outbred albino mice. The mice were divided into five groups. Groups 1 and 2 included intact (n=40) and control (n=60) animals, respectively. Other mice received intramuscular injections of 30 mg/kg xymedon four times a week at 24-h interval. Animals of groups 3 (n=20), 4 (n=40), and 5 (n=40) were injected for one week before irradiation, one week before and one week after irradiation, and one week after irradiation, respectively. The schedule of experiments on mice of the first and the second groups was similar except for these mice were injected with 100 μl physiological saline. Group 2-5 mice were subjected to whole-body irradiation (3 Gy) on a Kobal't-60 device.

The postradiation T-cell immune response was studied by using the delayed-type hypersensitivity (DTH) model. This reaction was determined by measuring the tail diameter 24 h after administration of the resolution dose of the antigen (sheep erythrocytes) [11]. The relative increase in the tail diameters in

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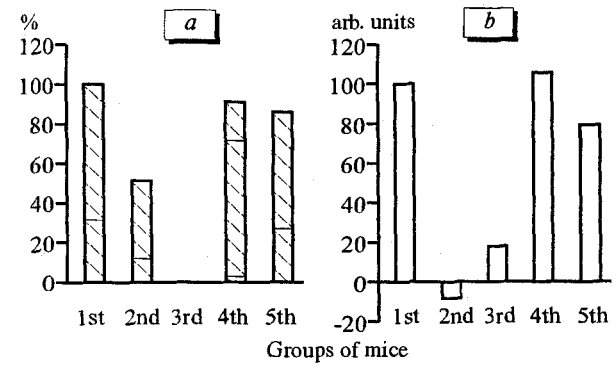

Fig. 1. Effects of various schedules of xymedon administration on *a*) the Ca^{2+}-ATPase activity and *b*) intensity of delayed-type hypersensitivity response under γ-irradiation.

group 1 mice was considered as a physiological level of DTH intensity (100 arbitrary units, arb. units).

CAA was determined 60 min after incubation of splenocytes (10^6 cells/ml) in the medium containing 50 mM Tris-HCl buffer (pH 7.5), 1 mM $CaCl_2$, 1.5 mM ATP, 50 μM $MgCl_2$, and 5 mM EDTA at 37°C. Cell suspension was obtained by homogenization of the spleen. The reaction was stopped by the addition of cold trichloroacetic acid to a final concentration of 5%. The inorganic phosphorus (P_i) concentration was measured [5], and CAA was expressed in μM $P_i/10^6$ cells/min. All measurements were performed in triplicate. The degree and the efficiency of CAA restoration (DR and ER, respectively) were calculated from the following formulas:

$$DR=(CAA_{exp}-CAA_{con})\times 100\%/(CAA_{ini}-CAA_{con})$$
$$ER=(CAA_{exp}-CAA_{con})\times 100\%/CAA_{con}$$

Splenocytes of intact CBA×C57B1 mice were used to determine the effect of xymedon on the relaxing activity of DNA topoisomerase-I *in vitro*. The cells were incubated in the RPMI-1640 medium containing 5% embryonal calf serum at 37°C for 3 h in the presence of xymedon or methyluracil at final concentrations of 10^{-3} M and 10^{-6} M, respectively. The activity of DNA topoisomerase-I was then measured in nuclear extracts of splenocytes [1]. Supertwisted DNA of the pUC19 plasmid served as the enzyme substrate. The change of its relaxation was a measure of the topoisomerase activity. DNA electrophoretograms were stained with ethidium bromide [3] and photographed in UV light. The enzyme quantity necessary for the relaxation of 1 μg DNA at 37°C for 1 h was taken as the activity unit. The activity was calculated at a 50% relaxation of plasmid DNA which was estimated by densitometry of the negatives of electrophoretograms in a GS-300 Scanning Densitometer. We conducted five series of experiments. All measurements of the preparation concentration were performed two or three times.

Data were statistically analyzed by Student's *t* test.

RESULTS

Whole-body irradiation (3 Gy) of mice decreased significantly the intensity of the DTH response on the 8th day of experiments (Table 1, Fig. 1). This inhibition of the T-cell-dependent immune response was probably due to predominant effects of irradiation on the pool of T effectors of DTH [11]. The effects of 30 mg/kg xymedon on the DTH response depended on the schedule of experiments. The intensity of T-cell immune response in group 3 mice was only 18.1 arb. units (compared with physiological level, $p<0.05$). However, this level was higher than that in group 2 mice (Fig. 1). In group 4 mice, DTH was restored to the level observed in unirradiated animals (Fig. 1). The intensity of the DTH response in group 5 mice was higher than that in group 2 and group 3 animals ($p<0.05$). However, the xymedon-stimulated immune response in these mice was only 79.6 arb. units compared with that in group 1 animals ($p<0.05$). Thus, injection of xymedon before and after irradiation had the most pronounced radioprotective effects.

TABLE 1. Effect of Xymedon on the DTH Response Intensity in Mice After Whole-Body Irradiation (*M*±*m*)

Groups of mice	Tail diameter, mm		Relative changes, %
	before administration of the antigen resolution dose	after administration of the antigen resolution dose	
1	3.25±0.032	3.50±0.046⁺	7.53±0.007**
2	3.09±0.079	3.07±0.057	—0.56±1.7*
3	2.88±0.062	2.92±0.144	1.25±0.405*,**
4	3.08±0.1	3.33±0.096⁺	8.99±3.9**
5	2.94±0.056	3.12±0.056⁺	6.1±0.65*,**

Note. Here and in Table 2: ⁺$p<0.05$ compared with results obtained before the administration; *$p<0.05$ compared with the fist and **$p<0.05$ compared with the second groups.

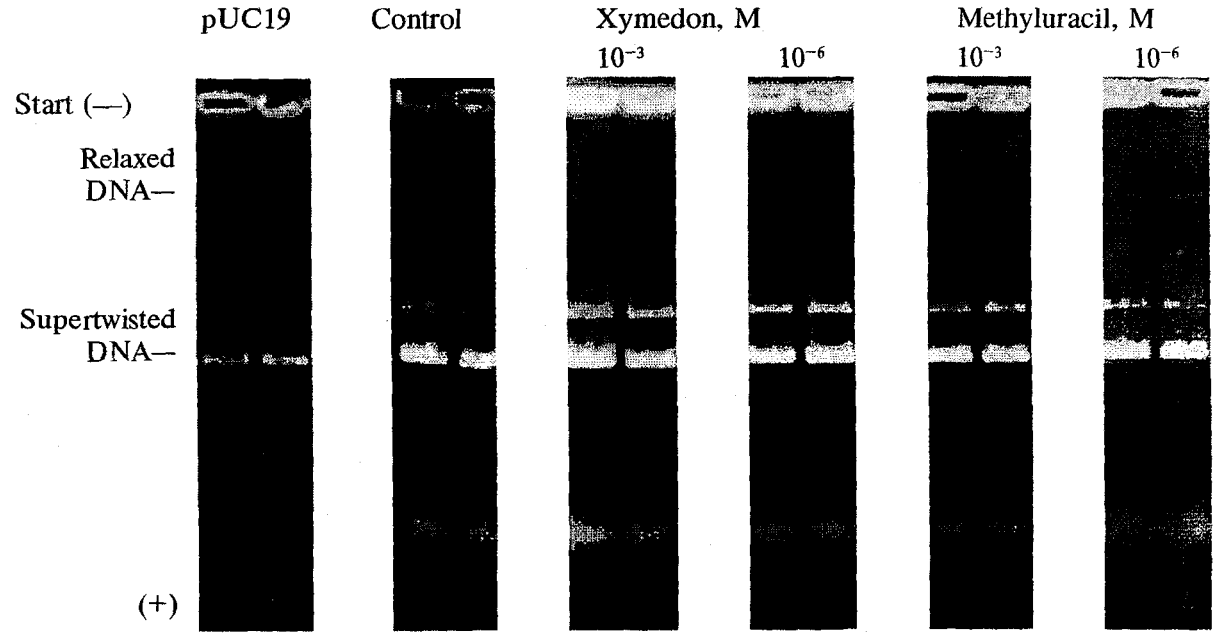

Fig. 2. Xymedon and methyluracil stimulate the activity of DNA topoisomerase-I. This results in relaxation of substrate molecules of plasmid pUC19 DNA manifesting in a decrease in their electrophoretic mobilities.

Inhibition of the T-cell-mediated immunity caused by γ-irradiation is associated with reproductive and interphase death of immunocompetent cells [4]. Damage to membranes, apparatus of division, and chromatin are involved in the mechanism of reproductive death [8]. Therefore, we examined the effects of xymedon on the membrane CAA and chromatinic DNA topoisomerase-I in mouse splenocytes.

Whole-body irradiation (3 Gy) of mice inhibited CAA by 1.9 times compared with the initial level (Table 2). The concentration of intracellular Ca^{2+} plays a key role in many biochemical processes which accompany the apoptotic cell death. CAA inhibitors induce apoptosis of thymocytes [17]. On the other hand, γ-irradiation stimulates apoptosis in mammalian cells [10]. These data suggest that a decrease in splenocytic CAA induced by γ-irradiation is a stage of pathological apoptosis (interphase death). CAA was not determined in group 3 mice (administration of the preparation before irradiation) because the protective effects of xymedon on DTH were minimal compared with those observed in animals of groups 4 and 5 (Table 1). In this case, CAA was of considerable interest. Administration of xymedon before and after irradiation (group 4) restored CAA (Table 2). The preparation injected after irradiation (group 5) induced a 1.7-fold increase in CAA compared with control. Thus, we think that the radioprotective effect of xymedon accompanied by an increase in CAA is realized through the inhibition of apoptosis.

The maximum restoration of CAAs was observed in group 4 mice. This correlated with the maximum restoration of the DTH response when the preparation was administered on this schedule. This probably reflects the completion of proliferation and differentiation of T effectors of DTH. This suggestion is supported by studies of the interrelation between the CAA and proliferation and differentiation of immunocompetent cells induced by xymedon [7].

Chromatin degradation is one of the major structural and biochemical changes in irradiated lymphoid cells [8]. Therefore, the influence of xymedon on the enzymes responsible for DNA reparation and topological reconstruction of chromatin is another possible mechanism of its radioprotective effects.

DNA topoisomerase-I participates in all processes of nucleic metabolism (replication, reparation, recombination, and transcription) [15]. The enzyme generates temporal DNA single-strand breaks and, therefore, the DNA molecule may rotate around the phos-

TABLE 2. Effect of Xymedon on the CAA of Mouse Splenocytes Under Whole-Body Irradiation with 3 Gy (*M*±*m*)

Groups of mice	CAA, μM $P_i/10^6$ cells/min	Indices of CAA stimulation under effects of xymedon, %	
		DR	ER
1	1.68±0.089		
2	0.864±0.061*		
4	1.53±0.1**	82	77
5	1.44±0.098**	70.5	67

TABLE 3. Effects of Xymedon and Methyluracil on the Activity of DNA topoisomerase-I in Splenocytes in CBAx C57B1 mice ($M \pm m$)

Experimental conditions		Enzyme activity, relaxed DNA optical density U/μg plasmid	Compared with control (100%)
Control		8.5±1.5	—
Xymedon,	10^{-3} M	23.2±3.9	272.9 ($p<0.01$)
	10^{-6} M	21.6±2.8	254.1 ($p<0.001$)
Methyluracil,	10^{-3} M	20.7±5.1	243.5 ($p<0.05$)
	10^{-6} M	20.3±4.8	238.8 ($p<0.05$)

phodiesterase bond located opposite the rupture, and the degree of supertwisting decreases. DNA topoisomerase-I then "ligates" this rupture.

Incubation of splenocytes with the pyrimidine derivatives xymedon and methyluracil increased the topoisomerase activity in nuclear extracts. At 10^{-3} M and 10^{-6} M, respectively, xymedon and methyluracil applied caused the appearance of a well-defined band of relaxed DNA. The electrophoretogram was fuzzy due to the spectrum of DNA molecules with various electrophoretical mobilities (Fig. 2). Xymedon and methyluracil at concentrations of 10^{-3} M displayed nearly similar activities (Table 3). Stimulatory effects of these preparations at concentrations of 10^{-6} M were less pronounced. Thus, this effect did not depend on the concentration of xymedon.

Studies of the role of topoisomerases in genetical homeostasis showed that inactivation of one or several enzymes of this group leads to destabilization of genome [16]. The DNA topoisomerase inhibitor, camptothecin (an anticancer preparation), generates DNA double-strand ruptures and apoptosis of proliferating [14] and resting [13] cells. On the other hand, γ-irradiation induces a wide range of genetic defects (single-bond and double-bond breaks in DNA, DNA-DNA cross-links, and DNA-protein cross-links). DNA topoisomerase-I and other enzymes are involved in reparation of DNA damage [12]. Thus, stimulation of the activity of DNA topoisomerase-I is another mechanism of radioprotective effects of xymedon.

Therefore, the radioprotective effect of xymedon can be realized by the following mechanism. The preparation inhibits γ-irradiation-induced apoptosis of proliferating (immature) and resting (mature) immunocompetent cells by Ca^{2+}-ATPase-dependent and DNA topoisomerase-I-dependent mechanisms and stimulates the proliferation and differentiation of lymphoid precursor cells.

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