REPAIR OF SINGLE-STRANDED DNA BREAKS IN CELLS OF MICE SENSITIVE TO ALKYLATING AGENTS

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It was shown previously that mice of the 101/H strain differ from CBA mice in the increased sensitivity of the chromosomes of their somatic cells to the mutagenic action of thiophosphamide and mitomycin C [4, 10], their higher embryonic mortality, and the higher yield of dominant lethal mutations induced in their ripe spermatozoa or spermatids [4, 6, 7]. It has been suggested that the increased sensitivity of 101/H mice to alkylating agents is due to mutation of one gene which determines the reduced efficiency of their intracellular repair processes [10].

Data confirming this hypothesis have recently been obtained. Excision repair of DNA lesions induced by the alkylating agents thiophosphamide and embichin has been shown to be disturbed in cells of 101/H mice [11]. It has also been shown that cells of this strain of mice repair DNA lesions induced in vaccinia virus by 4-nitroquinoline-1-oxide less effectively than cells of CBA and C57BL/6 mice, i.e., they behave as hcr^--mutants [3].

These data suggest that mice of the 101/H strain can be used as a model of a hereditary human disease with chromosomal instability and a defect of DNA repair, namely, Fanconi's anemia or xeroderma pigmentosum. Cells from patients with these diseases are known to repair DNA lesions induced by UV light or alkylating agents ineffectively [2, 5, 8], and are hcr^--mutants [12, 13], although they are capable of normal repair of DNA lesions induced by ionizing radiation [2, 8].

The object of this investigation was to study the ability of cells of 101/H mice to repair DNA lesions induced by γ-rays and bleomycin; like γ-rays, the latter causes the formation of single-stranded DNA breaks [1].

EXPERIMENTAL METHOD

In the experiments of series I, the ability of mast cells to repair DNA lesions induced by γ-rays was determined. The test material consisted of male 101/H and CBA mice aged 2-3 months and kept in the animal house under standard conditions. The mice were irradiated on the Gamma-cell-220 apparatus in a dose of 300 Gy (dose rate 7 Gy/min). Sedimentation analysis of alkaline cell lysates was carried out in an alkaline sucrose gradient. It was shown previously that repair of single-stranded DNA breaks induced by γ-rays in mouse hepatocytes takes place in the course of the first hour after irradiation [14]. The time for investigation was chosen in accordance with these data.

Immediately or 3 h after irradiation, the mice were killed and the liver removed and perfused with cold 0.15 M NaCl solution in 0.1 M EDTA. Samples of 0.1-0.2 g of liver were then homogenized in a glass-Teflon homogenizer in cold 0.075 M NaCl solution with 0.025 M EDTA and the suspension was cleared by centrifugation for 2 min at 200 rpm. The suspension, in a volume of 0.2 ml (about 2×10^5 cells), was applied to 0.6 ml of a lytic layer (0.5 M NaCl, 0.1 M EDTA) located above 15 ml of an alkaline sucrose gradient (5-20% sucrose in 0.8 M NaCl, 0.2 M NaOH, 0.01 M EDTA). Another 0.2 ml of the same lytic mixture was layered above the suspension. Lysis continued for 2-3 h at 29-30°C. The lysates were centrifuged for 3.5-4 h at 20°C and

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TABLE 1. MM of Single-Stranded DNA Fragments from Hepatocytes of 101/H and CBA Mice after Irradiation with γ-Rays in a Dose of 300 Gy ($MM_{1/2} \times 10^{-8}$ dalton)

Strain of mice	Intact mice		Mice after irradiation				% of repaired lesions
			immediately		3 h		
	n	$\bar{m} \pm S_{\bar{m}}$	n	$\bar{m} \pm S_{\bar{m}}$	n	$\bar{m} \pm S_{\bar{m}}$	
101/H	11	6,99±0,82	6	0,59±0,15	10	2,40±0,31	82,2±7,3
CBA	25	6,42±0,38	5	0,68±0,35	35	2,49±0,17	81,3±5,6

TABLE 2. MM of Single-Stranded DNA Fragments of 101/H Mouse Embryonic Fibroblasts Immediately and at Various Times after Treatment with Bleomycin ($MM_{1/2} \times 10^{-7}$ dalton)

Number of experiments	Mean value of $MM_{1/2}$				
	control	immediately after treatment	30 min later	3 h later	24 h later
3	8,56±0,4	3,10±0,57	8,31±0,35	9,26±0,67	8,08±0,16

24,000 rpm in the 6 × 16.5 rotor of an MSE Superspeed-65 centrifuge. Fractionation (23-25 fractions) was carried out by means of a three-channel **Multiperpex 2115 peristaltic micro-**pump (from LKB, Sweden). To determine sedimentation coefficients and molecular mass (MM) of the single-stranded DNA fragments, DNA of phage T4 and DNA of a Chinese hamster cell culture irradiated in a dose of 10 Gy were used as markers. Since sedimentation analysis of DNA of cells of a virtually nonproliferating population was carried out during the investigation a special method of determining DNA in fractions of gradients was developed, with the introduction of [^{3}H]glycine in the presence of formaldehyde [9]. Treatment of the fractions of the gradient, filtration, and counting the label on the filters followed by calculation of sedimentation coefficients and MM of the single-stranded DNA fragments were described previously [9]. Liver of unirradiated animals was used as the control.

In the experiments of series II, the ability of mouse cells to repair DNA lesions induced by bleomycin was determined. The test material consisted of primary cultures of embryonic fibroblasts of 101/H mice. Just as in the experiments of series I, sedimentation analysis of alkaline cell lysates was carried out in an alkaline sucrose gradient. The conditions of cell culture and methods of preparation of suspensions, lysis, centrifugation, and determination of MM of single-stranded DNA fragments were all described previously [12]. The cell culture was treated with a freshly prepared solution of bleomycin (bleocin), from Nippon Kagaku (Japan), containing bleomycin A_2 (55-70%) and bleomycin B_2 (25-32%) in a concentration of 40 μg/ml. Treatment continued for 30 min at 37°C. The cells were then washed with nutrient medium to remove bleomycin and incubated in fresh medium for 24 h at 37°C. During this time, the viability of the cultures, estimated from adhesion of the monolayer to glass, was unchanged. MM of fibroblast DNA was determined immediately after treatment with bleomycin, and also 3 and 24 h later. Intact cultures of fibroblasts served as the control.

EXPERIMENTAL RESULTS

The results of the experiments of series I showed (Table 1) that fragments of DNA in alkaline lysates of liver cells from intact 101/H and CBA mice are virtually indistinguishable as regards MM. The radiosensitivity of hepatocyte DNA from both strains of mice, expressed as the number of single-stranded breaks per unit dose of irradiation, also was practically identical at roughly 70×10^{12} daltons/Gy. As Table 1 shows, no differences likewise were found in the efficiency of repair of single-stranded DNA breaks in the hepatocytes of the two strains of mice: about 80% of lesions were repaired in 3 h.

The experiments of series II showed (Table 2) that immediately after treatment of 101/H mouse fibroblasts with bleomycin there was a significant decrease in MM of the single-stranded DNA fragments compared with the control ($P < 0.001$). When the bleomycin was removed after incubation of the cells for 30 min, the sedimentation rate of DNA was significantly increased,

almost up to the control values; differences between MM in the experiment and control were not statistically significant ($P > 0.05$). Practically normal sedimentation profiles of DNA were obtained also for cells incubated after exposure to bleomycin for 3 and 24 h. This means that cells of 101/H mice in culture can repair single-stranded DNA breaks induced by bleomycin very effectively.

A defect of DNA repair, manifested in 101/H mice as impaired ability to remove lesions of the DNA–DNA or DNA–protein cross-linkage type [11], thus does not affect the efficiency of repair of single-stranded DNA breaks. It can thus be taken as established that with respect to this feature, cells of 101/H mice do not differ from those of patients with chromosomal instability and with a defect of excision repair of DNA.

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COMPARISON OF THE FREQUENCY OF CHROMOSOMAL ABERRATIONS INDUCED BY THIOPHOSPHAMIDE IN RABBIT LYMPHOCYTES *IN VITRO* AND *IN VIVO*

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The question of comparability of data on mutagenesis obtained *in vivo* and *in vitro* and the possibility of extrapolating data from one system to the other has frequently been discussed in the literature. Most investigations devoted to comparison of the mutagenic action of chemicals *in vivo* and *in vitro* have been qualitative in character, i.e., devoted to the study of mutagenic activity of a certain substance *in vivo* and *in vitro*, but not concerned with the degree of manifestation of this activity in the two systems. This problem can be studied more fruitfully in the field of radiation mutagenesis by the use of the chromosomal aberrations (CA) test [1]. In chemical mutagenesis, the problem of comparison of the cytogenic

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