REPAIR OF DNA INJURIES INDUCED BY MUSTINE IN EMBRYONIC FIBROBLASTS OF 101/H AND CBA MICE

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DNA injuries induced by nitrogen mustard (mustine hydrochloride, the Soviet equivalent embikhin) in mammalian cells are eliminated by excision repair [6]. It has been found in the case of cells of Ehrlich's ascites mouse carcinoma *in vivo* sensitive and resistant to nitrogen mustard that the rate and efficiency of removal of alkylated bases from DNA are identical, but whereas the processes of excision and repair of DNA lesions were coordinated in time, in sensitive cells repair of the lesions did not take place and they persisted for at least 24 h [9]. It has been shown on sensitive Ehrlich's ascites mouse carcinoma cells *in vivo* that treatment with nitrogen mustard inhibits elongation of newly synthesized 9S-DNA fragments [5]. These facts are evidence that the sensitivity of mouse cells to nitrogen mustard may be connected with insufficiency of the ligase reaction.

This paper describes a study of the formation and repair of DNA lesions induced by mustine hydrochloride in cells of mice of the 101/H and CBA lines, which differ in their sensitivity to the mutagenic action of alkylating compounds [2].

EXPERIMENTAL METHOD

Primary cultures of embryonic fibroblasts from 101/H and CBA mice were used. The cells were cultured in Eagle's medium with the addition of glutamine, antibiotics, and 20% bovine serum and labeled with ^{3}H-thymidine (5 μCi/ml) for 24 h. The fibroblast cultures were treated for 30 min with mustine (final concentration $2.5 \cdot 10^{-5}$ M) at 37°C, the mustine was then washed off, and the cells were incubated in fresh medium for 24 h. During this time the survival rate of the cultures, estimated from adhesion of the monolayer to the glass, was unchanged. The molecular weight (mol. wt.) of the single-stranded DNA fragments was investigated before the action of mustine, immediately after its removal, and at various time intervals thereafter. Mol. wt. of the DNA was determined by a modified method [7]. Above a 5-20% alkaline sucrose gradient (0.7 N NaOH, 0.3 N NaCl, 0.01 M EDTA) with a volume of 4.8 were successively applied layers of 0.1 ml lytic mixture (1 N NaOH, 0.1 M EDTA), 0.1 ml of cell suspension ($5 \cdot 10^4$ cells in Hanks' solution), and 0.2 ml of the lytic mixture. Lysis of the cells took place for 2 h at 20°C. The products were centrifuged on an AH-650 rotor on a Beckman L-2-65 centrifuge at 35,000 rpm for 90 min at 20°C. The gradient was fractionated from the bottom. The radioactivity of the fractions was measured in Triton-toluene scintillator and converted into counts per minute against an external standard. The sedimentation coefficient was determined by the method [3]. Mol. wt. of DNA was calculated by the method [7]. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Three experiments were carried out with cell cultures from CBA mice and four with cell cultures from 101/H mice. The experimental results were reproducible. As Fig. 1 shows, sedimentation profiles of DNA from cells treated with mustine (Fig. 1: A, 2 and C, 2 respectively for lines 101/H and CBA) were appreciably modified by comparison with sedimentation profiles of DNA from intact cells (Fig. 1: A, 1 and C, 1 respectively for lines 101/H and CBA). Under these circumstances two DNA fractions appeared: sedimenting quickly and slowly. The formation of DNA cross-linkages may lead to an increase in the sedimentation

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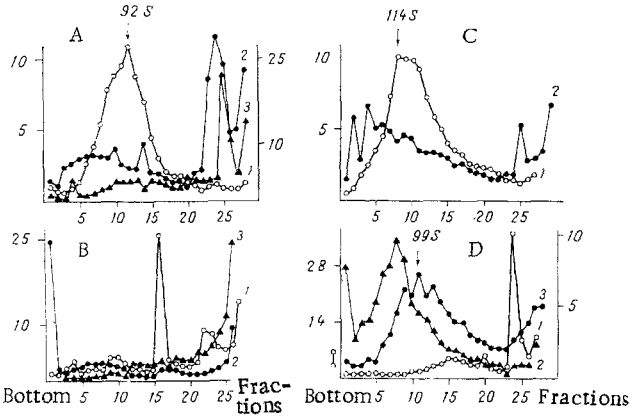

Fig. 1. Changes in mol. wt. of single-stranded DNA fragments from embryonic fibroblasts of 101/H (A and B) and CBA (C and D) mice in the course of incubation after exposure for 30 min to mustine ($2.5 \cdot 10^{-5}$ M). Sedimentation profiles of DNA in alkaline sucrose gradient. A: 1) Control, 2) immediately after exposure, 3) after incubation for 1 h; B: 1 and 2) after incubation for 4 h, 3) after incubation for 24 h; C: 1) control, 2) immediately after exposure; D: 1) after incubation for 1 h, 2) after incubation for 4 h, 3) after incubation for 24 h. Abscissa, Nos. of fractions of gradient; ordinate, percentage of total radioactivity.

rate of the DNA: cross-linkages within and between strands and also DNA–protein cross-linkages [1, 6]. Two processes evidently contribute to a decrease in the sedimentation rate of DNA. First, as a result of the direct action of mustine on DNA leading to the formation of alkali-labile DNA regions, followed by their conversion to breaks, and second, the appearance of DNA breaks as a result of excision of alkylation products [6]. The appearance of two DNA fractions — sedimenting quickly and slowly — after treatment of the cells with mustine was observed in mice of both lines. The ratio between quickly and slowly sedimenting fractions varied in different experiments for mice of both lines. The results are evidence that the character and degree of injury to fibroblast DNA by mustine is the same in mice of lines 101/H and CBA. Other workers also have noted an equal degree of alkylation of DNA in sensitive and resistant mouse cells by nitrogen mustard [4, 8].

Practically all the DNA from the fibroblasts of both lines of mice 1 h after removal of the mustine was in the slowly sedimenting fraction (Fig. 1: A, 3 and D, 1 respectively for lines 101/H and CBA). This suggests that removal of the DNA cross-linkages was complete by this time in cells of both lines 101/H and CBA. Sedimentation profiles of DNA similar to the control were obtained for CBA mouse cells after incubation for 4 h (Fig. 1: D, 2). In none of the three experiments were any DNA breaks found. However, the value of mol. $wt._{1/2}$ was higher than the control [$(1.71 \pm 0.07) \cdot 10^8$ and $(1.29 \pm 0.04) \cdot 10^8$ respectively, ($P < 0.01$, $n = 3$)]. For cells of the 101/H mice at this time an increase in the sedimentation rate of DNA also was observed compared with results obtained after incubation for 1 h, from a very small amount — sedimentation coefficient 62S and 68S (Fig. 1: B, 1) to a substantial proportion — 25-40% of the total radioactivity of DNA was detected on the bottom of the gradient (Fig. 1: B, 2). The results suggest that sedimentation profiles of DNA obtained after incubation for 4 h reflect two processes taking place simultaneously in mouse cells. First, ligation of the broken ends of the DNA strand, which takes place effectively in CBA mouse cells but much more slowly in 101/H mouse cells. Second, resumption of formation of DNA injuries, leading to an increase in the DNA sedimentation rate.

Normal DNA sedimentation profiles were obtained for CBA mouse cells 24 h after removal of the mustine (Fig. 1: D, 3): mol. wt.$_{1/2}$ was $(1.14 \pm 0.06) \cdot 10^8$ and $(1.29 \pm 0.07) \cdot 10^8$ for the experiment and control respectively ($P > 0.05$, $n = 3$). It can accordingly be concluded that repair of DNA injuries induced by mustine in CBA cells is complete after 24 h of incubation. The sedimentation profiles of fibroblast DNA from 101/H mice point to intensive fragmentation of DNA (Fig. 1: B, 3), indicating that integrity of the DNA strand is not restored in cells of this line of mice. The reason may be insufficiency of the last stage of excision repair, namely the ligase reaction, or the uncoordinated character of the endonuclease and ligase reactions. The results are evidence of the defective repair of DNA lesions induced by mustine in 101 H mouse cells.

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EFFECT OF WIDELY USED DRUGS ON FREQUENCY OF SISTER CHROMATID EXCHANGES IN CULTURED HUMAN LYMPHOCYTES

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As the number and variety of chemicals used by man increase, so also does the number of reports of undesirable effects of some of them and, in particular, of the mutagenic activity of drugs [1, 11]. The development of methods of differential staining of sister chromatids has made it possible to test chemical compounds for mutagenicity by analysis of sister chromatid exchanges [5, 6]. In some cases this method proved to be more sensitive than analysis of chromosomal aberrations or the micronuclear test [4, 9]. The cytogenetic action of several chemical agents has already been studied by analysis of the frequency of sister chromatid exchanges [7, 8, 10]. However, widely used drugs exhibiting weak mutagenic activity, or not exhibiting it at all in other tests, have been investigated very rarely.

The object of this investigation was to determine the frequency of sister chromatid exchanges (SCE) in cultures of human peripheral blood lymphocytes under the influence of widely used drugs.

EXPERIMENTAL METHOD

Five drugs were studied: dibazol*, diphenhydramine, caffeine sodium benzoate, magnesium sulfate, and procaine. These drugs are widely used in medical practice, available for the

*2-Benzylbenzimidazole hydrochloride.

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