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CORRELATION BETWEEN CHANGES IN DNA STRUCTURE AND SYNTHESIS INDUCED IN MOUSE L1210 LEUKEMIA CELLS BY 1-METHYL-1-NITROSOUREA AND 1,3-BIS-(2-CHLOROETHYL)-1-NITROSOUREA

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KEY WORDS: L1210 leukamia; DNA synthesis and structure; 1-methyl-1-nitrosourea; 1,3-bis-(2-chloroethyl)-1-nitrosourea.

Experimental data showing significant differences in the molecular mechanisms of action of the methyl and chloroethyl derivatives of N-nitrosourea, which differ in the spectrum of their antitumor activity, have been published in recent years. Methyl derivatives are most effective in the treatment of first generation spontaneous mouse mammary gland tumors, whereas chloroethyl derivatives are most effective in the treatment of experimental leukemias [6, 12].

Methyl and chloroethyl derivatives of 1-alkyl-1-nitrosourea (NAU) are nowadays widely used in combination chemotherapy of malignant neoplasms. Many years of systematic study of the mechanism of action of these compounds has shown that NAU in aqueous solutions, and under close to physiological conditions, are unstable and decomposed spontaneously to form chlorodiazohydroxides and isocyanates, which can alkylate and carbamoylate biomacromolecules [3, 6, 11]. In addition, the possibility of enzymic degradation of NAU (hydroxylation and denitrosation) has been demonstrated experimentally [2].

This paper describes a comparative study of changes in DNA structure and synthesis in mouse L1210 leukemia cells induced *in vitro* by 1-methyl-1-nitrosourea (MNU) and by 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU).

EXPERIMENTAL METHOD

Experiments were carried out on (C57BL × DBA2)F_1 hybrid mice weighing 18-21 g, inoculated intraperitoneally with mouse L1210 leukemia cells ($2 \cdot 10^5$-$3 \cdot 10^5$ cells per mouse). MNU was dissolved in 0.9% NaCl and BCNU in 10% ethanol immediately before injection. MNU was injected in doses of 20 and 80 mg/kg and BCNU in a dose of 8 or 20 mg/kg, by a single intraperitoneal injection on the 5th day of leukemia development. DNA synthesis was studied by recording incorporation of 2-^{14}C-thymidine or deoxy-2-^{14}C-uridine. The experiments and analysis of the results were carried out in accordance with the scheme described previously [1, 5]. Defects in the secondary structure of DNA (single breaks and alkali-labile regions) after injection of MNU and BCNU were determined by centrifugation in an alkaline sucrose gradient [13]. To analyze

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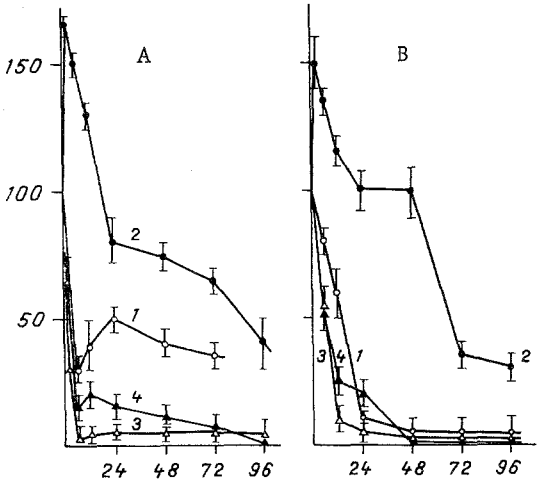

Fig. 1

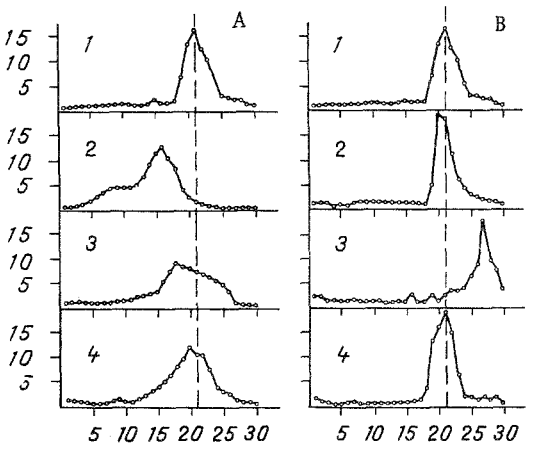

Fig. 2

Fig. 1. Effect of MNU (A) and BCNU (B) on incorporation of 2-^{14}C-thymidine (1, 2) and deoxy-2-^{14}C-uridine (3, 4) into DNA of L1210 leukemia cells. Abscissa, time after injection of compound; ordinate, specific radioactivity (in per cent). A: 1, 3) 80 mg/kg, 2, 4) 20 mg/kg; B: 1, 3) 20 mg/kg, 2, 4) BCNU, 8 mg/kg.

Fig. 2. Alkaline sucrose density gradient sedimentation of preformed DNA of L1210 leukemia cells after injection of MNU in a dose of 80 mg/kg (A) and BCNU in a dose of 20 mg/kg (B). 1) Intact cells; 2, 3, 4) 1, 4, and 18 h, respectively, after injection of the drug. Abscissa, fraction No., ordinate, per cent of total radioactivity.

disturbances in the structure of the pre-existing DNA, 2-^{14}C-thymidine (2 μCi per mouse) was injected on the 4th day after inoculation of the tumor cells, 18 h, and MNU and BCNU 1, 4, and 18 h, before sacrifice of the animals. The newly synthesized DNA was analyzed 1 h after injection of 2-^{14}C-thymidine; MNU and BCNU were injected 30 min and 1, 4, and 24 h before sacrifice of the animals. Specimens for counting radioactivity were prepared and the results analyzed by methods described previously [5].

EXPERIMENTAL RESULTS

Kinetic curves of changes in incorporation of 2-^{14}C-thymidine and deoxy-2-^{14}C-uridine into DNA of leukemic cells after injection of MNU and BCNU are given in Fig. 1. The degree of inhibition of incorporation of 2-^{14}C-thymidine into DNA of L1210 leukemia cells after injection of MNU and BCNU in single therapeutic doses (80 and 20 mg/kg, respectively) differed, and correlated with their antitumor activity on this experimental model (Fig. 1, 1). When the preparations were injected on the 5th day after inoculation of the tumor cells MNU increased the mean life span of the animals by 150%, and BCNU by more than 300%, with cure of 30% of the animals with tumors. Under these conditions of administration MNU inhibited DNA synthesis maximally after 24-72 h, by 50%, and BCNU by 90-100%. The inhibitory effect was reduced if the MNU concentration was reduced to 20 mg/kg and the BCNU concentration to 8 mg/kg (Fig. 1, 2). The initial activation of incorporation of 2-^{14}C-thymidine into DNA after injection of small doses of the preparations will be noted. In this case inhibition of DNA synthesis did not begin until 24-48 h after injection of MNU and BCNU.

Both doses of the compound caused profound and lasting inhibition of incorporation of deoxy-2-^{14}C-uridine into DNA of the tumor cells (Fig. 1, curves 3, 4).

The results are evidence of the greater sensitivity of DNA synthesis *de novo* to the harmful action of MNU and BCNU, probably due to inactivation of thymidylate synthetase, compared with DNA synthesis by the reserve pathway, involving thymidine kinase. Disturbances observed in DNA synthesis may be connected with injuries in the structure of the DNA template and the newly synthesized DNA [5, 6, 11]. We know that methyl derivatives mainly induce lesions of the single break type and alkali-labile regions. Under the influence of chloroethyl derivatives, lesions of these types are quickly manifested as intra- and intermolecular DNA-DNA and DNA-protein cross-linkages [5, 10].

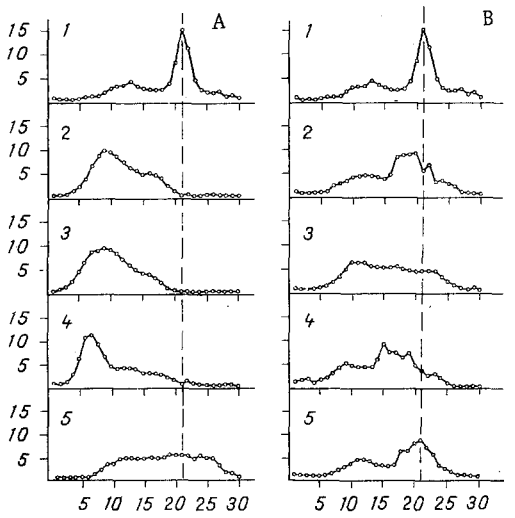

Fig. 3. Sedimentation of newly synthesized DNA of L1210 leukemia cells in an alkaline sucrose gradient after injection of MNU in a dose of 80 mg/kg (A) and of BCNU in a dose of 20 mg/kg (B). 1) Intact cells; 2, 3, 4, 5) 30 min and 1, 4, and 24 h, respectively, after injection of preparation. Abscissa, fraction no.; ordinate, per cent of total radioactivity.

To study the duration of existence of injuries of the single break type and of alkali-labile regions in the leukemia cells after a single injection of the NAU in therapeutic doses, changes in the DNA structure of whole cell lysates were analyzed by centrifugation in an alkaline sucrose gradient.

Sedimentation profiles of pre-existing and newly synthesized DNA of L1210 leukemia cells at different times after injection of MNU and BCNU are shown in Figs. 2 and 3. The defects, namely single breaks and alkali-labile regions in the preformed DNA, induced by MNU, were shown to exist for a long time, and even after 18 h the DNA structure was not completely restored, for the sedimentation profile showed a marked degree of polydispersity (Fig. 2A). Unlike MNU, BCNU induced the formation of aggregates of greater molecular weight than in samples of the original DNA as early as after 4 h (see Fig. 2, B: the sedimentation peak is shifted from fraction 21 to fraction 27). However, after 18 h the DNA structure was restored. It was shown previously that MNU induces activation of template activity of chromatin in a system with exogenous RNA-polymerases I and II between 4 and 24 h after injection into mice with leukemia L1210, probably due to the formation of breaks in the DNA. Under similar conditions, during the first 24 h after its injection into mice with leukemia L1210, BCNU changes the template structure of chromatin negligibly [2].

Sedimentation profiles of newly synthesized DNA of L1210 leukemia cells 30 min and 1, 4, and 24 h after injection of MNU and BCNU in therapeutic doses are illustrated in Fig. 3. The first point to be noted is the greater sensitivity of the structure of the newly synthesized DNA to the injurious action of MNU and BCNU *in vivo*. At all times after injection (Fig. 3A) MNU induced numerous single breaks. The heterogeneity of the DNA sedimentation profile 24 h after injection of MNU, in all probability, was evidence of preservation of a certain number of single breaks formed 1-4 h after injection of the compound. Lesions of this type may also arise as a result of repair processes [4]. Our observations showing greater sensitivity of newly synthesized DNA of leukemic cells to the injurious action of MNU compared with pre-existing DNA are in agreement with data showing predominant formation of methylated adducts under the influence of MNU in actively replicated DNA compared with the whole mass of DNA of the cells [7]. The effects observed may be the result of greater accessibility of single-stranded regions of the replication fork to the modifying action of MNU, or to intensive incorporation of methylated deoxyribonucleotide triphosphates, which are methylated 190-13,000 times more rapidly than DNA [15]. The formation of breaks in the newly synthesized DNA under the influence of BCNU also was found in experiments *in vitro* on a culture of L1210 leukemia cells, but with higher doses of the compound; however, the authors cited did not demonstrate the greater sensitivity of the newly synthesized DNA compared with preformed DNA to the injurious action of BCNU [8, 13, 14].

Thus BCNU induces long-lasting lesions of the single break type and alkali-labile regions in the DNA-replicating system *in vivo*. Realization of injuries of this type as DNA-DNA or DNA-protein cross-linkages may be hindered by steric factors or by differences in the kinetics of DNA replication *in vivo* and *in vitro*.

Differences in the character of lesions in the DNA structure of leukemia cells induced by MNU and BCNU confirm the view that there are differences in the molecular mechanisms of action of these preparations [3, 11] but that cross-resistance between them is absent [9].

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