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INDUCTION OF LATENT LIVER DAMAGE BY CYCLOPHOSPHAMIDE

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Ionizing radiation is known to induce latent damage in the intact liver, which is manifested in the course of induced proliferation in the form of certain biochemical and morphological changes, more especially by inhibition of DNA synthesis and of mitotic activity, and also by chromosomal aberrations [3, 5, 6, 9]. A similar effect is given by certain chemical substances (cytostatics). Cyclophosophamide is one of the most frequently used cytostatics. Although the mechanisms of their action differ at the molecular level, the eventual results of damage to hematopoietic and lymphatic organs after exposure to ionizing radiation and to cyclophosphamide are identical in many respects [10, 11, 13].

The aim of this investigation was to discover whether cyclophosphamide can induce latent damage in nonproliferating tissues also. For this purpose we studied the effect of preoperative administration of cyclophosphamide on some cytological indicators of damage in the rat liver regenerating after partial hepatectomy (PHE).

EXPERIMENTAL METHOD

Experiments were carried out on adult male Wistar albino rats with a mean weight of 250 g. The animals were kept under standard conditions in the animal house with free access to food and water. Before PHE (two-thirds of the liver) all the animals were divided into three groups: 1) control, operation only; 2) animals receiving cyclophosphamide, 100 mg/kg intraperitoneally, 2 h before the operation; 3) rats receiving 200 mg/kg cyclophosphamide intraperitoneally 2 h before the operation.

Cyclophosphamide, the diamide ester of N,N-bis(β-chloroethyl)-N',O-propylene phosphoric acid + NaCl (East Germany), was synthesized as the nontoxic transport form of the alkyl derivative $C_7H_{15}Cl_2N_2O_2P$. Cyclophosphamide is inactive in vitro. It is activated in vitro by liver oxidases, with the formation of biologically active metabolites, possessing marked mutagenic properties. PHE was performed by the standard method [7] and the animals were studied 24, 30, and 48 h and 3, 7, and 14 days after the operation. At each time interval five animals in each group were tested. The animals were killed between 6 and 8 a.m. in order to reduce the influence of circadian rhythms. Sections were cut from the regenerating liver tissues and stained by Feulgen's method. During analysis of 50,000-60,000 cells in each group studied, all mitotic figures and chromosomal aberrations in postmetaphase were noted. These data served as a basis for calculation of the mitotic index (MI; the number of mitotic figures per 1000 cells), the ratio of the number of metaphase to the number of prophases, and the number of chromosomal aberrations as a percentage of the total number of postmetaphase figures found among all the cells examined. The statistical significance of the results were estimated by the t test.

EXPERIMENTAL RESULTS

Mitotic figures were rare in the regenerating liver of the control animals until 24 h after PHE (Fig. 1). Later MI rose sharply to reach a maximum (27.291 ± 0.310‰) at the 30th

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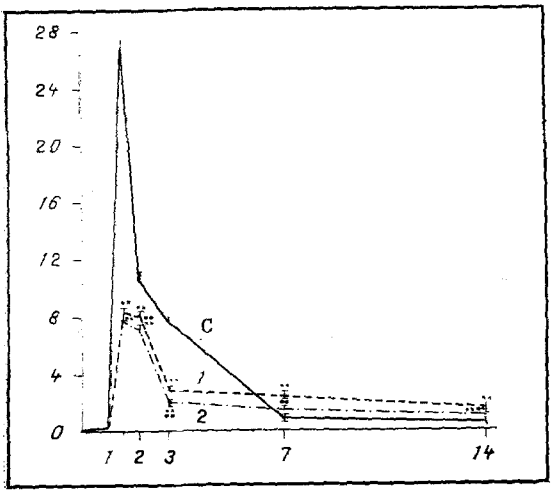

Fig. 1. MI in regenerating liver of control animals and after injection of cyclophosphamide. Abscissa, days after PHE; ordinate, MI (in %). C) Control. Injection of cyclophosphamide in a dose of 100 mg/kg (1) and 200 mg/kg (2).

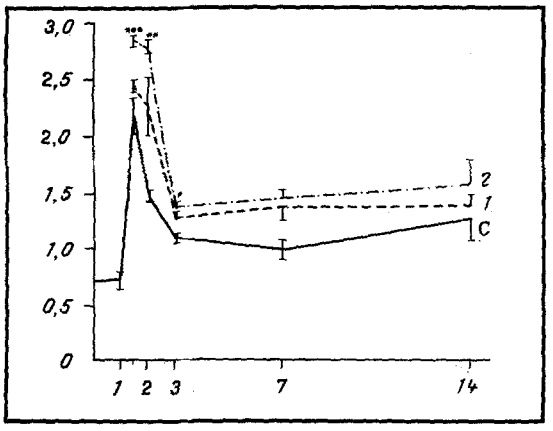

Fig. 2. Ratio of number of metaphases to number of prophases (M/P) in regenerating liver of control animals and after injection of cyclophosphamide. Ordinate, ratio M/P. Remainder of legend as to Fig. 1.

hour, in agreement with observations made by other workers [1, 5, 6]. Later it fell quickly, and from the 7th day after the operation it was closely similar to values in the intact liver.

In animals receiving cyclophosphamide, just as after a single session of acute x-ray or γ-ray irradiation, no mitotic figures were observed before 24 h after PHE. Thus, cyclophosphamide, in both doses used, delayed mitosis in the regenerating liver by ~6 h [2, 11], whereas after irradiation in a single dose of 5.742-6.300 Gy before PHE, inhibition of mitotic activity in the regenerating liver likewise was observed for 4-6 h. The degree of inhibition of proliferation depended on dose. In animals receiving cyclophosphamide in a dose of 100 mg/kg (CP100) the number of dividing cells 30 h after PHE was ~3.5 times less than in the regenerating liver of the control animals, and it remained at this level (8.130 ± 0.180‰) until 48 h after the operation. In the course of 3 days mitotic activity fell quickly, but it was still statistically significantly higher on the 14th day after PHE than in the corresponding control. After injection of cyclophosphamide in a dose of 200 mg/kg (CP200) changes in MI were similar in character, but its values varied at a statistically significantly lower level, which was observed throughout the period of observation.

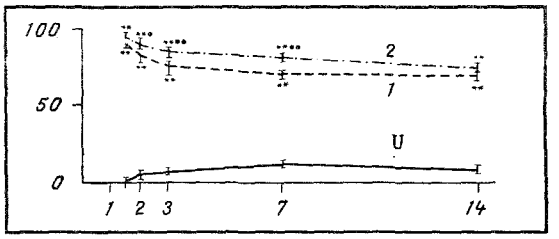

Fig. 3. Chromosomal aberrations in postmetaphase in regenerating liver of control animals and after injection of cyclophosphamide. Ordinate, % of chromosomal aberrations. Asterisks indicate significant differences between control and group receiving cyclophosphamide; filled circles indicate that differences between experimental groups are significant Remainder of legend as to Fig. 1.

It can be concluded from planimetric measurement of the area of the figures that injection of cyclophosphamide in a dose of 100 mg/kg inhibited mitotic activity in general by 24%, but in a dose of 200 mg/kg mitotic activity was inhibited by 43% compared with the control. These results are comparable with those of a single acute exposure to x-ray irradiation in a dose of 2.871 and 5.742 Gy, which causes a reduction of mitotic activity by 15 or 39% respectively compared with the unirradiated control [9]. However, a single acute irradiation in a dose of 5.742 Gy also affected the first wave of mitosis, so that the largest number of mitotic figures was not observed until 48 h after PHE, i.e., 18 h later than in the absence of irradiation (control). Cyclophosphamide did not inhibit entry of the cells into the first wave of mitosis more clearly than this. The ratio M/P in the control animals on the 30th days after PHE was ~3 times higher than in the intact liver. This is evidence that most cells, after entering the first wave of mitosis, are already in metaphase of the cell cycle. An increase in relative substitution of the number of metaphases for the number of prophases was observed in the regenerating liver of rats irradiated with x rays or γ rays [8, 9] and, on a limited scale, also in the control after partial hepatectomy alone, and in the later periods after the operation (Fig. 2).

In the regenerating liver of the control animals there was 18 ± 1.3% of aberrant postmetaphase figures. Administration of cyclophosphamide led to a sharp rise in the number of chromosomal aberrations – even up to 90% (CP100) or 95% (CP200), which was observed until the 30th hour after PHE. Until the 3rd day after PHE some aberrant postmetaphases were eliminated, but at this time we also observed 75% (CP100) for 85% (CP200) of aberrant postmetaphase cells.

Later the percentage of chromosomal aberrations fell slowly – to 70-74 on the 14th day after the operation. These results agree with data in the literature [12] and our own previous investigations [9] in which 80-95% of chromosomal aberrations were observed after a single session of acute x-ray irradiation in a dose equivalent to 6 Gy (Fig. 3).

To conclude, administration of cyclophosphamide 2 h before PHE caused latent damage to the rats' liver similar in character to damage after x-ray or γ-ray irradiation. The damage was manifested after PHE by delay of regeneration, as proved by the reduction of MI and the increase in the frequency of chromosomal aberrations.

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