EFFECT OF A SINGLE INJECTION OF HYDROXYUREA ON CELL DIVISION IN ESOPHAGEAL AND LINGUAL EPITHELIUM IN MICE

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Hydroxyurea (HU) is known to block the passage of cells temporarily from the G_1 period of the mitotic cycle into the S period, and also to inhibit DNA synthesis [1-6]. Nevertheless, the dependence of the effect of the compound on dose has so far received little study [3-6]. The action of HU also has been studied without regard to circadian rhythms of cell proliferation.

The object of this investigation was to study the effect of various doses of HU on the kinetics of the epithelial cells of the esophagus and tongue. The time of administration of the compound was chosen on the basis of diurnal fluctuations in proliferative activity of the cells in these organs.

EXPERIMENTAL METHOD

Experiments were carried out on 85 male noninbred mice (weighing 25 g) kept in the animal house for 14 days under conditions of 12 h daylight and 12 h darkness: daylight from 8 a.m. to 8 p.m. Control animals were killed at 10 p.m. and 1, 4, 7, and 10 a.m. The experimental mice were given a single injection of HU (from Serva, West Germany) at 10 p.m. in doses of 100, 250, or 500 mg/kg body weight and investigated after 3, 6, 9, and 12 h (at 1, 4, 7, and 10 a.m.). All the animals were given an injection of ^{3}H-thymidine 45 min before sacrifice in a dose of 0.75 μCi/g body weight (specific activity 10.1 Ci/mole). Paraffin sections of the tongue and esophagus 5 μ thick were prepared and coated with type M (Photographic Chemical Research Institute) emulsion and exposed for 21 days. The mitotic indices (MI) and indices of labeled nuclei (ILN) were determined after examination of 5000-10,000 esophageal and lingual epithelial cells and expressed in pro mille. Nuclei were considered

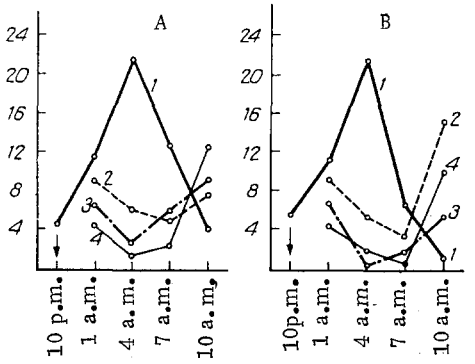

Fig. 1. Changes in MI in esophageal (A) and lingual (B) epithelium of mice after a single injection of different doses of HU and in the control. 1) Control; 2) 100 mg/kg, 3) 250 mg/kg, 4) 500 mg/kg HU. Abscissa, time of day; ordinate, MI (in %). Arrow indicates time of injection of HU.

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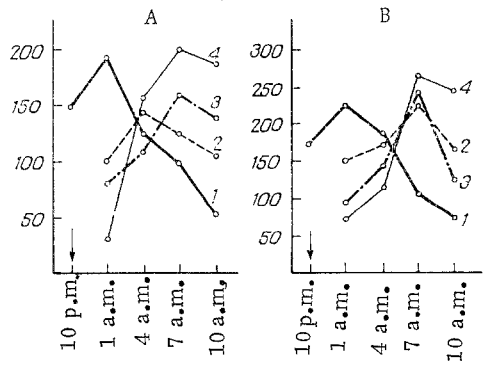

Fig. 2

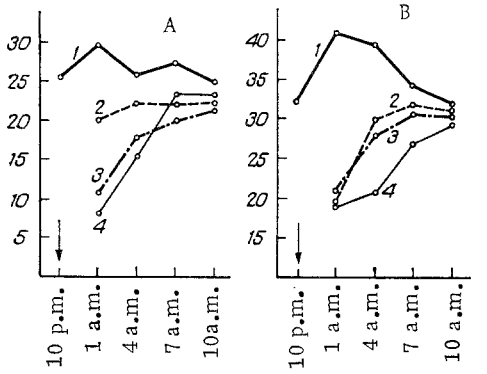

Fig. 3

Fig. 2. Changes in ILN in esophageal (A) and lingual (B) epithelium of mice after a single injection of different doses of HU and in control. Ordinate, ILN (in %). Legend as in Fig. 1.

Fig. 3. Changes in intensity of labeling of nuclei of esophageal (A) and lingual (B) epithelium in mice after a single injection of different doses of HU and in control. Ordinate, intensity of labeling of nuclei (number of grains above nuclei). Remainder of legend as in Fig. 1.

to be labeled if they had three or more grains of silver above them. Student's t test was used for statistical analysis. Differences were considered to be significant at the $P = 0.05$ level.

EXPERIMENTAL RESULTS

Maximal values of ILN and MI in the esophageal and lingual epithelium of the control animals occurred at 1 and 4 a.m., reflecting changes in the levels of these indices in the circadian rhythm of proliferative activity (Figs. 1 and 2).

HU was injected at 10 p.m., i.e., shortly before the peak of ILN in the circadian rhythm of the control animals. A decrease in ILN was observed 3 h after injection of HU, the degree of which correlated positively with the dose of the compound (Fig. 2). For instance, a dose of 100 mg reduced ILN by 1.8 and 1.5 times, a dose of 250 mg reduced it by 2.4 times, and a dose of 500 mg by 11.8 and 3.0 times (the first numbers relate to the esophageal epithelium, the second to the lingual epithelium; $P = 0.001$–0.015). By 9-12 h after the beginning of the experiment (7-10 a.m.) ILN was significantly (by 1.7-3.3 times) higher than the control level; with the larger dose of the compound a greater increase was observed in ILN.

Similar results were obtained during the investigation of MI in the experimental animals (Fig. 1). Three hours after injection of HU the value of MI was reduced in the esophageal and lingual epithelium by 2.0 and 1.6 times respectively for a dose of 250 mg and by 3.0 and 2.5 times for a dose of 500 mg ($P = 0.001$–0.009). When a dose of 100 mg was used, no significant decrease in MI was observed after 3 h. Maximal inhibition of mitosis occurred 6 h after injection of HU, and the larger dose of the compound had a stronger effect (Fig. 1). After 12 h of the experiment MI was higher than in the control both in the esophageal and in the lingual epithelium (by 2.0-10.0 times; $P = 0.015$).

A study of the intensity of labeling of the nuclei showed (Fig. 3) that in the experimental animals in both types of epithelium it was significantly lower (by 1.45-3.0 times) as early as 3 h after injection of HU ($P = 0.035$), and it was close to the control values only toward the end of the experiment. With respect to both ILN and MI, the influence of HU on the intensity of labeling of the cell nuclei depended on dose. The reduction in the intensity of labeling was more marked when HU was given in a dose of 500 mg.

Administration of HU to the animals thus caused a decrease in ILN and MI and in the intensity of labeling of the nuclei with ^{3}H-thymidine during the first few hours of the experiment. After 9-12 h of the experiment ILN and MI were increased and exceeded the control values, whereas the intensity of labeling returned to normal. The degree of the change in cell kinetics depended on the dose of HU, but with all doses used inhibition of cell proliferation due to the action of HU on passage of the cells from G_1 period into the S period of the mitotic cycle and on DNA synthesis in the S period was temporary and reversible.

Under the influence of HU a small proportion of esophageal epithelial cells (2-14%) was characterized morphologically by a sharp change in nuclear structure. However, as the results show, injuries in the cells caused by HU have no significant effect on proliferative processes.

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