TISSUE-SPECIFIC INHIBITION OF CELL PROLIFERATION IN EHRLICH'S ASCITES TUMOR

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Extract of Ehrlich's ascites tumor cells (chalone) and its cell-free fluid have a marked inhibitory action on proliferation of these tumor cells 4 h after injection. The effect is tissue specific, it is more marked in the extract, and it depends on the dose of the agent. Mitotic activity in the tumor 8 h after injection of the extract or cell-free fluid is higher than in the control, evidence of a short-term effect of the chalone on the G_2 phase of the mitotic cycle and on synchronization of cell division.

KEY WORDS: chalone; Ehrlich's ascites tumor; mitotic index.

The study of the tissue mechanisms of regulation of cell proliferation is one of the most urgent problems in experimental biology. In 1964, Bullough and co-workers [2] observed inhibition of division of epidermal cells in mice by an aqueous extract of their epidermis. Tissue-specific inhibitors of cell division (chalones) have now been found in many tissues of animals and man [1]. The study of this problem in relation to tumor tissues is particularly interesting.

In this investigation the effect of an extract of Ehrlich's ascites tumor cells and the cell-free fluid from it on the proliferative activity of this tumor was studied.

EXPERIMENTAL METHOD

Male noninbred albino mice aged 1.5-2 months were used. A diploid strain of Ehrlich's ascites tumor* was transplanted by intraperitoneal injection of 0.2 ml of ascites fluid. Mice with 13-day-old tumors were used to obtain the cell extract and cell-free fluid. The latter was separated by centrifugation (1000 rpm, 5 min). The cells were washed by a single centrifugation in physiological saline. The residue was resuspended in distilled water in the proportions of 20 volumes to 1 volume of residue. The cells were incubated at 37°C for 30 min and then homogenized for 20 min in a rotatory homogenizer at 0°C. The homogenate was then centrifuged for 20 min at 3000 rpm. The supernatant was used in the experiments. Supernatant was injected intraperitoneally in a volume of 1 ml into the experimental animals of series I, whereas the control animals received 1 ml of physiological saline. The mice were killed 4 h later and ascites fluid and a piece of the small intestine was taken from them for preparing specimens for cytological analysis. In series II the animals of group 1 were injected with undiluted supernatant (as in series I). The mice of groups 2 and 3 received injections of the same fluid but diluted two- and fourfold, respectively. The animals of group 4 received the cell-free fluid. In both series animals with 5-day-old tumors were used. In the specimens from Ehrlich's ascites tumor 5000 cells were examined and the mitotic index (MI) calculated in promille. In the specimens of intestine cells were examined in 50 crypts and MI also was calculated in promille. The results were subjected to statistical analysis by Student's method.

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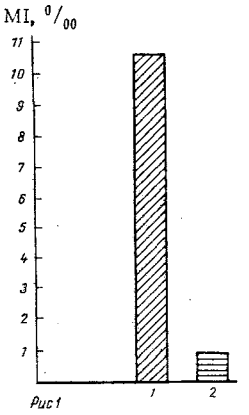

Fig. 1

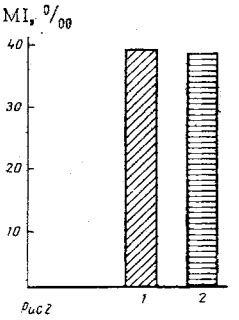

Fig. 2

Fig. 1. MI in Ehrlich's ascites tumor 4 h after injection of extract from its cells: 1) control; 2) experiment.

Fig. 2. MI of epithelium of small intestine 4 h after injection of extract of Ehrlich's ascites tumor cells: 1) control; 2) experiment.

EXPERIMENTAL RESULTS

The results given in Figs. 1 and 2 (the results of series I) show that extract of tumor cells caused marked (up to 91%) inhibition of mitotic activity in the tumor ($P = 0.01$). Meanwhile, MI of the epithelium of the small intestine was unchanged after injection of the extract (Fig. 2). The results thus indicate that extract of Ehrlich's ascites tumor has a tissue-specific inhibitory action on proliferation of the cells of this tumor.

The results given in Fig. 3 (results of series II) confirm the presence of this inhibitory effect 4 h after injection of the extract; both the undiluted extract and its two- and fourfold dilutions had inhibitory activity. The undiluted extract reduced MI in the tumor by 90.5% ($P = 0.001$), the twice-diluted extract reduced it by 66% ($P = 0.01$), and the fourfold diluted extract by 41% ($P = 0.07$). Compared with the undiluted extract, the inhibitory activity of the diluted extract on cell division was thus reduced in the first case by 33% and in the second by 50%; i.e., the inhibitory effect depended on the degree of dilution and, consequently, on the dose of the inhibitory agent. Incidentally, in these experiments on Ehrlich's ascites tumor a much higher degree of inhibition was obtained than has been reported by other workers [2, 5].

The cell-free fluid also inhibited MI in Ehrlich's ascites tumor. The degree of inhibition was 38% ($P = 0.07$), in agreement with data in the literature [4]. Comparison of the inhibitory effects in the cell-free fluid and undiluted extract showed that the action of the extract on proliferative activity of Ehrlich's ascites tumor was 2.4 times stronger than that of the fluid ($P = 0.001$). In the system of Ehrlich's tumor cells, both the extract of its cells and the cell-free fluid thus possessed inhibitory activity. This raises the question of whether the inhibitors in the extract and in the cell-free fluid are identical.

Marked inhibition of MI was observed 4 h after injection of the extract, indicating that it acts on the G_2 period of the mitotic cycle. Considering also the tissue specificity of the extract and the dependence of its effect on dosage, it can be concluded that it satisfies all the accepted requirements of those inhibitors of cell proliferation known as chalones.

After 8 h (Fig. 3) an increase in MI was observed in all the experimental groups of series II compared with the control. The degree of this increase was inversely proportional to the dilution and, consequently, directly proportional to the dose of the inhibitory agents. When undiluted extract was used MI was increased by 57% ($P = 0.01$), the twofold dilution increased it by 45% ($P = 0.07$), and the fourfold dilution by 34% ($P = 0.1$). It was found that the greater the increase in MI of the tumor cells after 8 h, the more sharply reduced mitotic activity was in the animals of the corresponding groups after 4 h. Mitotic activity in the tumor also was increased 8 h after injection of the cell-free fluid. MI was increased by 31% ($P = 0.05$). The observed increase in mitotic activity is evidence that the inhibitory effect of a single injection of the chalone occurred during the next few hours, after which the $G_2 \rightarrow M$ block was removed and the delayed cell population began mitosis synchronously.

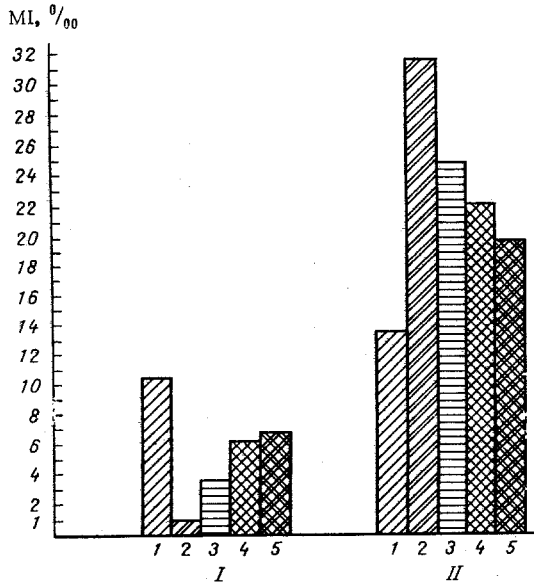

Fig. 3. MI in Ehrlich's ascites tumor 4 h (I) and 8 h (II) after injection of undiluted and diluted extract and cell-free fluid: 1) control; 2) undiluted extract; 3) twofold diluted extract; 4) fourfold diluted extract; 5) cell-free fluid.

The question of the possible synchronizing action of the chalone on the cell population has been discussed in the literature on the regulation of cell division [3], but as yet the existence of this phenomenon has not been confirmed experimentally.

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