COLCHICINE-LIKE ACTION OF SEVINE ON HUMAN EMBRYONIC FIBROBLASTS IN VITRO

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UDC 615.285.7.015.45:611-018.2.085.23

Sevine inhibits mitotic activity of human embryonic fibroblasts in vitro, delays division at the metaphase stage, and leads to the formation of up to 97% of pathological mitoses. The colchicine-like and toxic actions of sevine are clearly dependent on the dose of the compound and the duration of exposure.

Sevine is a pesticide belonging to the carbaminic acid group which is nowadays widely used in agriculture. Its general toxic properties have been closely studied [2, 3, 5-7].

The writers' experiments have shown that the compound possesses cytotoxic properties which are of interest not only to the toxicologist but also to the cytologist.

EXPERIMENTAL METHOD

Cultures of human embryonic fibroblasts were used. Skin and muscle tissue was trypsinized in the usual way [4]. The cells were grown on cover slips in tubes in medium No. 199 (50%) with lactalbumin hydrolysate (30%) and bovine serum (20%). Penicillin and streptomycin were added in doses of 100 units/ml medium. Sevine (a technical product containing 84% of the active substance) was added to the nutrient medium in which it forms a fine grain suspension. To keep the product constantly in a suspended state both the experimental and control tissues were kept in a rotating drum and incubated at 37°C. The cover slips were taken from the tubes after 6, 24, and 48 h, rinsed with warm physiological saline, fixed in Shabadash's mixture, and stained with hematoxylin–eosin.

To assess the effect of sevine on the culture, besides straight microscopic examination of the stained preparations, mitotic activity was determined and pathological forms of mitoses sought. To analyze the pathological forms of mitoses, Alov's classification [1] was used.

EXPERIMENTAL RESULTS

A series of preliminary experiments showed that sevine in a concentration exceeding 100 μg/ml nutrient medium is absolutely toxic for fibroblast cultures, causing degeneration of the cells and their separation from the glass during the first few hours of exposure. The lowest concentration of sevine causing degeneration of up to 50% of the cells (++) after 48 h was taken as the toxic dose. In these experiments this dose was found to be a concentration of 80 μg/ml. Doses of 40 and 20 μg/ml also were tested.

TABLE 1. Change in Phase Index of Mitosis under the Influence of Various Doses of Sevine

Dose of compound (in μg/ml)	Duration of exposure (in h)		
	6	24	48
Control	2,82	1,9	1,78
20	4,43	2,27	1,86
40	11,2	6,2	2,0
80	63,5	300	—

The number of cells in the monolayer in cultures treated with sevine was reduced, although no conglomeration was observed. Pycnosis of the nuclei, eosinophilic granulation of the cytoplasm, and the number of

Laboratory of Immunomorphology, Moldavian Research Institute of Hygiene and Epidemiology, Kishinev. (Presented by Academician of the Academy of Medical Sciences of the USSR A. A. Smorodintsev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 6, pp. 91-93, June, 1972. Original article submitted September 22, 1971.

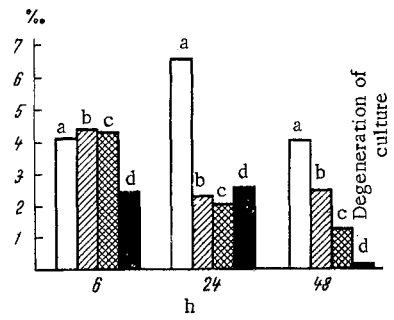

Fig. 1

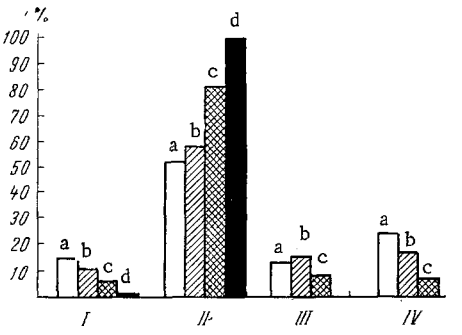

Fig. 2

Fig. 1. Dynamics of mitotic activity in a culture of human embryonic fibroblasts treated with various doses of sevine. Here and in Fig. 3: a) control; b) 20 μg/ml; c) 40 μg/ml; d) 80 μg/ml sevine.

Fig. 2. Change in ratio between phases of mitosis 24 h after treatment of the culture with sevine. I, II, III, and IV: pro-, meta-, ana-, and telophases respectively.

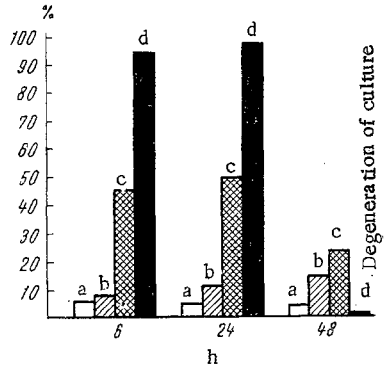

Fig. 3. Relationship between number of pathological forms of mitosis, dose of sevine, and duration exposure.

degenerating cells all increased with an increase in the dose of the compound and the duration of exposure to it.

At the beginning, after exposure to 6 h, a decrease in mitotic activity was observed only under the influence of a toxic dose of sevine. Later, after 24 and 48 h, a decrease in mitotic activity was produced by all doses used (Fig. 1).

A change in the ratio between the phases of mitosis was observed 6 and 24 h after treatment of the cultures: there was a progressive (depending on the dose of the product) decrease in number of pro-, ana-, and telophases, but a marked increase in the number of metaphases (Fig. 2). The ratio between the phases was equalized 48 h after exposure in cultures treated with sevine in concentrations of 20 and 40 μg/ml. In a dose of 80 μg/ml, however, the compound completely inhibited mitotic activity after 48 h.

Even more demonstrative results (Table 1), indicating delay of division in metaphase, were obtained by comparing the phase index: 6 h after the beginning of the experiment the number of the first two phases was considerably increased and the number of the next two phases correspondingly reduced.

The same relationship continued after 24 h. The normal phase index was restored only by the end of the 2nd day of exposure, and then only in culture treated with doses of 20 and 40 μg/ml.

Pathological forms of mitoses were studied in the same specimens. On the average in the control cultures there were up to 6% of pathological forms of mitosis, and in the first few hours after a change of medium this percentage was slightly higher than after 24 and 48 h (Fig. 3). Sevine induced a sharp increase in the number of pathological mitoses (up to 97%). The great majority of pathological forms consisted of c-mitoses, and there were fewer degenerative forms.

It can be concluded from the results of these experiments that sevine has a marked colchicine-like action on human fibroblasts in culture. This action is manifested by delay of cell division in metaphase and in the appearance of pathological forms of mitosis. At the same time, unlike colchicine, sevine prevents cells from commencing mitosis, i.e., it acts not only as a metaphase poison, but also as a "preprophase" poison.

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