ACTION OF HEPARIN ON SOME MITOTIC INDICES OF TRANSPLANTABLE CULTURES OF HUMAN AMNION CELLS (LINE A1)

L. I. Ul'yanova, V. N. Blyumkin, and A. E. Gosheva

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The effect of heparin was studied on the mitotic indices of transplantable monolayer cultures of human amnion cells (strain A1). Inhibition of mitotic activity of the cultures was found to be directly proportional to the heparin concentration in the medium. High doses of heparin blocked mitosis at the stage of transition from late telophase to interphase of the next cell cycle. Meanwhile numerous amitoses appeared. The cells blocked in late telophase or which took part in amitosis underwent pycnosis and destruction.

KEY WORDS: heparin; mitotic activity; amitosis; pycnosis; destruction.

Heparin has found clinical application in recent years not only in the prevention of blood clotting [3, 12], but also as an immunodepressant [4, 5, 7, 13]. The need has thus arisen for a comprehensive study of heparin and, in particular, of its cytological and cytostatic properties. These properties are clearly revealed by the use of monolayer cell cultures as the test object.

This paper describes a study of the effect of heparin on some mitotic indices of transplantable cultures of human amnion cells (line A1), namely their mitotic activity and phases of mitosis. The paper also gives information on amitoses in the cell cultures treated with heparin and the relationship between disturbances of cell division and the subsequent destructive processes.

EXPERIMENTAL METHOD

A suspension of transplantable human amnion cells (line A1) in medium No. 199 with 10% normal bovine serum was poured into penicillin flasks with cover slips. The concentration of the suspension on seeding was $1.5 \cdot 10^5$ cells/ml. Incubation continued at 37°C. After 48 h, when a continuous monolayer of cells was formed, the growth medium was replaced by medium No. 199 containing heparin (Polfa) in concentrations of 10, 50, 200, and 500 units/ml or medium No. 199 without heparin (control). The cell cultures were fixed with 96° ethanol 5, 24, 48, and 72 h later. The preparations were stained with hematoxylin and eosin. The mitotic activity of the A1 cultures was expressed per thousand cells (%). In each case from 3000 to 7000 cells were counted. The results were subjected to statistical analysis by the Fisher–Student method. The distrubution of the phases of mitoses among 100 dividing cells was determined. The number of amitoses per thousand cells also was calculated in the control and experimental cultures. In the final stages the process of destruction of the cell cultures was studied.

EXPERIMENTAL RESULTS

In cultures of line A1 cells treated with heparin the mitotic activity was reduced (Table 1). After 5 h of the experiment the mitotic activity of the control cultures was 38.6 ± 1.2%. In cultures treated with

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TABLE 1. Mitotic Activity (in %) of A1, Cultures Treated with Heparin, andof Control Cultures (M ± m)

Heparin concn. (units/ml)	Time of expt. (h)			
	5	24	48	72
10	37,2±1,2 $P>0,2$	21,67±0,3 $P<0,01$	12,0±0,57 $P>0,5$	2,54±0,7 $P<0,001$
50	22,0±1,0 $P<0,001$	16,33±0,33 $P<0,001$	8,33±0,3 $0,1>P>0,05$	1,5±0,3 $P<0,001$
200	18,5±0,6 $P<0,001$	14,33±0,1 $P<0,001$	2,33±0,3 $P<0,01$	0 Beginning of destruction
500	7,75±0,4 $P<0,001$	5,57±0,6 $P<0,001$	0 Pycnosis and destruction	0
Control*	38,6±1,2	25,75±0,8	11,33±0,8	9,66±0,9

*The decrease in mitotic activity of the control cultures was accounted for by their physiological aging [2].

TABLE 2. Distribution of Phases of Mitosis in Cultures of A1 Cells Treated with Heparin and in Control Cultures (100 mitoses counted in each case)

	Time of expt. (h)																			
	5					24					48					72				
	P	M	A	T	TG_1	P	M	A	T	TG_1	P	M	A	T	TG_1	P	M	A	T	TG_1
10	17	37	4	16	26	31	30	0	10	29	32	41	4	9	14	22	55	3	4	16
50	9	31	4	19	37	29	13	0	16	42	15	31	5	9	40	Mitotic activity close to 0				
200	18	21	4	13	44	10	21	2	24	43	8	25	2	28	47	0	0	0	0	0
																Beginning of destruction				
500	6	35	1	10	48	2	17	0	0	81	0	0	0	0	0	0	0	0	0	0
																Pycnosis and destruction				
Control	28	38	4	16	14	17	33	5	25	20	22	41	3	14	20	25	56	5	6	8

Legend: P) prophase, M) metaphase, A) anaphase, T) telophase, TG_1) transition from telophase to interphase of the next cell cycle.

a small dose of heparin (10 units/ml) the decrease in the number of mitoses with not statistically significant. Higher doses of heparin (50, 200, and 500 units/ml) caused a statistically significant decrease in mitotic activity. The decrease was greater still 24 h after addition of heparin to the medium. By 48 h, in cultures treated with heparin in a concentration of 500 units/ml mitoses disappeared completely and destruction of the cell monolayer began, whereas in cultures treated with a dose of 200 units/ml the mitotic activity was down to 2.33 ± 3% compared with 11.3 ± 0.8% in the control. Mitoses disappeared completely after 72 h in cultures treated with heparin in a concentration of 200 units/ml and almost completely in cultures treated with smaller doses of heparin.

A distinguishing feature of the cultures treated with a high dose of heparin (500 units/ml) was the delay of mitosis in late telophase, or more exactly in the period of transition from telophase to the next interphase (in the terminology adopted, period TG_1) (Table 2).

Many of the cells held up in period TG_1 underwent pycnosis and destruction. Smaller concentrations of heparin delayed mitosis in telophase but a complete blocking of the cells in the TG_1 period was not observed.

Simultaneously with disappearance of mitoses in the A1 cultures treated with high doses of heparin, many amitoses appeared. Whereas in the control cultures of line A1 the number of amitoses did not exceed 10%, in the cultures treated with heparin (500 units ml) this index reached almost 100% (Table 3). The subsequent fate of cells passing through amitosis was studied. Like cells blocked in the TG_1 period of

TABLE 3. Number of Amitoses in Cultures of Transplantable Line A1 Treated with Heparin, and in Control Cultures (in %)

Heparin concn. (units/ml)	Time of expt. (h)			
	5	24	48	72
200	6	8	18	28 Beginning of destruction
500	8	59	98 Beginning of destruction	Pysnosis and destruction
Control	8	8	10	8

mitosis, they underwent pycnosis and destruction, particularly marked 72 h after the addition of heparin to the medium.

The results thus obtained confirmed the view that cell cultures are a promising tool for the study of interaction between immunodepressants and cells [1]. By using monolayer cell cultures more extensive information can be obtained on the effect of heparin on mitosis [8, 10, 12] and clearer ideas can be formed about the eliminating action of heparin on dividing cells. The experiments showed that high doses of heparin have an inhibitory action on the mitotic activity of transplantable line A1 of human amnion cells. The degree of inhibition of mitosis is directly dependent on the heparin concentration in the medium. Heparin delays mitosis on the border between late telophase and the T_1 period of the next interphase. If a high concentration of heparin was used, the cells blocked in this period underwent pycnosis and destruction. From this point of view the action of large doses of heparin was similar, morphologically speaking, to the effect observed after infection of sensitive cell cultures with poliomyelitis [6] and vesicular stomatitis [10] viruses. These viruses also cause delay of mitosis in telophase and death of the daughter cells in the G_1 period. Osunkoya and David–West [10] link this phenomenon with the severe disturbances of cell metabolism induced by virus infection, making normal cytotomy and protein synthesis in the daughter cells impossible. In addition, besides the delay of the cells in the TG_1 period, a marked increase in the number of amitoses also was observed in cultures treated with heparin. This phenomenon reflects the ability of cell cultures treated with heparin to compensate for a limited period of time their lost ability to divide by mitosis. Disturbance of the process of cytotomy, the destruction of cells blocked in the TG_1 period of mitosis, and death of the cells after amitosis are evidently important features of the eliminating action of high concentrations of heparin on cells in culture.

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