Effect of Complex Isoniazid on Mononuclear Cells in a Tuberculous Granuloma

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, No. 5, pp. 559-561, May, 1996
Original article submitted March 22, 1995

Light and electron microscopy shows that the use of dextran matrix in a complex with isoniazid ensures its lysosomotropism, prolonged action, and the capacity to stimulate plastic processes and accelerate the differentiation of cells — monocyte derivatives — in epithelioid cells in tuberculous granulomas.

Key Words: *tuberculous granuloma; epithelioid cells; dextran; isoniazid*

Previously, complex isoniazid (CI) on dextran matrix was shown to be capable of accumulating in tissues [8]. Hence, when used in the treatment of animals with chronic tuberculous inflammation in doses 3 times lower with regard to isoniazid (IS), it was more effective than IS alone [8]. The lysosomotropism of dextran and its capacity to stimulate plastic processes in cells [6] prompted us to propose that the higher activity of CI is due to increased bactericidal activity of macrophages (the place where *Mycobacterium tuberculosis* persist) in the granuloma, this increase being due to specific properties of the dextran matrix.

This study was aimed at investigating the effects of CI by comparing the changes in the number of epithelioid cells and their ultrastructural organization under conditions of spontaneous development of an inflammatory process treated with pure IS and CI.

MATERIALS AND METHODS

A chronic tuberculous process was induced in BALB/c mice weighing 20 to 25 g by a single intraperitoneal injection of BCG vaccine (Research Institute of Vaccines and Sera, Tashkent) in a dose of 50 μg/g b.w. [5]. One month later the animals were intraperitoneally injected IS or CI on dextran matrix whose molecular mass was 30 to 40 kD. The complex was prepared as described previously [8]. A single dose of both agents in terms of IS was equal to 14 mg/kg b.w.

The mice were divided into 5 groups, 15 animals in each. Group 1 (intact mice) was the control; in group 2 the animals were daily injected IS in the above dose; in group 3 IS was injected intermittently [4] at 3-day intervals [8]; in group 4 the animals were administered CI daily; in group 5 CI was administered similarly as IS in group 3. The treatment was administered for 3 months in all groups. Liver samples for examination under the light and electron microscope were collected 1, 2, and 3 months after treatment was started.

The animals were sacrificed under ether narcosis by dislocation of the cervical vertebrae. For light optic examination liver samples were fixed in 10% neutral formalin [1] and embedded in paraffin, and the slices were stained with hematoxylin and eosin. For electron microscopy the samples were fixed in 1% OsO_4 in phosphate buffer. Ultrathin slices were stained with a 1% aqueous solution of uranyl acetate and lead citrate and examined under a JEM-100S electron microscope. The morphology of granulomas and ultrastructure of epithelioid cells were studied using test grids of squares [7]. Differences between the compared mean values were considered reliable at $p<0.05$ (Student's t test).

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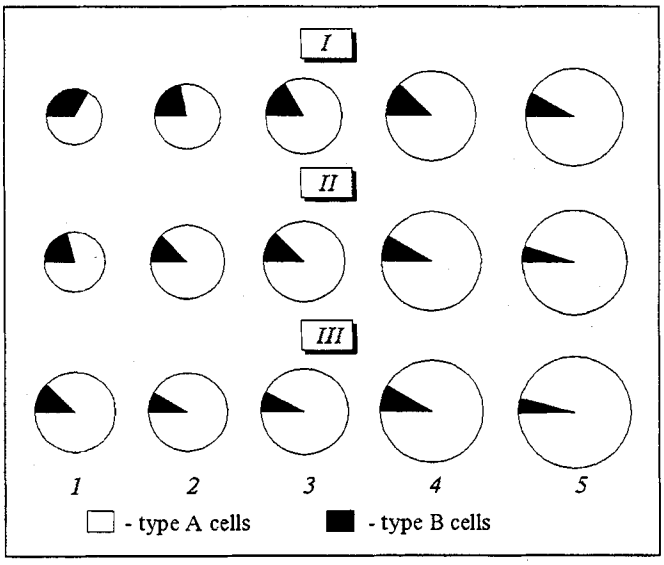

Fig. 1. Counts of type A and B epithelioid cells in liver granulomas. The diameter of the circle is proportional to the relative number of epithelioid cells of both types in a granuloma. The total number of cells of different types is taken as 100%. *1*) untreated animals challenged with BCG vaccine; *2*) animals administered IS daily; *3*) animals injected IS twice a week; *4*) animals injected CI daily; *5*) animals injected CI twice a week; *I*, *II*, and *III*) duration of treatment, months.

RESULTS

Epithelioid cells and mononuclears with an expressed phagocytic capacity, predominating in the granuloma, were subdivided into 2 types: A (cells with a well-developed cytoplasm and characteristic cytoplasmic outgrowths) and B (cells with just a few ultrastructures and secretory granules) [2,3]. In the case of spontaneous development of an inflammatory process the ratio of A and B cells was 3:1, and as the granulomas progressed, the share of type B cells decreased by 64% (Fig. 1). Since the share of B cells fell to just 3% of their total count in the course of treatment with CI (Fig. 1), type A epithelioid cells were the object of electron microscopy. We did not observe the death of type B cells, and hence we proposed that these cells differentiate into type A cells. This process was particularly well expressed during therapy, more so in CI treatment. For this reason, when analyzing the changes in the structure of type A epithelioid cells we paid special attention to the morphological signs indicating their increased functional activity, level of plastic processes, and special function — endocytosis (phagocytosis) as an indicator of differentiation.

A tuberculous granuloma consists mainly of mononuclear cells, which differentiate from monocytes to epithelioid cells [2,3]. The rate and degree of their differentiation may vary and therefore affect the dissemination and outcome of the inflammatory process. The treatment with both agents was in fact associated with a more dynamic increase in the count of type A epithelioid cells than during a spontaneous course of the inflammatory process (Fig. 1).

The level of plastic processes in type A epithelioid cells was higher in the treated animals, which was evident from the number of ribosomes and total concentration of cytoplasmic organelle membranes (Fig. 2, Table 1). It was highest in the animals treated with CI according to the intermittent scheme (Fig. 2, Table 1). The increase in the volume of the vacuolar apparatus and in the share of

TABLE 1. Results of Morphometry of Type A Epithelioid Cells

Parameter		Untreated animals	IS daily	IS intermittently	CI daily	CI intermittently
ΣS_v	1	1.51±0.12	1.86±0.14	2.46±0.16	3.75±0.19*	4.51±0.21*
	2	1.62±0.13	1.95±0.14	2.92±0.17	4.06±0.20*	5.03±0.22*
	3	2.14±0.15	2.03±0.14	3.62±0.19	4.79±0.22*	6.49±0.26*
V_{v1}	1	1.03±0.10	1.44±0.12	1.94±0.14	3.45±0.19*	3.81±0.19*
	2	1.35±0.12	1.65±0.13	2.04±0.14	3.61±0.16*	3.98±0.20*
	3	1.47±0.12	1.88±0.14	3.16±0.18	3.70±0.19*	4.47±0.21*
V_{v2}	1	3.36±0.18	6.65±0.26	6.94±0.25	8.79±0.29*	11.94±0.35*
	2	4.56±0.21	8.40±0.29	9.97±0.32	12.55±0.35*	13.50±0.35*
	3	8.40±0.28	12.19±0.35	12.37±0.35	15.23±0.39*	16.47±0.41*
$\Sigma V_{v1,2}$	1	4.39±0.21	8.09±0.28	8.38±0.29	12.24±0.35*	15.75±0.40*
	2	5.91±0.24	10.05±0.32	12.01±0.35	16.16±0.39*	17.48±0.41*
	3	9.87±0.31	14.07±0.38	15.53±0.39	18.94±0.44*	20.94±0.46*

Note. ΣS_v: total concentration of membranes of the granular endoplasmic reticulum, Golgi complex, and external mitochondrial membrane; volume density of primary (V_{v1}) and secondary (V_{v2}) lysosomes; $\Sigma V_{v1,2}$: total index of the volume density of primary and secondary lysosomes. 1, 2, and 3: time elapsed from the beginning of therapy, months. *Reliable differences from the parameters in respective groups of mice treated with isoniazid after the same protocol.

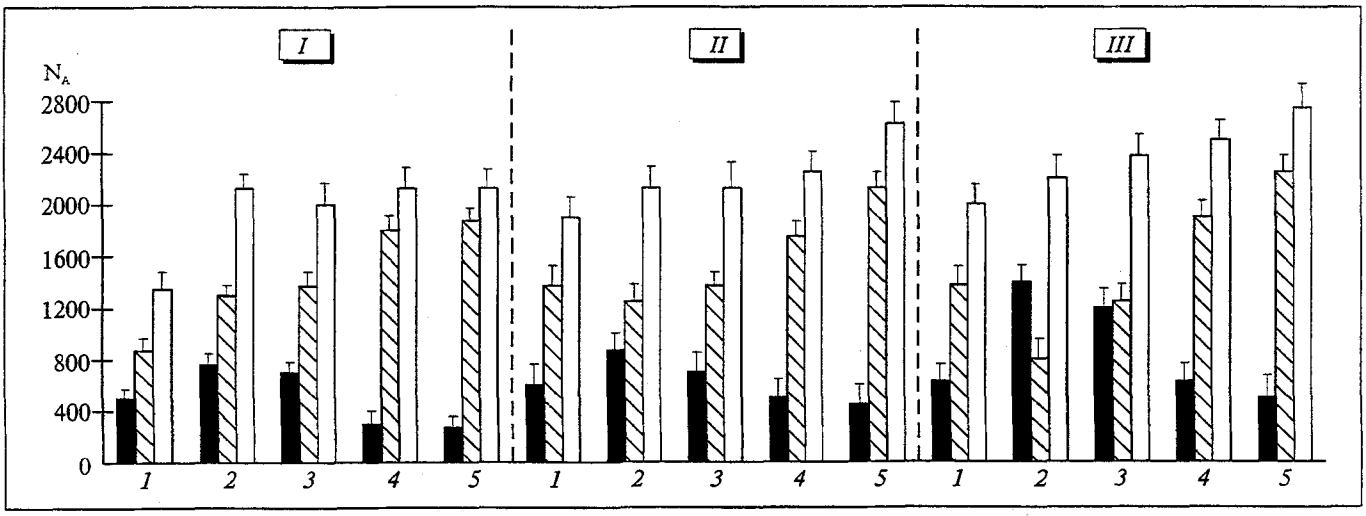

Fig. 2. Numerical densities of free and attached ribosomes in type A epithelioid cells of mouse liver granulomas. Dark bars: free ribosomes; cross-hatched bars: attached ribosomes; white bars: total number of ribosomes of both types.

secondary lysosomes as an indicator of the activity of endocytosis processes was more notable (Table 1). Together with the decreased count of monocytes in the granuloma [8] and the increase of the total count of epithelioid cells and of the share of type A cells (Fig. 1), this indicates intensification of the synthetic processes in them, as the basis of accelerated differentiation of mononuclears and their increased functional activity. All this, as was previously demonstrated [8], was realized by a decrease in the number of granulomas and of their diameter. On the other hand, whereas during therapy with CI the boosting of both these processes could be attributed to the effect of the dextran matrix, during therapy with IS we might ascribe the similar effect, though less expressed, to mycobacteria degradation products.

Previous findings [8] indicate that the principle of prolonging the antibacterial effect of a drug by endowing it with expressed lysosomotropic properties may be useful in the development of agents for the treatment of chronic inflammatory processes, when their course is due to the persistence of the agent. Our data indicate that the desired effect may be attained by reducing the total dose of the bacteriostatic and using lysosomal matrices capable of stimulating plastic processes in the cells.

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