LUMINESCENCE MICROSCOPIC STUDY OF THE ACTION OF DEXAMETHASONE ON MACROPHAGAL LYSOSOMAL MEMBRANES

M. Ya. Korn, A. V. Gavril'chak, and Kh. M. Isina

UDC 615.357.453.015.45:612.112.9

The effect of dexamethasone on lysis of the red cytoplasmic granules (lysosomes) of macrophages, fluorochromed intravitally with acridine orange, under the influence of antilysosomal sera was studied. Treatment of the cells with minimal concentrations of dexamethasone before and during incubation with antilysosomal serum inhibited its lytic action, but with an increase in the concentration of dexamethasone the lytic action was strengthened. The results confirm the hypothesis that the lytic action of antilysosomal serum in the presence of complement on the red cytoplasmic granules is the result of changes in the lysosomal membranes.

Previous experiments [2] showed that antilysosomal sera, in the presence of complement, produce lysis of the red cytoplasmic granules (RCG) of macrophages. These granules are lysosomes containing absorbed fluorochrome [1, 5]. It has been postulated that lysis of the RCG takes place as a result of an increase in the permeability or destruction of the lysosomal membranes [2]. This could be confirmed by weakening of the lytic action of the antilysosomal sera on the RCG after treatment of the cells with substances stabilizing lysosomal membranes.

Many anti-inflammatory preparations, including corticosteroids, are known to have this action [7-11].

The action of dexamethasone, one of the most active of the synthetic corticosteroids, on lysis of the macrophagal RCG by antilysosomal serum in the presence of complement was studied in this investigation.

EXPERIMENTAL METHOD

The method of obtaining, cultivating, and fluorochroming the macrophages was fully described previously [2-4]. A solution of dexamethasone (LEK, Yugoslavia) was added to a 4-5-day culture of macrophages fluorochromed with acridine orange. The final concentration of dexamethasone was from 0.1 to 10 μg/ml. Simultaneously with dexamethasone, antilysosomal (antimembrane) serum and complement were added. The technique of treating the macrophages with antilysosomal serum and complement also was described previously [2]. The macrophages were incubated with dexamethasone, antilysosomal serum, and complement for 3 h at 37°C and examined in the ML-2 luminescence microscope; the percentage of normal, unchanged cells containing luminescent RCG was counted and compared with the percentage of the same cells in preparations incubated under the same conditions with antilysosomal serum and complement but without dexamethasone.

In other experiments fluorochromed macrophages were preincubated with dexamethasone in the same concentrations at 37°C for 1 or 18 h, washed twice with medium No. 199, and then incubated with antilysosomal serum and complement for 3 h at 37°C.

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten Eksperimental'noi Biologii i Meditsiny, Vol. 77, No. 4, pp. 70-73, April, 1974. Original article submitted April 2, 1973.

TABLE 1. Percentage of Unchanged Macrophages after Simultaneous Incubation of Cells with Dexamethasone and Antilysosomal Serum (M ± m)

Concentration of dexamethasone (in μg/ml)	Percent of cells with red granules
0,1	68,25±2,3
0,5	70,5±2,25
1	53,3±2,48
5	21,5±2,05
10	14±1,72
Control with normal rabbit serum and without dexamethasone	100
Control with antilysosomal serum and without dexamethasone	19,3±2,0

A fluorochromed culture of macrophages, incubated under the same conditions with normal rabbit serum and complement, and also with dexamethasone in the presence of complement (without antilysosomal serum) was used as the control.

At least three repetitions of all experiments were carried out. To determine the percentage of unchanged macrophages containing RCG, 400 cells were counted in parallel preparations.

EXPERIMENTAL RESULTS AND DISCUSSION

On luminescence microscopy of the control preparations cells with the typical morphology, with a dull green cytoplasm containing many RCGs and with a weakly luminescent green nucleus with brighter nucleoli, were seen. In preparations incubated with dexamethasone for 18 h (without antilysosomal serum) rounded cells were rather more numerous, although they showed normal luminescence of their cytoplasm and nucleus and contained the same number of RCGs as the untreated cells. Most cells in cultures incubated with antilysosomal serum and complement were rounded in shape, without granules, and with a bright green pycnotic nucleus. Some cells were indistinguishable in their morphology and the character of their luminescence from the controls or they contained fewer RCGs.

Addition of dexamethasone in minimal concentrations simultaneously with antilysosomal serum and complement increased the percentage of unchanged cells containing RCG (Table 1). The number of these cells rose to a certain limit with an increase in the concentration of dexamethasone in the medium. With a further increase in the dexamethasone concentration the number of unchanged macrophages in the preparation began to fall and ultimately, instead of being inhibited, the lytic action of the antilysosomal serum was intensified (Fig. 1). This character of the action of dexamethasone could be explained either by its effect on the lysosomal membranes or by its effect on the activity of the antilysosomal serum and complement taking part in the reaction.

To solve this problem experiments were carried out in which the macrophage culture was first treated with dexamethasone and then incubated with antilysosomal serum and complement.

The results of these experiments (Table 2) show that the same principles were observed under these conditions as before: inhibition of the lytic action of the antilysosomal serum on incubation with lower concentrations of dexamethasone and a decrease in this inhibition, or even potentiation of the action of the antilysosomal serum with an increase in the dose of dexamethasone and in the duration of incubation. These results indicate that dexamethasone acts on the lysosomal membranes.

It is interesting to note that with an increase in the duration of preincubation with dexamethasone to 18 h the change in the character of its action occurred at a lower concentration. This points to the cumulative character of its action, evidently through absorption of dexamethasone and its accumulation in the lysosomes [5].

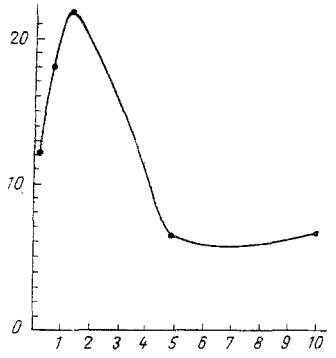

Fig. 1. Action of different concentrations of dexamethasone on changes in macrophages under the influence of antilysosomal sera. Abscissa, dexamethasone concentration (in μg/ml); ordinate, percentage of cells with red granules.

The results of these experiments confirm the view expressed before by the writers, namely that the lytic action of antilysosomal serum in the presence of complement on the macrophagal RCGs is the result of changes in the lysosomal membranes.

Meanwhile certain differences in the effects of dexamethasone on the lysosomal membranes were revealed depending on its concentration in the medium and the duration of incubation. With the lengthening of incubation or an increase in the dexamethasone concentration there was at first an increase in the stabilizing action, but this was followed by a decrease and, finally, by a change to a destabilizing action. The same effect of corticosteroids has been observed with isolated liver lysosomes [7]. The results of the present experiments show that it applies equally to the effect of corticosteroids on lysosomal membranes in the living cell.

TABLE 2. Percentage of Unchanged Cells after Preliminary Contact between Macrophages and Dexamethasone before Addition of Antilysosomal Serum (incubation for 1 and 18 h at 37°C; M ± m)

Concentration of dexamethasone (in μg/ml)	Percent of cells with red granules	
	1h	18 h
0,1	44,5±2,48	30,5±2,38
0,5	60±2,4	39,75±2,45
1	64,5±2,35	10,25±1,52
5	55±2,5	
Control with normal rabbit serum and without dexamethasone	100	100
Control with antilysosomal serum and without dexamethasone	37±2,4	16,25±1,85

It may be that other substances affecting the stability of the lysosomal membranes may exhibit their action differently under different conditions. In particular, the conflicting data regarding the action of chloroquine, which caused stabilization of the membranes in preparations of isolated lysosomes and autophagy (probably connected with the destabilization of these membranes), by its action on living macrophages [6], may be connected with this dependence on concentration.

An advantage of the luminescence-microscopic technique suggested by the writers is that the action of the various substances on the lysosomal membranes of the living cell can be estimated quantitatively.

LITERATURE CITED

1. A. V. Zelenin, Izvest. Akad. Nauk SSSR, Seriya Biol., No. 6, 977 (1965).
2. Kh. M. Isina and M. Ya. Korn, Byull. Eksperim. Biol. i Med., No. 9, 83 (1973).
3. M. Ya. Korn and M. A. Grigor'eva, in: Proceedings of the 15th All-Union Congress of Microbiologists, Epidemiologists, and Infectious Diseases Specialists [in Russian], Moscow (1970), Part 11, p. 161.
4. M. Ya. Korn, Abstracts of Proceedings of an All-Union Conference of the Use of Luminophores [in Russian], Stavropol' (1971), p. 67.
5. A. C. Allison and M. R. Young, Life Sci., No. 3, 1407 (1964).
6. M. E. Fedorko, J. G. Hirsch, and Z. A. Cohn, J. Cell Biol., 38, 377 (1968).
7. D. A. Lewis, A. M. Symons, and K. I. Ancill, J. Pharm. Pharmacol., 22, 902 (1970).
8. A. M. Symons, D. A. Lewis, and R. I. Aneill, Biochem. Pharmacol., 18, 25 (1969).
9. L. Weiss, Exp. Cell Res., 37, 540 (1965).
10. L. Weiss and L. Dingle, Ann. Rheum. Dis., 23, 57 (1964).
11. G. Weissmann, Fed. Proc., 23, 1038 (1964).