MICROBIOLOGY AND IMMUNOLOGY

Effects of Hydrocortisone, Retabolil, and Their Combination on the Phagocytic Activity of Rat Blood Neutrophils

A. V. Shurlygina, T. A. Obut, Ya. Sh. Schvarts, and V. A. Trufakin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 2, pp. 179-180, February, 1995
Original article submitted June 16, 1994

Effects of retabolil on the phagocytic index, phagocytic number, and the nitro blue tetrazolium reduction test with rat blood neutrophils were studied. The drug was found to reduce these parameters. Pretreatment with retabolil arrested or attenuated the depression of neutrophil phagocytic activity caused by hydrocortisone.

Key Words: *hydrocortisone; retabolil; phagocytosis; neutrophils*

Glucocorticoids are known to cause immune depression in the organism [3]. This effect is similar to the immunodepressive syndrome in stress states. On the other hand, we demonstrated a stress-limiting effect of weak androgens in animals, which manifests, among other things, by a negligible increase of the glucocorticoid level in the blood in response to stress. It seemed important to clarify in this connection how weak androgens influence the manifestation of the glucocorticoid-induced immunosuppressive effect.

The purpose of this research was to elucidate the effect of retabolil, a synthetic analog of reticular adrenocortical androgens, on the phagocytic activity and oxidative metabolism of blood neutrophils in male rats at rest and under conditions of suppression of the phagocytic function by an injection of exogenous hydrocortisone.

MATERIALS AND METHODS

Adult male Wistar rats weighing 160 to 180 g were used in the experiments. The animals were divided into 4 groups, 5 animals in each: group I intact animals, group II animals intramuscularly injected retabolil in a dose of 50 mg/kg 9 days before sacrifice, group III intraperitoneally injected hydrocortisone acetate (Gedeon Richter) in a dose of 100 mg/kg 2 days before sacrifice, and group IV injected hydrocortisone after retabolil, the drug being administered at the same times and in the same doses as in groups II and III. Three experiments were carried out. The animals were sacrificed by decapitation. Blood was collected in centrifuge tubes with heparin (about 10 units/ml).

The phagocytic activity of neutrophils was assessed by absorption of heat-killed bacterial corpuscles of *Staphylococcus aureus* (*S. aureus* vaccine produced by the Kharkov enterprise for the manufacture of bacterial preparations, Ukraine).

Incubation was carried out in 96-well disposable plates for immunologic tests with whole heparin-treated blood (about 10 units/ml heparin) at 37°C for 1 hour. Bacterial corpuscles were added at the rate of 100 corpuscles per cell. The mixture was carefully and thoroughly pipetted before and after incubation, and smears were made on slides, dried, fixed with methanol, and stained with azure-eosin. Then the percentage of phagocytically active cells (phagocytic index) and the mean num-

Institute of Clinical and Experimental Lymphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

0007-4888/95/0002-0171$12.50

TABLE 1. Phagocytic Activity of Rat Blood Leukocytes after Injection of Hydrocortisone, Retabolil, and Their Combination ($M \pm m$)

Group	Drugs injected	Phagocytic index	Phagocytic number	Spontaneous NBT test	Induced NBT test
I	Control	36.64±1.79	5.71±0.12	4.82±0.74	12.00±1.12
II	Hydrocortisone	18.13±2.07*	5.60±0.16	2.10±0.44*	4.03±1.11**
III	Retabolil	20.21±2.36*	5.72±0.27	6.28±1.30+	8.64±1.02**,+
IV	Hydrocortisone + retabolil	34.93±3.57°,+	5.54±0.15	4.20±0.52+	8.27±0.93*,+

Note. One asterisk shows $p<0.01$, two asterisks $p<0.05$ reliability of difference from the control; the plus and circle show $p<0.01$ in comparison with groups II and III, respectively.

ber of bacteria phagocytized by a single phagocytically active neutrophil (phagocytic number) were counted in 200 cells under a microscope at ×1000. Neutrophils which phagocytosed at least 3 bacterial corpuscles were considered as phagocytically active.

Oxidative metabolism of neutrophils was assessed by the level of the spontaneous and induced nitro blue tetrazolium (NBT) test. For this test, 0.025 ml Hanks solution and 0.025 ml 0.2% NBT were added to 0.05 ml heparin-treated blood. For the induced NBT test Hanks solution was replaced by 0.025 ml of 0.005% prodigiosan. The mixture was carefully pipetted, after which the wells of the plate were covered with a lid in which a filter moistened with normal saline was fitted, and incubated at 37°C for 30 min. The incubation mixture was then again pipetted, smears were prepared, fixed in methanol, and stained with carmine, and the percentage of neutrophils containing diformazan granules was estimated under the microscope at ×1000.

The reliability of differences was assessed using Wilcoxon-Mann-Whitney's test.

RESULTS

The data of three experiments are presented in Table 1. Injection of hydrocortisone (group II) led to a reduction of all the parameters studied except the phagocytic number. Retabolil also lowered the phagocytic index and the results of the NBT test, but to a lesser degree than hydrocortisone. While reducing the number of phagocytically active cells, the agent had almost no effect on their phagocytic activity or the baseline level of oxidative metabolism (spontaneous NBT test). This is to a certain extent in line with the data on the existence of a positive correlation between the concentrations of true androgens in the blood and the results of the NBT test (data not presented).

It is possible that retabolil causes a redistribution of phagocytically active cells from the blood in the tissue without appreciably influencing the function of still circulating leukocytes. The reduction of the phagocytosis parameters might have been due to the toxic effect of the agent, as has been observed *in vitro* under the effect of high testosterone doses [6]. However, in such a case the capturing capacity and baseline level of oxidative metabolism should have been reduced, which was not observed in our experiments.

In group IV animals, to which both hormones were injected, arrest or attenuation of the depressive effect of hydrocortisone was observed in the majority of cases. This is consistent with the report [2] that androgens of the reticular zone of the adrenal cortex, a synthetic analog of which is retabolil, can increase the body's resistance at least to viral infections on the systemic level.

It is possible that such an effect of retabolil is due to nonspecific cross-binding of this steroid hormone to the glucocorticoid receptors and their partial blocking. An indirect feedback type of effect of the agent mediated by the central endocrine component cannot be ruled out either. Specifically, pituitary hormones capable of influencing the receptor system and metabolic potential of phagocytizing cells [1,4,5] modify their reaction to hydrocortisone.

Hence, we revealed that retabolil injected in parallel with hydrocortisone prevents the hydrocortisone-induced reduction of the phagocytic activity of blood leukocytes. These results call for further investigation of retabolil as an agent preventing immunodepression caused by glucocorticoids.

This study was sponsored by the Russian Foundation for Basic Research.

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