BENZO(a)PYRENE HYDROXYLASE ACTIVITY OF IMMUNOCOMPETENT CELLS

Academician A. V. Bogatskii,*
T. O. Filippova, I. E. Kovalev,
S. A. Andronati, N. Ya. Golovenko,
B. N. Galkin, and L. A. Litvinova

UDC 612.017.1.015.1:577.152.193.1]014.46:615.276.4

KEY WORDS: benzo(a)pyrene hydroxylase activity; immunocompetent cells; immunostimulators.

The hypothesis has recently been put forward that the immunologic system is functionally connected with the cytochrome P-450 system. Interaction between these two systems maintains chemical homeostasis in the body [3]. There are data in the literature on the inhibitory effect of interferon-inducing agents and, in particular, of the low-molecular-weight immunostimulator tilorone [7], on mono-oxygenase activity in the liver. Consequently, these compounds have a reciprocal action on immunity and on liver mono-oxygenases.

The object of this investigation was to study the effect of compounds with immunostimulant properties on mono-oxygenase activity of immunocompetent cells and hepatocytes. Several workers have studied [4] activity of benzo(a)pyrene hydroxylase in human blood lymphocytes and monocytes or mouse peritoneal cells [6]. However, these investigations were carried out as a rule on cell cultures stimulated by phytohemagglutinin, in order to study transformation of xenobiotics in man on accessible material or on cell homogenates. We, however, were concerned with the activity of this enzyme in immunocytes of different origin and with completely preserved function: thymus and spleen cells and macrophages, which play different roles in the immune response.

EXPERIMENTAL METHOD

Experiments were carried out on female CBA mice. Isolated hepatocytes were used for comparison, for the liver is an organ with the most highly developed mono-oxygenase system in its cells, and which affects the development of the immune response. Thymus and spleen cells were obtained by the usual methods and hepatocytes were isolated by the method in [2]; adherent peritoneal exudate cells were used as macrophages. Enzyme activity was determined by the method in [5]. The cells were incubated for 1 h at 37°C in Hanks' solution without Ca^{++} and glucose, in the presence of an NADPH-generating system. The quantity of 3-hydroxybenzo(a)-pyrene formed was determined on a Specol-10 (East Germany) instrument with fluorometric attachment.

The different cells from intact mice differed in their level of aromatic hydrocarbon hydroxylase activity and can be arranged in the following order: macrophages > hepatocytes $\gg$ thymocytes > splenocytes (Table 1). Activity of the enzyme in lymphocytes was an order of magnitude lower than in liver cells and macrophages. Lymphocytes of different origin also differ in this respect. Thymocytes oxidize benzo(a)pyrene 4 times faster than splenocytes. The study of macrophages yielded rather unexpected results. Benzo(a)pyrene hydroxylase activity in them, when expressed for equal numbers of cells, was 3 times higher than in liver cells.

The effect of immunostimulators (tilorone and two of its analogs – IS-23 and IS-33), whose immunotropic activity was reported previously [1], on benzo(a)pyrene hydroxylase activity also was studied. The substances were injected for 3 days in a daily dose of 50 mg/kg. The results showed that the cells tested differed not only in their level of mono-oxygenase activity, but also in the character of its change under the influence of immunotropic agents. Benzo(a)pyrene hydroxylase activity in macrophages increased by 30-80%. Activity of the enzyme

*Academy of Sciences of the Ukrainian SSR.

Laboratory of Psychotropic Drugs, I. I. Mechnikov Odessa University. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 7, pp. 23-24, July, 1983. Original article submitted February 4, 1983.

0007-4888/83/9607-0905 $07.50

TABLE 1. Aryl Hydrocarbon Hydroxylase Activity (in pmoles 3-hydroxybenzo(a)-pyrene/min · 10^7 cells) in Immunocompetent Cells (M ± m, n = 6-10)

Experimental conditions	Thymocytes	Splenocytes	Macrophages	Hepatocytes
Control	0,61±0,15	0,15±0,02	5,33±0,75	1,77±0,25
Tilorone	0,77±0,04	0,07±0,02*	9,01±0,85*	1,23±0,14
IS-23	0,84±0,24	0,05±0,01*	7,03±0,67	1,20±0,23
IS-33	0,66±0,15	0,06±0,01*	9,64±1,75*	1,15±0,21

Legend. *P < 0.05.

in spleen cells was reduced by 2-3 times. Although the changes in the thymus and hepatocytes were not significant, a tendency for activity to increase (by 10-40%) was observed in the thymocytes, and a tendency for it to decrease (by 30-40%) in the liver, in agreement with results obtained on the microsomal fraction after administration of tilorone to animals [7].

There is evidence in the literature [8] of the inhibitory action of inducers (tilorone and its analogs) on liver microsomal enzyme activity. The results now obtained show that their effect depends on the type of cells, for in macrophages, besides a high level of activity, an increase in that activity also was observed under the influence of immunostimulators.

LITERATURE CITED

1. S. A. Andronati, K. P. Balitskii, I. G. Veksler, et al., in: Abstracts of Proceedings of the 6th Congress of Oncologists of the Ukrainian SSR [in Russian], Kiev (1980), pp. 232-234.
2. I. P. Kanaeva, A. V. Karyakin, G. V. Alenicheva, et al., Tsitologiya, 17, 545 (1975).
3. I. E. Kovalev, Khim.-Farm. Zh., No. 12, 3 (1977).
4. R. S. Lake, M. R. Pezzutti, M. L. Kropko, et al., Cancer Res., 37, 2530 (1977).
5. D. W. Nebert and H. V. Gelboin, J. Biol. Chem., 243, 6242 (1968).
6. K. Ptashne, L. Brothers, S. G. Axline, et al., Proc. Soc. Exp. Biol. (New York), 146, 585 (1974).
7. K. W. Renton and G. Y. Mannering, Biol. Fate Chem., 4, 223 (1976).
8. K. W. Renton and G. Y. Mannering, Biochem. Biophys. Res. Commun., 73, 343 (1976).