EFFECT OF LIPOPOLYSACCHARIDES FROM HIGHER PLANTS (PHYTOLIPOPOLYSACCHARIDES) ON REGENERATION OF THE LIVER IN MICE

(UDC 612.36-003.93-02:615.32)

I. N. Yashina, Z. A. Ryabinina, and B. N. Gladyshev

Laboratory of Growth and Development (Head, Professor L. D. Liozner),
Institute of Experimental Biology (Director, Professor I. N. Maiskii),
AMN SSSR and A. N. Bakh Institute of Biochemistry (Director, Academician A. I. Oparin),
AN SSSR, Moscow
(Presented by Active Member AMN SSSR A. V. Lebedinskii)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 58, No. 9,
pp. 116-119, September, 1964
Original article submitted July 4, 1963

Hexosamine-containing compounds of liposaccharide type (phytolipopolysaccharides), belonging to the same group of compounds as the bacterial lipopolysaccharides, have been found in the tissues of higher plants [1-3]. Bacterial lipopolysaccharides (i. e., endotoxins of Gram-negative bacteria of the enteric group) are known to possess high biological activity over a broad spectrum [4-7].

It has been shown that lipopolysaccharides from higher plants, from the point of view of their chemical structure analogues of the bacterial lipopolysaccharides, also have a marked influence on the bone marrow. For instance, a preparation of the lipopolysaccharides from tea leaves stimulated regeneration of the marrow of rats exposed to the action of ionizing radiation, as revealed by stimulation of erythro- and thrombocytosis [8]. It has been suggested that lipopolysaccharides from tea leaves may also stimulate the regeneration of other animal tissues. In the present investigation the effect of lipopolysaccharides from tea leaves on regeneration of the liver in mice was studied.

EXPERIMENTAL METHOD

Experiments were carried out on albino mice weighing 30-45 g. The left lateral lobe of the liver (30% of the liver tissue) was removed from the animals. On the 2nd day after the operation the mice received a subcutaneous injection of physiological saline containing lipopolysaccharides from tea leaves as follows: the 7 mice of group 1 received 10 μg lipopolysaccharides and the 10 mice of group 2—100 μg. Control groups consisted of 16 mice from which the left lateral lobe of the liver was removed, but which did not received lipopolysaccharides, and 7 mice with the liver intact. The animals of group 1 were sacrificed on the 3rd day after the operation, and those of group 2 on on the 4th day. Control mice were sacrificed at the same times. The regenerating liver was weighed and fixed in cold 80% ethyl alcohol and embedded in paraffin wax, and sections were cut from it to a thickness of 5-7μ. The sections were stained with hematoxylin-eosin, methyl green—pyronine, and by Feulgen's method.

The mitoses in the parenchymatous (per 6000 cells) and nonparenchymatous (per 4000 cells) cells were counted in the liver sections. Calculations were made of the number of nonparenchymatous cells (cells of sinusoids and bile duct, Kupffer cells) and also the numbers of mono-, bi-, and trinuclear liver cells counted per 1000 parenchymal cells and the total number of the latter in a field of vision. For examining the sections and counting the cells a 90 × objective and 10 × ocular with a square window in the diaphragm measuring 7 × 7 mm were used.

EXPERIMENTAL RESULTS

Injection of lipopolysaccharides, even in a small dose, resulted in an improvement in the general condition of the hepatectomized mice. This was shown, in particular, by a smaller weight loss of the mice after the operation. For example, the weight loss on the 4th day after operation was 1.9% in the experimental mice of group 2, compared with 8.4% in the control mice. The difference between the experimental and control findings was statistically significant.

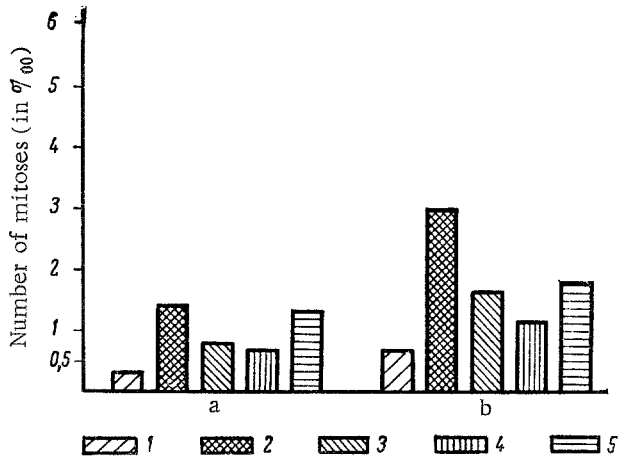

Fig. 1. Effect of lipopolysaccharides on mitotic activity of parenchymatous (a) and nonparenchymatous (b) cells of the regenerating liver of mice. 1) Intact liver; 2) control: regenerating liver on 3rd day after operation; 3) experiment: regenerating liver on 3rd day after administration of 10μ lipopolysaccharides; 4) control: regenerating liver on 4th day after operation; 5) experiment: regenerating liver on 4th day after operation administration of 100μg lipopolysaccharides.

When lipopolysaccharides were injected into mice after resection of the left lateral lobe of the liver, the weight of the regenerating liver increased. In group 1 the differences between the weights of the liver in the experimental and control animals were random in character (P = 0.162). In the animals of group 2 the absolute weight of the regenerating liver increased by 16.5% and its mean value in the experimental animals was 1.682 g, and in the controls 1.357 g. The relative weight of the regenerating liver increased by 12% (4.45% in the experimental animals, 3.97% in the controls). The difference between the experimental and control values is statistically significant (P < 0.01) (see table). Hence, the subcutaneous injection of lipopolysaccharides in an adequate dose (100μg) had a marked effect on the weight of the hepatectomized animals and also on the weight of the regenerating liver.

Experiments on different mammals have shown that regeneration of their liver follows the course of regeneration hypertrophy. The weight of the liver is restored during the first 1-2 weeks after the operation, as a result of an increase in the number of cells and of their hypertrophy.

Our experiments were conducted on old mice, and the mitotic activity in the liver during the daytime was therefore slight. The number of mitoses in the parenchymatous cells of the liver in the intact mice was 0.5‰. On the 3rd day after the operation the number of mitoses in the liver of the mice not receiving lipopolysaccharides was 2.1‰, and on the 4th day—1.0‰; the difference between the values obtained on the 3rd and 4th days after operation was statistically significant (P = 0.013). The number of mitoses in the nonparenchymatous cells of the liver of the intact mice was 1.0‰. On the 3rd day after the operation it rose to 5.5‰ (P = 0.004), and on the 4th day it fell to 2.0‰, i. e., the difference between the number of mitoses on the 3rd and 4th days of regeneration in the control mice was 3.6‰ (P = 0.01).

Hence, the maximum of mitotic activity of both nonparenchymatous and parenchymatous cells in the regenerating liver of the animals not receiving lipopolysaccharides was observed on the 3rd day after the operation (Fig. 1). Administration of lipopolysaccharides from tea leaves in a dose of 10μg lowered the mitotic activity in the regenerating liver on the 3rd day post operation. The number of mitoses in the liver cells of these mice was 1.4‰ (compared with 2.0‰ in the controls). The difference was close to statistically significant (P = 0.03). The number of mitoses in the nonparenchymal cells of the experimental mice was 2.7‰, and in the control mice 5.5‰ (P = 0.042). Hence, after administration of the preparation of lipopolysaccharides in a dose of 10μg a tendency was observed for the mitotic activity of both the parenchymatous and the reticulo-endothelial cells of the liver to be depressed.

After administration of lipopolysaccharides in a dose of 100 μg, an increase in the mitotic activity of the liver cells was observed, to reach a level of 2.3‰ on the 4th day after operation, compared with 1.0‰ in the controls (P = 0.07). The number of mitoses in the parenchymatous cells of the regenerating liver of the mice receiving 100μg of lipopolysaccharide was 0.31‰, and 0.20‰ in the control mice, but this difference was not statistically significant.

Because of the fact that the differences between the mitotic activity of the parenchymatous cells of the mice receiving 100μg of lipopolysaccharides and of the control mice on the 4th day of regeneration were not statistically significant, it may be concluded that the lipopolysaccharides had no effect on the mitoses in the nonparenchymatous elements of the regenerating liver. Counts of the nonparenchymatous cells in the liver of the experimental and control animals showed that their number increased during regeneration. Whereas in the intact liver it amounted to 407 (per 1000 parenchymatous cells), or 28.9% of the total number of cells; on the 4th day of regeneration it had reached 576, or 36.6% (P = 0.0001). On the 3rd day after the operation the number of nonparenchymatous cells of the regenerating liver was 490, or 33.5%.. The difference between the values relating to the regenerating and intact livers was close to statistically significant (P = 0.025). After administration of lipopolysaccharides in a dose of 100μg,

Effect of Injection of Lipopolysaccharides in a Dose of 100μg on Weight of Regenerating Liver on 4th Day after Operation

Mouse No.	Experiment		Control	
	Absolute wt. (in g)	Relative wt. (in %)	Absolute wt. (in g)	Relative wt. (in %)
1	2,210	4,7	1,420	3,8
2	1,050	3,7	1,180	3,7
3	1,610	4,7	1,470	3,8
4	1,800	5,0	1,350	4,5
5	1,650	4,6	1,500	4,2
6	1,450	4,3	1,220	3,7
7	1,550	4,2		
8	1,320	4,3		
9	1,200	4,3		
10	1,300	4,7		
Mean	1,682	4,45	1,357	3,97

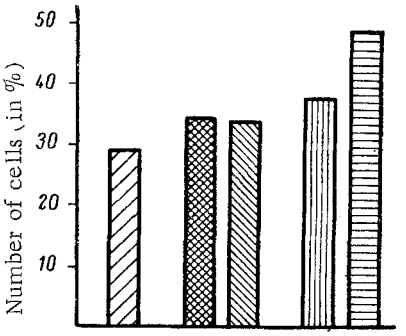

Fig. 2. Effect of lipopolysaccharides on number of nonparenchymatous cells in the regenerating liver in mice. Legend the same as Fig. 1.

the number of nonparenchymatous cells in the liver on the 4th day of regeneration was 907, or 47.9% of the total number of cells (576 in the controls, or 36.6%). There was no transgression of the indices obtained in the experimental and control series. We concluded from these findings that the lipopolysaccharides, when injected in a dose of 100 μg, undoubtedly affected the mitotic activity of the nonparenchymatous cells of the regenerating liver, but the maximum of this activity lay, not on the 4th day, but sooner (Fig. 2).

To discover the cause of the increase in weight of the regenerating liver after injection of lipopolysaccharides in a dose of 100 μg, we counted the number of liver cells in a field of vision. No statistically significant difference could be found between the results obtained in the experimental and control series. Administration of lipopolysaccharides likewise had no effect on the number of bi- and trinuclear cells in the regenerating liver. After administration of the preparation in a dose of 10μg, the percentage of binuclear cells in the regenerating liver (on the 3rd day after resection) had a mean value of 10.1, compared with 9.9 in the controls, and after administration of lipopolysaccharides in a dose of 100μg (4th day of regeneration) the corresponding values were 19.2 and 17.5.

The results show that liposaccharides from tea leaves, when administered in a dose of 100 μg on the 2nd day after operation, have a stimulant action on regeneration of the liver. This effect is shown by the increase in weight of the regenerating liver and by the increased intensity of proliferation of both parenchymatous and reticulo-endothelial cells. The nonparenchymatous elements of the liver undergo the most marked changes.

In contrast to bacterial lipopolysaccharides, those from tea leaves give rise to no toxic manifestations, and indeed have a beneficial action on the organism, as shown, in particular, by the stabilization or even increase in the body weight of the animal undergoing operation.

LITERATURE CITED

1. B. N. Gladyshev. In book: Abstracts of Sectional Proceedings of the 5th International Biochemical Congress [in Russian], Moscow, 15, 24, 98 (1962).
2. B. N. Gladyshev. Doklady Akad. Nauk SSSR, 145, 4, 929 (1962).
3. B. N. Gladyshev. Biokhimiya, 2, 210 (1963).
4. V. A. Sondak, E. P. Gracheva, B. N. Gladyshev, et al., Doklady Akad. Nauk SSSR, 146, 4, 925 (1962).
5. H. Fricke, V. Probst, and G. Schumacher, Schweiz. med. Wschr., 88, 691 (1958).
6. I. Fromme, O. Lüderitz, and A. Nowotny. In book: Pharm. acta helv., 33, 391 (1958).
7. H. Göing, Klin. Wschr., 38, 1069 (1960).
8. O. Westphal, Ann. Inst. Pasteur, 98, 789 (1960).