USE OF PRODIGIOSAN TO ISOLATE EXTRACTS STIMULATING PROLIFERATION IN THE RESECTED LIVER

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Macrophages (Mph) are involved in proliferative processes taking place in the resected liver [3, 6]. The lipopolysaccharide prodigiosan, which increases the number of Kupffer cells and advances the time of appearance of the peak of mitoses in the hepatocytes of rats after resection of the liver, is an exogenous activator of Mph [4]. It was shown previously that ischemia of the liver, resection of 70% of the liver, and in vitro culture of liver explants give rise to the formation of proliferation-stimulating factors (PSF) [1, 2]. However, the possibility of using prodigiosan as a stimulator of Mph in order to obtain extracts containing PSF (PSE) in the intact and resected liver has not yet been studied. The investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on 126 noninbred male rats weighing 160-180 g. In Part I of the investigation activity of proliferation in the liver was studied 24 and 48 h after resection of 70% of the organ or after injection of prodigiosan, and also after a combination of 70% resection with simultaneous injection of prodigiosan. Prodigiosan was injected intraperitoneally in a dose of 0.25 mg/kg. Intact animals served as the control. In Part II of the investigation, after the same procedures and at the same times, PSE were obtained from the liver and their activity studied on a model of resection of 30% of the liver. The PSE were injected simultaneously with resection, and the effect of the PSE was evaluated 24 h after their injection. Animals with resection of 30% of the liver and not receiving PSE served as the control. Resection of the liver was carried out under ether anesthesia by removal of the central and left lobes in the experiments with 70% resection, and of the left lobe of the liver in the experiments with 30% resection. PSE were obtained by the method in [5] in the modification in [1], in supernatant obtained after centrifugation of a liver homogenate, heated to 100°C for 15 min, at 23,000*g* for 20 min. The PSE were kept at −20°C. The preparation was injected intraperitoneally in a volume of 5 ml immediately after the end of resection. The protein concentration in the preparation was 7 mg/ml.

Activity of proliferative processes of the liver in both parts was assessed by measuring incorporation of ^{3}H-thymidine into DNA of the liver cell nuclei. The ^{3}H-thymidine was injected intraperitoneally into the animals 2 h before decapitation, in a dose of 0.5 μCi/g body weight. Methods of isolating the nuclei and determining the content and radioactivity of DNA were described by the writers previously [1, 2].

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TABLE 1. Specific Radioactivity of DNA (cpm/mg) of Nuclear Fraction of Rat Liver Cells (M ± m)

Series	Experimental conditions	Time of investigation, h	Part I (after same procedure)	Part II (after injection of PSE into rats with 30% resection of liver)	p_3
1a	Intact rats (control I)		10 207±966	12 337±888	>0,05
1b	30 % Resection of liver (control II)	24	17 443±645*	—	—
2	70 % Resection of liver	24	16 663±371**	38 428±1425**	<0,01
		48	14 290±481**	35 733±1528**	<0,01
3	Injection of prodigiosan into intact rats	24	10 712±389 p_1<0,01	44 619±904** p_1<0,01	<0,01
		48	8 805±643 p_1<0,01	32 377±980**	<0,01
4	70 % Resection of liver + prodigiosan	24	17 461±375** p_2<0,01	47 909±811** p_1, p_2<0,01	<0,01
		48	12 990±358** p_2<0,01	26 624±563** $p_{1,2}$<0,05	<0,01

Legend. *p < 0.01 – Significance of differences between controls of Parts I and II; **p < 0.01 – significance of differences between experimental series and control, p_1) significance of differences between series 3 and 4, and series 2; p_2) significance of differences between series 4 and 3; p_3) significance of differences between corresponding series of Parts I and II. Each series consisted of 6-12 rats.

EXPERIMENTAL RESULTS

Analysis of the results of Part I of the investigation (Table 1, Part I), showed that 30% (series 1b) and 70% resection of the liver (series 2) led to significant intensification of DNA biosynthesis in the nuclear fraction of the liver cells. After injection of prodigiosan into the intact animals (series 3) no stimulation of DNA biosynthesis could be found. Injection of prodigiosan together with resection of the liver (series 4) likewise did not increase the stimulating effect of resection on DNA biosynthesis. Thus prodigiosan, when injected into intact animals or into animals simultaneously with 70% resection of the liver, did not stimulate proliferative processes in the liver, recorded as incorporation of ^{3}H-thymidine into DNA of the nuclear fraction of the cells. Stimulation of proliferation in the liver after 70% resection in series 2 and 4 was more marked than after 48 h.

In Part II of the investigation, assessment of activity of PSE obtained 24 and 48 h after the same procedures showed (Table 1, Part II) that the PSE obtained from the liver of intact animals (series la) did not possess a stimulating action on DNA biosynthesis, whereas PSE obtained from animals with 70% resection (series 2) had a marked stimulating effect on DNA biosynthesis in the liver, in agreement with our previous data [1, 2].

PSE isolated from the liver of rats in experiments with injection of prodigiosan into intact animals (series 3) also had a marked stimulating effect on DNA biosynthesis in the liver cell nuclei. Incorporation of ^{3}H-thymidine into DNA of the cell nuclei in these experiments after 24 and 48 h was 258 and 182% respectively of the level in the control animals (30% resection); after 48 h it did not differ from, but after 24 h it was significantly higher than the value obtained in the experiments with injection of PSE obtained from resected rats. PSE obtained from the liver of animals after the combined procedure (series 4) likewise had a marked DNA stimulating action, and after 24 h the effect of prodigiosan injection and 70% resection was added to it.

These results are evidence that administration of prodigiosan under these experimental conditions did not activate proliferative processes in the intact liver and did not intensify proliferation in the rat liver after 70% resection. Meanwhile prodigiosan induces the formation of active PSE in both intact and resected liver, injection of which into animals with 30% resection of the liver caused marked stimulation of proliferation; more active PSF were formed if prodigiosan was injected after 24 h.

The question of the mechanisms of the effect of prodigiosan on proliferative processes is not absolutely clear. It may perhaps be realized due to the fact that activated MpH secrete lysosomal proteases [9], glycosidases [10], collagenase [7], and also substances of nonenzymic nature (in particular, prostaglandins E [6], which participate in proliferative process).

This effect was not found in vivo (Part I of the investigation), in all probability because of the presence or activation of inhibitors of proliferation. Heating the homogenate during preparation of the PSE (Part II) may perhaps inactivate the inhibitors, thereby leading to absence of the direct but presence of the indirect effect of Prodigiosan.

The results thus show that a single injection of prodigiosan into intact thermostable factors stimulating proliferation in the liver. This method of obtaining animals can be used to obtain biologically active PSF without surgical intervention means that the livers of cattle, intended for slaughter, can be used, thereby greatly simplifying the task of obtaining PSF in large quantities.

The further study of this problem likewise is important for revealing the mechanisms of involvement of macrophages in proliferative processes [11].

REFERENCES

1. O. Yu. Abakumova, N. G. Kutsenko, S. R. Karagyulyan, et al., Vopr. Med. Khim., No. 69 (1989).
2. M. V. Bilenko, L. A. Seregina, P. G. Komarov, and O. Yu. Abakumova, Vopr. Med. Khim., No. 2, 83 (1989).
3. D. N. Mayanskii and V. I. Shcherbakov, Byull. Éksp. Biol. Med., No. 9, 69 (1978).
4. D. N. Mayanskii, Patol. Fiziol., No. 4, 80 (1985).
5. P. Davies, R. Bonney, J. Humes, and F. Kuchl, J. Invest. Derm., **74**, 292 (1980).
6. B. Fisher, M. C. Gebhardt, E. A. Saffer, and E. R. Fisher, Cancer Res., **39**, 1361 (1979).
7. K. Fujiwara, T. Sakai, and T. Oda, Biochem. Biophys. Res. Commun., **54**, 531 (1973).
8. W. J. Johnson, S. V. Pizzo, M. J. Imber, and D. O. Adams, Science, **218**, 574 (1982).
9. D. R. La Brecque and A. Pesch, J. Physiol. (London), **248**, 273 (1975).
10. J. Schyder and M. Baggiolini, J. Exp. Med., **148**, 435 (1978)
11. S. Shimaoka, T. Nakamura, and A. Ichihara, Exp. Cell Res., **172**, No 1, 228 (1987).