EFFECT OF HOMOLOGOUS HEPATIC RNA ON LIVER COLLAGEN AND TRYPTOPHAN PYRROLASE IN RATS POISONED WITH CCl_4

A. M. Chernukh, E. D. Vyshepan, T. F. Shukalova, N. N. Alekseeva, and I. L. Razumova

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Fresh cytoplasmic RNA from the liver of rats was injected into rats during chronic CCl_4 poisoning and in the period of regeneration after poisoning for 2 months. In the second case, after the end of CCl_4 poisoning, administration of RNA reduced the collagen content and restored the normal tryptophan pyrrolase activity in the liver. RNA had no effect if administered simultaneously with CCl_4.

A previous investigation [5] showed that injection of heterologous (rat) hepatic RNA into mice during CCl_4 poisoning prevented death of the animals, but at the same time accelerated connective tissue development in the liver.

In view of reports in the literature of differences between the actions of heterologous and homologous RNA in regenerative processes [1], it was decided to investigate the action of homologous RNA on the liver during CCl_4 poisoning. Besides biochemical and histological investigation of connective tissue proliferation, the activity of tryptophan pyrrolase (TP) was determined. The activity of this enzyme is a good index of the state of the protein-synthesizing system of the liver cells during CCl_4 poisoning [9].

EXPERIMENTAL METHOD

Male Wistar rats were used. In all experiments CCl_4 was injected subcutaneously in a dose of 0.12 ml/100 g body weight, and RNA was injected intraperitoneally in a dose of 0.5 mg/100 g body weight.

Cytoplasmic RNA was isolated from the liver of rats which had previously received an injection of hydrocortisone to increase the content of messenger RNAs for the synthesis of a number of enzymes, including TP, in their liver [11, 12]. The pattern of administration of CCl_4 and RNA was varied, for according to information in the literature [4, 10], the fate of exogenous RNA in the cell after administration of CCl_4 may differ depending on the liberation of nucleases by the action of the poison.

In the experiments of series I, rats weighing 80-100 g received injections of CCl_4 twice a week for 2 months. On the day after each injection of CCl_4 the control rats were injected with physiological saline and the experimental rats with RNA.

In the experiments of series II, rats weighing 130-140 g were injected with CCl_4 only for 2 months, after which, when injections of CCl_4 had ceased, for the next 3 weeks they were injected twice a week either with physiological saline (control rats), or with RNA (experimental rats).

In the experiments of series III, by contrast with II, the administration of CCl_4 was continued during the 3 weeks when physiological saline or RNA was being injected.

Institute of Normal and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow.
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TABLE 1. Results of Injection of RNA, Depending on Conditions of CCl_4 Administration

Series of experiment	Animals	No. of animals	Collagen content (in % of normal)	Histological observations	TP activity (in % of activity)
I	Control	3	257 p = 0.001	Well-marked proliferation of connective tissue	75 p = 0.02
	Experimental	6	228 P > 0.05	No difference from control apparent	70 P > 0.05
II	Control	5	123 p = 0.001	Well-marked proliferation of connective tissue	76 p = 0.001
	Experimental	5	93 P = 0.02	Proliferation of connective tissue only slight (mainly around blood vessels)	94 P = 0.001
III	Control	5	183 p = 0.001	Well-marked proliferation of connective tissue	80 p = 0.02
	Experimental	5	191 P > 0.05	No difference from control apparent	74 P > 0.05

Note. P – compared with control animals; p – compared with intact animals.

In each series of experiments four intact rats also were used in order to determine the levels of the relevant indices in normal animals. Because of diurnal fluctuations in TP activity, it was thought advisable to sacrifice the rats at intervals of not more than 10 min, alternating the animals of the different groups: intact, control, experimental, and so on.

RNA was obtained by Georgiev's method [2] at zero temperature from the liver of male Wistar rats which had received an injection of 3 mg hydrocortisone/100 kg body weight 2 h previously. The preparation, precipitated by alcohol at −20°C, was dissolved in physiological saline and used on the same day.

Insoluble collagen in the fat-free dry tissue after extraction with hot TCA was estimated as hydroxyproline [8].

TP activity was determined in the whole homogenate by Knox's method [6].

In intact rats the following values were obtained for these indices: collagen content, 0.43%, TP activity 2 μmoles kynurenin (in g/h).

Pieces of tissue for histological investigation were fixed in 12% formalin and stained by Van Gieson's method.

EXPERIMENTAL RESULTS AND DISCUSSION

Injection of homologous RNA on the day after injection of CCl_4 (for 2 months) did not affect either the collagen content or the TP activity. Injection of RNA likewise did not prevent or delay the development of the pathological process. However, unlike the injection of rat RNA into mice [5], injection of this RNA into rats did not increase the already high collagen content.

In the experiments of series II, 3 weeks after the end of CCl_4 administration the collagen content in the control animals was raised, but the TP activity still remained low. Administration of RNA during regeneration significantly reduced the collagen content (and the proliferation of the connective tissue correspondingly) in the liver, while at the same time it increased the TP activity. According to reports in the literature [9], the activity of this enzyme in liver damaged by CCl_4 is reduced sooner and restored to normal later than the activity of many other enzymes. It is thus evident that administration of homologous RNA in the experiments of series II speeded up the regeneration of the liver.

In the experiments of series III, administration of RNA during continuing poisoning of the animals had no effect on any of the indices studied. The impression is obtained that, if RNA was injected 24 h after injection of the CCl_4, it appears to lose its ability to influence liver metabolism.

Judging from the literature [4], lysosomal enzymes are liberated about 8 h after intraperitoneal injection of CCl_4, while necrosis of the liver cells begins after 18 h. With the passage of time, activity of the

free nucleases was evidently reduced, so that RNA, if injected during the period of regeneration (as was the case in the experiments of series II), could function in the polymer state.

Since in experiments on mice [5], rat RNA was injected 1-2 h after subcutaneous injection of CCl_4, it could also have functioned for some time without undergoing degradation. The positive effect of heterologous RNA on the survival rate of the mice in these experiments agrees with observations on the effect of RNA from cow's liver on regeneration of the rat liver after partial hepatectomy. However, it still remains unexplained why, in the present investigations, injection of rat RNA in the polymer form into rats in the period of regeneration of the liver caused a decrease in the collagen content of that organ, while its injection into mice, on the other hand, led to an increase in the collagen content. One possible explanation would be the differences between the effects of CCl_4 on different animals, because it is known that although CCl_4, for example, is carcinogenic to mice, it is not carcinogenic to rats. Another possibility is that heterologous RNA stimulates only collagen synthesis in mice, and the resulting more rapid replacement of necrotic liver tissue by connective tissue reduces the severity of the toxic effects at the stage of poisoning at which most of the mice die. According to new evidence [7], during CCl_4 poisoning in rats the collagenolytic activity of their liver tissue relative to insoluble collagen is reduced, while during regeneration this activity is restored. It is possible that the decrease in the collagen content observed in the experiments of series II was related to the more rapid synthesis of the collagenolytic enzyme.

By destroying the endoplasmic reticulum, CCl_4 separates the messenger RNA from the polysomes. Free RNA is rapidly degraded, and the protein-synthesizing ability of the ribosomes can be restored by binding other RNAs to them [4, 10, 13]. Preliminary injection of CCl_4 can thus lead to an exchange of information for protein synthesis. This could therefore be why the effects of exogenous RNA could be observed in the period of regeneration of the liver after poisoning with CCl_4, whereas numerous attempts to obtain this effect in intact animals and after poisoning with actinomycin D were unsuccessful.

The writers consider that the mechanism of action of homologous hepatic RNA during regeneration of the liver may be identical with the mechanism of action of preparations of bone RNA during regeneration of bones [1]. The work of A. M. Belous et al. has shown that cytoplasmic RNA can stimulate protein synthesis and the synthesis of the characteristic cellular messenger RNA, although to a lesser degree than nuclear messenger RNA. Such stimulation of the synthesis of messenger RNAs after partial hepatectomy may be the explanation of the observation made by Sarkisov and Rubetskii [3] that this operation can lead to regression of the cirrhosis of the liver after CCl_4 poisoning.

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