EFFECT OF CYTIDINE AND URIDINE ON REGENERATION OF THE LIVER IN RATS POISONED WITH CARBON TETRACHLORIDE

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The effect of uridine and cytidine on the course of repair processes in the liver of rats with experimental hepatitis due to CCl_4 was studied. Injection of uridine or cytidine simultaneously with CCl_4 over a period of 7 days did not prevent damage to the liver by the poison. Further treatment with the nucleosides (up to 15 and 20 days) accelerated, although to different degrees, the course of repair processes after discontinuation of CCl_4. Cytidine, for instance, caused marked hypertrophy of regenerating hepatocytes, combined with proliferation of mesenchymal cells, which, however, was not accompanied by restoration of the conjugating and excretory functions of the liver. Unlike cytidine, uridine led to more rapid normalization of the above-mentioned functions, although restoration of the structure of the organ in this case was less complete.

KEY WORDS: liver; regeneration; cytidine; uridine.

The effect of pyrimidine nucleosides on the course of repair processes, including those associated with liver damage, is an increasingly popular subject for investigation. It has been shown, in particular, that uridine has a marked prophylactic and therapeutic effect in experimental D-galactosamine hepatitis [8-10, 12, 14]. A combination of cytidine, uridine, malate, and arginine has been used with success in the clinical treatment of acute and chronic hepatitis [7, 13].

The object of the present investigation was to study the effect of uridine and cytidine on the dynamics of restoration of the structure and certain functions of the liver in CCl_4-induced hepatitis.

EXPERIMENTAL METHOD

Experiments were carried out on 156 male rats weighing 120-160 g. Carbon tetrachloride was injected subcutaneously (0.2 ml of a 50% oily solution/100 g body weight) daily for 7 days. Uridine or cytidine was injected subcutaneously in doses of 51 and 27 mg/kg respectively (0.01 LD_{50}) for 7, 15, and 20 days from the beginning of the experiment. Animals receiving subcutaneous injections of CCl_4 and of 0.85% NaCl solution and also intact rats (receiving 0.85% NaCl solution alone, served as control. The excretory and conjugating functions of the liver were tested 48 h after the end of treatment by determining the rate of decrease of the plasma concentration of intravenously injected bromsulfthalein [4]. The animals were then killed and pieces of liver were fixed in 10% neutral formalin and Carnoy's fluid. Sections were stained with hematoxylin-eosin. Glycogen was detected by the PAS reaction with amylase control, RNA by staining by Brachet's method with RNase control, and neutral lipids by staining frozen sections with a mixture of Sudans III and IV. The intensity of liver damage and the dimensions of the hepatocytes and their nuclei were determined by a dot counting method [1, 3].

EXPERIMENTAL RESULTS

At short times after the beginning of the experiments (9th day) no difference was observed in the character or degree of liver damage in the animals of the control and the experimental groups. Centrilobular foci of

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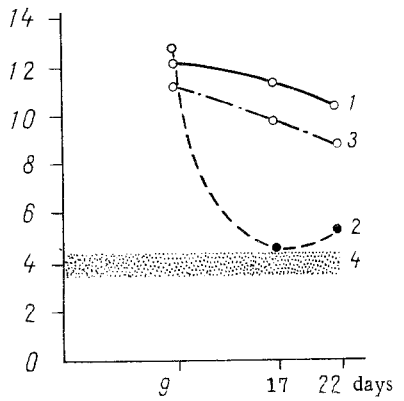

Fig. 1. Plasma bromsulfthalein concentrations in control and experimental rats 15 min after intravenous injection of the dye (50 mg/kg). Abscissa, time of investigation (in days); ordinate, bromsulfthalein concentration (in mg%). 1) CCl_4 + 0.85% NaCl solution; 2) CCl_4 + uridine; 3) CCl_4 + cytidine; 4) 0.85% NaCl solution. Filled circles denote significant differences between results and those for control (CCl_4 + 0.85% NaCl), empty circles the same compared with results for intact animals (0.85% NaCl solution).

necrosis and infiltration by large droplets of fat in the mediolobular zones affected up to 45 and 60% of the lobules. Glycogen was absent in the liver, no RNA could be detected in the affected hepatocytes, but in the hepatocytes of the periportal zones the RNA content was sharply reduced compared with that in the liver of intact animals. During this period the conjugating and excretory functions of the livers of the experimental rats (treated and untreated) were sharply reduced, on average by more than two-thirds compared with those in intact animals (Fig. 1).

Hence neither uridine nor cytidine, if injected simultaneously with CCl_4, protected against liver damage produced by the poison.

By the 17th day signs of regeneration of the parenchyma were already visible, mainly in the centers of the lobules, where groups of large hepatocytes with basophilic cytoplasm appeared. Starting from the 17th day differences were clearly observed in the indices studied in the different groups of animals. For instance, in the control animals a considerable proportion of the hepatocytes at this time were still in a state of granular, vacuolar, or balloon degeneration, with small foci of necrobiosis and necrosis present. This was reflected in an increase in the mean volume, a considerable decrease in the nucleocytoplasmic ratio, and a decrease in the percentage of binuclear hepatocytes, compared with intact rats (Table 1).

In the rats receiving uridine the degenerative changes were much less marked than in the control animals, and this was confirmed by a significant decrease in the mean volume of the hepatocytes and an increase in the nucleocytoplasmic ratio (Table 1). The glycogen and RNA content in the regeneration hepatocytes also was higher than in the control, but was not yet back to normal.

On the 22nd day differences in the liver morphology in the different groups of animals were more pronounced still. In the control rats, despite the undoubted regression of the degenerative changes in the hepatocytes, accompanied by a decrease in their mean volume and an increase in the nucleocytoplasmic ratio, and despite some stimulation of regeneration (for example, an increase in the number of binuclear cells), the RNA and glycogen content in the hepatocytes remained low and, on the whole, the morphological picture of the liver still showed evidence of severe damage (Fig. 2A).

In the animals receiving uridine, by the 22nd day the structure of the liver was much closer to normal, although in the mediolobular and peripheral zones of the lobules there were still many hepatocytes in a state of moderately severe vacuolar or granular degeneration (Fig. 2B). Whereas in the control the increase in the nucleocytoplasmic ratio of the hepatocytes took place purely on account of a decrease in the volume of their

TABLE 1. Results of Morphometric Investigation of Liver in Rats with Hepatitis Induced by CCl_4 (control and experiment) and in Intact Rats (M ± m)

Indices	Groups of animals						
	0.85% NaCl solution	CCl_4 + 0.85% NaCl solu.		CCl_4 + uridine		CCl_4 + cytidine	
		time of investigation, days					
		17th	22nd	17th	22nd	17th	22nd
Volume of hepatocyte	100±8,78	214,1±12,99	162,4±10,52	172,7±11,36	181,4±10,54	175,3±10,42	127,0±5,49
P_i		<0,001	<0,001	<0,001	<0,001	<0,001	<0,01
P_c				<0,05	>0,1	<0,05	<0,01
Volume of hepatocyte nucleus	100±5,83	134,2±10,52	131,0±4,88	141,6±4,08	157,9±10,08	153,4±6,89	156,3±10,27
P_i		<0,01	<0,001	<0,001	<0,001	<0,001	<0,001
P_c				>0,5	<0,02	>0,1	<0,05
Nucleocytoplasmic ratio	0,101±0,005	0,060±0,004	0,079±0,003	0,081±0,007	0,080±0,004	0,087±0,008	0,124±0,004
P_i		<0,001	<0,001	<0,05	<0,01	>0,1	<0,002
P_c				<0,01	>0,5	<0,01	<0,001
Binuclear hepatocytes, %	21,8±2,00	13,1±0,72	15,9±1,91	12,0±1,96	23,9±3,00	25,1±2,75	30,7±1,58
P_i		<0,002	<0,05	<0,01	>0,5	>0,25	<0,002
P_c				>0,5	<0,05	<0,01	<0,001

Legend. P_i and P_c) significance of differences relative to intact and control rats respectively.

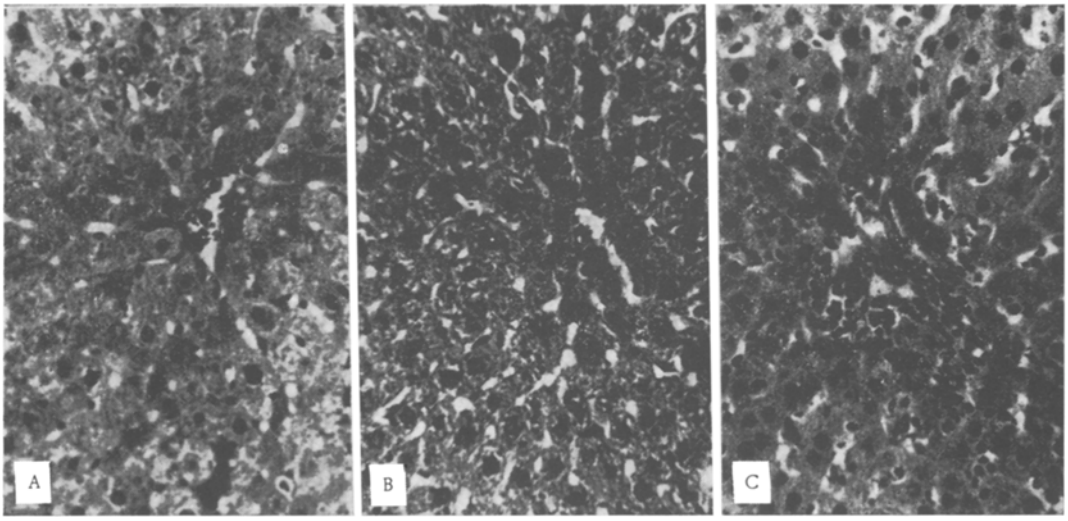

Fig. 2. CCl_4-induced hepatitis in rats on 22nd day after beginning of experiment (A – control, B – uridine, C – cytidine). Explanation in text. Hematoxylin-eosin, 200×.

cytoplasm, the mean volume of the nuclei remaining stable, in the rats receiving uridine a proportional increase was observed in the mean volume of both nuclei and cytoplasm, and combined with normalization of the structure of the hepatocytes and of the number of binuclear cells and with a further increase in their RNA and glycogen content, must be regarded as evidence of their intracellular regeneration. The conjugating and excretory functions of the liver in animals receiving uridine returned to normal on the 17th and 22nd days of the experiment respectively, whereas in the control animals they were still at the same low level as on the 9th day.

Somewhat different results were obtained with rats receiving cytidine. Although on the 17th day a few degenerated hepatocytes were still present in the periportal zones of these animals, on the whole recovery of the structure of their liver was more complete than in the rats receiving uridine. Because of the greater increase in the mean volume of the nuclei in these animals than in the latter, the nucleocytoplasmic ratio was closer to normal and the number of binuclear hepatocytes was actually above normal. The properties of this group were revealed more clearly still on the 22nd day, when together with eradication of the last evidence of degeneration of the hepatocytes a marked proliferative reaction of the connective-tissue cells was observed for the first time (Fig. 2C). Although the mean volume of the hepatocytes in the "cytidine" rats at this time was significantly smaller than in the control and the "uridine" rats it was nevertheless 27% higher than in the

intact animals. In the absence of degenerative changes and with a high (0.124) nucleocytoplasmic ratio [2], this index could serve as a measure of hypertrophy of the hepatocytes. Evidence of activity of repair processes and the intensity of plastic activity of the parenchymatous cells also was given by the very high RNA and glycogen levels in their cytoplasm and the increased (almost twofold) number of binuclear hepatocytes. It is evidently along these lines that the proliferation of the mesenchymal cells noted above must be evaluated, namely, as a necessary accompanying reaction when there is intensive regeneration of the parenchyma. This view is in agreement with data of Mayanskii et al. [5], who showed that Kupffer cell blockade inhibits regeneration of the liver after its resection.

Of the two nucleosides tested, cytidine thus has the stronger action by stimulating regeneration of the damaged liver. The results of the function test were therefore all the more unexpected, for in the "cytidine" animals it was just as low as in the control rats on both the 17th and the 22nd days (Fig. 1). Thus, at first sight, surprising disparity between the structure and function of the regenerating liver in the "cytidine" rats can evidently be regarded as further evidence in support of Wilson and Spelsberg's hypothesis [15], developed further by Uryvaeva and Faktor [6], according to which any stimulation of regenerative (proliferative) processes in the liver leads to depression of its specific functions on account of the switching of cell metabolism to provision for growth. The possibility likewise cannot be ruled out that the rapid recovery of the conjugating function of the liver in "uridine" rats was due not only to the early maturation of the regenerating hepatocytes, but also to the direct participation of uridine, in the composition of UDP-glucuronate, in bromsulfthalein binding [11].

Hence, neither uridine nor cytidine, in the doses used, had any protective effect in experimental CCl_4 hepatitis. However, both nucleosides clearly, although differently, stimulated repair processes in the damaged liver. The more rapid recovery of the structure and hypertrophy of the cells under the influence of cytidine were not, however, accompanied by recovery of the conjugating and excretory functions of the organ, possibly on account of antagonism between syntheses maintaining growth and the specific functions of the liver tissue. Unlike cytidine, uridine leads to the more rapid normalization of liver function, although restoration of the structure of the organ after treatment with uridine appeared less complete.

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