EFFECT OF GLUCOCORTICOIDS ON α-FETOPROTEIN SYNTHESIS AND LIVER STRUCTURE OF MICE WITH ACUTE CARBON TETRACHLORIDE POISONING

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UDC 615.917:547.412.133].015.4:612.35

Experiments on male C57BL mice showed that inhalation of CCl_4 for 15 min in a concentration of 0.05 ml/4 liters air causes severe degenerative changes in the liver with features of necrobiosis. However, phenomena of regeneration and cell infiltration arise as early as after 24 h and are accompanied by the secretion of α-fetoprotein into the blood stream. After 4 days the foci of necrobiosis disappear and are totally replaced by lymphocytes with large hepatocytes at the periphery. Processes of regeneration in animals receiving hydrocortisone (20 mg/kg) or dexamethasone (2 mg/kg) therapeutically or prophylactically were less marked and the number of animals producing α-fetoprotein was reduced from 92 to 60-65%.

KEY WORDS: *glucocorticoids*; *α-fetoprotein*; *liver*; *carbon tetrachloride*.

The hepatotoxic action of carbon tetrachloride (CCl_4) is accompanied in some animals by the appearance of α-fetoprotein in the blood [2]. This protein is also found in the blood serum of adult animals and human patients with hepatoma [1, 6, 8]. Administration of glucocorticoids, corticotropin, or sex hormones lowers, whereas thyroid extract raises the level of this protein in the blood serum of newborn rats [5].

The object of this investigation was to study the possible effect of hydrocortisone and dexamethasone on α-fetoprotein production and on the structure of the liver of adult mice poisoned with CCl_4.

EXPERIMENTAL METHOD

Experiments were carried out on male C57BL mice weighing 18-23 g and divided into 5 groups: group 1) control, group 2) mice receiving a subcutaneous injection of hydrocortisone (20 mg/kg) 24 and 2 h before exposure to CCl_4 and thereafter once a day, group 3) mice receiving hydrocortisone in the same doses 10 min after exposure to CCl_4 and daily thereafter; the mice of groups 4 and 5 received dexamethasone (2 mg/kg) subcutaneously: group 4) by the scheme of group 2, and group 5) by the scheme of group 3. To produce CCl_4 poisoning the mice were kept for 15 min in a closed 4-liter exsiccator on the floor of which 0.05 ml CCl_4 was introduced. The serum α-fetoprotein level of each mouse was determined 1, 2, 3, 4, 6, and 8 days later by an immunochemical agar-diffusion method with the aid of a monospecific test system [1, 10]. The mice were killed 5 at a time, after 1, 2, 3, 4, 6, and 8 days in groups 1 and 2 and after 4 days in groups 3-5. The liver tissue was cut into sections in a cryostat and embedded in celloidin. The sections were stained with hematoxylin–eosin and Sudan III, the PAS reaction (with amylase control) and reaction for alkaline and acid phosphatase and esterase were carried out, and sections were impregnated by Foot's method.

EXPERIMENTAL RESULTS

No α-fetoprotein could be found in the blood serum of the animals 24 h after CCl_4 poisoning. It appeared in some animals after 48 h, and its production reached a maximum in the control group after 3-4 days. By 6-8 days α-fetoprotein synthesis had completely ceased.

Department of Pharmacology, Astrakhan Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 2, pp. 151-153, February, 1977. Original article submitted April 14, 1976.

TABLE 1. Production of α-Fetoprotein in Mice Poisoned with CCl_4 and Treated with Hydrocortisone and Dexamethasone

Group of animals	No. of animals in group	No. of mice producing α-fetoprotein on 3rd-4th day		P
		abs.	%	
1	30	28	92	
2	30	18	60	<0,01
3	10	6	60	<0,05
4	20	13	65	<0,05
5	20	12	60	<0,01

Multiple small foci of necrosis mainly around the central veins were found 24 h after poisoning in the liver of the control group of mice. At the periphery of the foci of necrosis a marked cellular response consisting of polymorphs mixed with a few lymphocytes and plasma cells was observed. Diffuse fatty infiltration of the cytoplasm of the hepatocytes and an almost total disappearance of glycogen were revealed. Alkaline phosphatase was absent in the sinusoids but was found in the cytoplasm of the hepatocytes. During the next 2 days the intensity of cellular infiltration increased at the site of the necrotic foci, with the appearance of large hepatocytes with a pale cytoplasm and hyperchromic nucleus, or sometimes with two nuclei, at the periphery. The fatty degeneration persisted throughout. The glycogen content was increased outside the foci of necrosis, especially in the large and binucleate hepatocytes. Alkaline phosphatase began to appear in the sinusoids. After the 4th day the foci of necrobiosis could no longer be seen and clusters of lymphocytes appeared, surrounded by groups of binucleate and large hepatocytes with hyperchromic nuclei. Fatty infiltration of the cytoplasm and a high content of glycogen were observed in individual hepatocytes. Activity of alkaline and acid phosphatases and of nonspecific esterase was normal. On the 6th-8th day the sites of the former foci of necrosis were marked only by nodules of infiltrating lymphocytes. Virtually no fatty degeneration could be seen. The content of glycogen and enzymes was within normal limits.

After administration of hydrocortisone and dexamethasone to the experimental animals, the number of animals producing α-fetoprotein during CCl_4 poisoning was statistically significantly reduced (Table 1).

In the liver of mice with CCl_4 poisoning treated with hydrocortisone and dexamethasone the development of the necrotic foci and reactive changes was somewhat delayed and normalization of the structure took place later. For instance, on the 4th day in mice receiving hydrocortisone foci of necrobiosis and diffuse fatty degeneration of the hepatocytes were still present. Massive necrotic areas with a well marked cellular response and evidence of fatty degeneration were observed at the same time in the mice receiving dexamethasone. Evidence of regeneration was less intensive, especially in animals with no α-fetoprotein.

Poisoning of mice with CCl_4 thus causes disturbances of the protein, lipid, and carbohydrate functions of the liver accompanied by severe cellular degeneration, and necrosis of hepatocytes; these changes are accompanied after the first day by regenerative changes in the liver in agreement with data in the literature [7]. The appearance of α-fetoprotein in the blood serum is an indicator of these processes [3, 6]. The decrease in α-fetoprotein production during the action of glucocorticoids may suggest the inhibitory effect of these hormones on regeneration. Glucocorticoids also were found to have a weakening or "delaying" effect on the processes of cellular infiltration, and this evidently is responsible for increasing the toxicity of CCl_4. Intensification of the harmful action of CCl_4 on the liver by glucocorticoids has also been observed by other workers [4, 9]. The question of the effect of glucocorticoids in toxic liver lesions is of practical interest, for they are widely used in clinical practice for the treatment of hepatitis.

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ABSORPTION OF CARBOHYDRATES AND FATS IN AN ALLOGENEIC GRAFT OF THE SMALL INTESTINE

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UDC 616.341-089.843-092.9-07: 616.341-008.6-072.7

The results of a study of the absorptive function of an allogeneic graft of the small intestine, using D-xylose loading and electron-microscopic investigation of neutral fat transport in the intestinal wall, are described. An unusual increase in the absorption of D-xylose was shown to take place in association with structural changes in the wall of the intestine and disturbance of fat transport.

KEY WORDS: *transplantation; absorption; electron microscopy.*

Several attempts have now been made outside the USSR to perform allogeneic transplantation of the small intestine in man [3, 6, 10, 12]. The unsuccessful results of these operations have shown the importance of the study of the functional state of the graft. In particular, the absorptive function of an allogeneic intestinal graft has received very little study and such investigations as have been made have been mainly on an isolated segment of the small intestine [4, 5, 7, 8, 11].

The object of this investigation was to study the absorption of carbohydrates and fats in dogs after total allotransplantation of the small intestine.

EXPERIMENTAL METHOD

Functional and morphological investigations were carried out on 25 mongrel dogs with an allogeneic small intestine. The control consisted of 20 intact animals and 35 dogs with an autologous intestine (the results of these investigations were published previously [1]). The operation of total orthotopic transplantation of the small intestine was carried out by the generally accepted technique [2]. Loading with D-xylose (5 g in 300 ml water) was carried out after starvation for 12 h and emptying of the urinary bladder. In the course of 5 h, urine was collected from the dogs by catheterization of the bladder and the concentration of D-xylose in it was determined [13]. To study lipid transport the dogs were fed with sunflower oil at the rate of 5-6 ml/kg body weight. The animals were killed 1 h after loading and a portion of the graft was removed 5-10 cm distally to the proximal anastomosis for histological (staining with hematoxylin–eosin and Sudan III) and electron-microscopic investigation.

EXPERIMENTAL RESULTS AND DISCUSSION

Control tests showed that the quantity of D-xylose excreted with the urine of the intact

Central Research Institute of Gastroenterology and Department of Operative Surgery and Topographical Anatomy, Patrice Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. Kh. Vasilenko.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 2, pp. 153-156, February, 1977. Original article submitted June 21, 1976.