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BIOCHEMICAL SIGNS OF EARLY REJECTION OF LIVER ALLOGRAFTS IN DOGS

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Biochemical alterations during early rejection of allogeneic liver transplants were studied in 17 dogs. The animals were divided into 3 groups: 1) orthotopic liver transplantation with immunosuppressive therapy, 2) orthotopic liver transplantation without immunosuppressive therapy, 3) immunosuppressive therapy as in group 1, but no transplantation. Results of biochemical studies indicate that the most typical sign of early rejection of the liver is an increase in serum biblirubin level. High serum bilirubin points to advanced and usually irreversible alterations in the liver parenchyma. Efforts to mitigate the rejection process with immunosuppressive drugs thus far are usually ineffective.

Preparations for clinical transplantation of the liver have been carried out in our laboratory for the past two years. They included studies on liver preservation, early diagnosis of transplant rejection and immunosuppressive therapy. The purpose of the present communication has been to follow the biochemical alterations developing in the recipient of a liver allograft during the early phase of rejection.

METHODS

Experiments were carried out on 17 dogs divided into 3 groups. In group 1 an orthotopic liver transplant was performed in 9 dogs according to the technique described previously (Olszewski et al., 1973). The total ischemia time of the liver ranged between 45 and 80 min. The following immunosuppressive regimen was applied: days + 1 to + 3 immuran 4 mg/kg and hydrocortisone 4 mg/kg days + 4 to + 10 immuran 3 mg/kg and prednisone 1 mg/kg: day + 11 until the death of the recipient a chronic dose of immuran and prednisone 1 mg/kg each. In group 2 of 6 dogs, orthotopic liver transplantation was carried out with the same technique as in group 1, but no immunosuppressive therapy was given. In group 3 of 3 dogs, the animals were given immunosuppressive drugs according

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to the above described schedule, but there was no transplantation. The following biochemical tests were performed daily: serum bilirubin concentration, serum AspAT and AlAT activity, blood alkaline phosphatase activity, WBC and platelet count, and hematocrit. At autopsy special attention was paid to the patency of vascular and biliary anastomoses. Specimens of the transplanted liver and recipient's kidneys, lymph nodes, spleen, and lungs were taken for histology.

Table 1. Survival time, biochemical data, and cause of deaths of dogs in group 1

No. of dog	Survival time	Biochemical values at death				Cause of death
		Bilirubin mg%	AspAT u.	AlAT u.	Alkaline phosphatase	
379	6	6·1	504	1412	140	invagination
473	7	11·0	144	616	212	necrosis of the gall-bladder
423	8	13·6	458	1070	168	liver insufficiency
478	10	4·6	76	286	220	intestinal perforation
408	14	0·8	42	616	100	wound dehiscence
443	16	6·6	170	308	164	?
412	17	4·0	696	316	258	?
532	30	4·5	80	328	264	duodenal ulcer perforartion
657	48	4·8	112	164	160	liver insufficiency

RESULTS

Group 1. The survival time of liver recipients ranged between 6 and 48 days, on the average 17·5 days. Serum bilirubin concentration rose steadily (Table 1) parallel to the increase in alkaline phosphatase activity. The higher and more rapid was the increase in serum bilirubin concentration, the shorter was the survival time of the recipient. An increase in serum bilirubin level was found in 2 dogs already on the 1st day after transplantation, in 2 on the 2nd day, in 2 on the 6th day, in 1 on the 11th day, and in 2 on the 19th day. There were marked differences in the serum bilirubin concentration and time of its increase in spite of the same dosage of immunosuppressive drugs per kg of body weight.

Serum transaminase activity was high an the first two days after transplantation, reaching 1500 u. for AspAT and for AlAT, accounted for by mechanical and ischemic damage of the liver during transplantation. The increase in aminotransferase activity was transitory and did not parallel

the increase in serum bilirubin concentration. At the time of death of the animals it was low, except in two dogs with necrosis of a lobe of the liver.

The cause of death of recipients in 2 cases was coma, jaundice and cachexia, in another 2 perforation and bleeding from the duodenal ulcer, in others necrosis of the gallbladder, intestinal invegination and wound dehiscence. Two dogs died for unknown reasons. There was no bile in the biliary of the transplanted livers.

Table 2. Survival time, biochemical data, and causes of death of dogs in group 2 and 3

No. of dog	Survival time	Biochemical values at death				Cause of death
		Bilirubin mg%	AspAT u.	AlAT u.	Alkaline phosphatase	
			Group 2			
1	2	0·5	612	1248	254	liver insufficiency
2	3	0·2	47	116	164	liver insufficiency
3	6	5·5	560	1200	200	liver insufficiency
4	7	4·5	1150	1280	258	liver insufficiency
5	7	5·0	850	1020	258	liver insufficiency
			Group 3			
1	21	0·1	15	85	24	
2	21	0·1	83	490	30	
3	29	1·9	200	455	12	

Histologically, small scattered foci of necrosis were found in the liver panrenchyma in 7 of 9 dogs. In only 2 of 9 dogs, mononuclear cell infiltrates were seen in the portal areas. In two dogs there was evident bile stasis in bile canaliculi, and in 5 accumulation of bile pigment in the hepatocytes.

Group 2. The survival time of recipients ranged between 2 and 7 days, on the average 5 days (Table 2). From the 3rd day onward serum bilirubin concentration and alkaline phosphatase activity rose rapidly, accompanied by an increase in serum transaminase activity. All the dogs died in coma. At autopsy, enlarged friable, dark brown liver with no bile in the biliary tree was found in all cases. Histologically, necrosis of hepatocytes around the central vein and mononuclear infiltrates were found in the portal areas as typical signs of liver rejection.

Group 3. Serum bilirubin concentration and alkaline phosphatase activity remained normal throughout the whole period of study. There was a slight increases in serum aminotransferases activity, but it never

rose above 200 u. for AspAT and 500 for AlAT. At the autopsy performed on the 21st day of immunosuppressive therapy no gross changes in the liver were observed. Histologically, dissociation of liver trabeculae was observed, but there was no necrosis, infiltration or bile stasis.

DISCUSSION

Recognition of early rejection of liver allografts remains difficult for two reasons. Firstly, it is not known which of many liver functions is mostly affected by the rejection process. None of the presently known biochemical liver tests have proved useful for rejection recognition. Secondly, liver ischemia during preservation and transplantation is followed by more or less pronounced insufficiency of that organ. It makes impossible distinction between these two forms of insufficiency due to rejection and ischemia. The immediate posttransplantation period is characterized by increased serum aminotransferase activity, lactic dehydrogenase activity, and high hematocrit. Alkaline phosphatase activity and serum bilirubin levels remain normal. Biochemical changes due to initial liver ischemia usually subside within 2—3 days, unless ischemia was prolonged for more than 1·5 hr. In this situation, ischemic changes last several days and are superimposed on liver insufficiency due to the rejection process.

Liver allografts without immunosuppression were rejected within 7 days. The rejection process was characterized by increased serum bilirubin level, high alkaline phosphatase, aminotransferase and lactic dehydrogenase activities. Histologically, two typical changes were found: mononuclear infiltrates around portal ares and central veins, and necrosis around central veins (Porter, 1969; Starzl et al., 1969). In this type of rejection, not modified by immunosuppressive therapy, necrosis of hepatocytes with high serum aminotransferase activity predominates.

In recipients treated with pharmacological (imuran, steroids) or biological (ALG) immunosuppressive agents, or combination of both, the rejection process is attenuated, prolonged and difficult to detect by routine methods. Early rejection of the liver may be classified clinically into 3 types (Starzl et al., 1969). The first type — unicteric rejection — is characterized by normal serum bilirubin concentration, transitory bilirubinuria, and decreased fecal urobilinogen. There may be transitory increase in alkaline phosphatase and transaminase activity. The second type — rejection crisis — may be recognized by sudden increase in serum bilirubin concentration within 24—48 hours to rather bigh values. This may be preceded by rapid rise of alkaline phosphatase activity. No major changes in serum transaminase activity were observed. The third type of rejection is refractory to immunosuppressive therapy.

Two special phenomena may be seen in liver allografts undergoing early rejection. In one, hepatocytes become damaged with subsequent intracellular and intracanalicular bile stasis and clinical picture of obstructive jaundice (Hunt, 1967; Porter, 1969, Starzl et al., 1969; Myburhg et al., 1970). In the other, liver segments or even lobes become necrotic, and serum aminotransferase activity rises to high values (Birtch et al., 1969; Starzl et al., 1969). Immunological factors are responsible for liver tissue necrosis, as has been proved histologically and with immunofluorescent techniques (Starzl et al., 1969).

The results of experimental studies indicate that the process of liver allograft rejection in dogs, pigs, and monkeys is similar to that in man. Experimental observations may prove helpful in the evaluation of human liver transplants. The first stage of our liver transplantation program included experimental studies on biochemical changes in the liver recipient during early rejection phase, and correlation of their intensity and survival time of recipients. Our experiments revealed that the most constant change during rejection was an increase in serum bilirubin concentration. Late increase in serum bilirubin levels correlated well with prolonged survival of recipients. Hyperbilirubinemia, however, was not a good early index of liver rejection. It occurred at the time of advanced liver insufficiency, when trials of controlling the process with immunosuppressive drugs were inneffective. The dogs died within a few days. Serum aminotransferase activity was not very helpful in detecting early rejection. Aminotransferase activity was high immediately after transplantation. At the time of death of animals it remained relatively low. Immunosuppressive therapy to some extent damages liver cells also in the normal untransplanted organ. However, this was never accompanied by intracellular or intracanalicular bile stasis.

It may be concluded, basing on the results of our studies, that better understanding of the mechanism of impaired bile production in the liver allograft is needed to work out a reliable biochemical test for rapid detection of early liver transplant rejection.

CONCLUSIONS

1. Early rejection of liver allograft in dogs is characterized by increasing serum bilirubin concentration. Other biochemical tests are less reliable.

2. High serum bilirubin concentration is an index of advanced histological changes. Efforts to control the rejection process at that time are ineffective.

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