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# SIGNS OF EARLY REJECTION OF LIVER TRANSPLANT IN DOGS

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Biochemical alterations developing during the early rejection of the allogenic liver transplant in the dog were studied. Experimental animals were divided into 3 groups: I — orthotopic liver transplantation without immunosuppressive therapy, II — orthotopic liver transplantation with immunosuppressive therapy, III — no transplantation, the same immunosuppressive regimen as in group II. Results of the studies indicate that the most characteristic sign of early rejection of the liver transplant was an increase in serum bilirubin level. High serum bilirubin levels indicate irreversible alterations in the liver parenchyma. Any trials to modify the immunosuppressive regimen at that time remain usually ineffective.

Preparations for the clinical transplantation of the liver have been carried out in the Department of Experimental Surgery and Transplantology Laboratory in Warsaw for the last 2 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 during the early rejection of the transplanted organ. Proper knowledge of the mechanism of biochemical changes developing during rejection would be extremely helpful in early diagnosis of that process and also in institution of an appropriate immunosuppressive regimen.

## METHODS

Experiments were carried out on 17 dogs divided into 3 groups. In group I an orthotopic liver transplant was performed in 9 dogs according to the technique described previously for allogenic liver transplantation (6). The period of total ischemia ranged between 45 and 80 min. The following immunosuppressive regimen was applied for dogs of that group: 1st—3rd day — imuran 4 mg/kg and hydrocortizone 4 mg/kg, 4th—10th day, imuran 3 mg/kg and prednison 1 mg/kg, and since the 11th day on, chronic dose of imuran and prednison 1 mg/kg each. In group II of 6 dogs orthotopic liver transplantation was carried

out with the same technique as in group I, but no immunosuppressive therapy was instituted. In group III of 3 dogs the animals were given immunosuppressive drugs according to the above described schedule, but without transplantation. Following biochemical tests were performed daily in all the animals: serum bilirubin concentration, AspAT and AlAT activity in the serum, alkaline phosphatase activity in the blood, WBC, platelet count and hematocrit. At autopsy special attention was paid to the patency of vascular and biliary anastomoses. Specimens were taken for histology from the transplanted liver and recipients own organs like kidneys, lymph nodes, spleen and lungs.

Table I

Survival time, biochemical data, and causes of death of dogs in Group I

| No. of dog | Survival time | Biochemical values at death | | | | Cause of death |
|---|---|---|---|---|---|---|
| | | bilirubin mg% | AspAT u | AlAT u | alkaline phosphate | |
| 379 | 6 | 6·1 | 504 | 1412 | 140 | invagination |
| 473 | 7 | 11·0 | 144 | 616 | 212 | gallbladder necrosis |
| 423 | 8 | 13·6 | 458 | 1070 | 168 | liver insufficiency |
| 478 | 10 | 4·6 | 76 | 286 | 220 | small bowel perforation |
| 408 | 14 | 0·8 | 42 | 616 | 200 | eventration |
| 443 | 16 | 6·6 | 170 | 308 | 164 | ? |
| 412 | 17 | 4·0 | 96 | 316 | 258 | ? |
| 432 | 30 | 4·5 | 80 | 328 | 264 | duodenal ulcer perforation |
| 657 | 48 | 4·8 | 112 | 164 | 160 | liver insufficiency |

## RESULTS

Group I. The survival time of liver recipients ranged between 6 and 48 days, on the average 17·5 days. Serum bilirubin concentration rose steadily (Table I) concomittantly with the increase of alkaline phosphatase activity. The higher and more steep was the increase in serum bilirubin concentration, the shorter was the survival time of the recipient. The 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 11 day, and in 2 on the 19 day. There were major differences in the bilirubin concentration and time of its increase despite of the same dosage of immunosuppressive drugs per kg of body weight.

Serum transaminases activity was rather high during the first 2 days after transplantation and amounted to 1500 u for AspAT as well as for AlAT. It diminished considerably thereafter with upper levels of 500 u for AspAT and 1500 u for AlAT. Increase in transaminases activity was usually transitory and did not parallel the increase in serum bilirubin concentration. At the time of

death of the animals it was low, except of two dogs with necrosis of a lobe of the liver.

The cause of death was in 2 cases coma, jaundice and cachexia, in another 2 perforation and bleeding from the duodenal ulcer, in all the others necrosis of the gallbladder, intestinal invagination and wound dehiscence. Two dogs died because of unknown reasons. In none of the dogs could bile be found in the biliary tree.

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

Table II

Survival time, biochemical data, and causes of death of dogs

| No. of dog | Survival time | Biochemical values at death | | | | Cause of death |
|---|---|---|---|---|---|---|
| | | bilirubin mg% | AspAT u | AlAT u | alkaline phosphatase I u | |
| | | Group II | | | | |
| 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 III | | | | |
| 1 | 21 | 0·1 | 15 | 85 | 24 | |
| 2 | 21 | 0·1 | 83 | 490 | 30 | |
| 3 | 29 | 1·9 | 200 | 455 | 12 | |

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

Group III. Serum bilirubin concentration and alkaline phosphatase activity remained normal throughout the whole period of study. There was a slight increase in serum transaminases activity, but is never exceeded 200 u for

AspAT and 500 u for AlAT. At autopsy performed on the 21st day of the study no gross changes in the liver could be found. Histologically dissociation of liver trabeculae was observed, but there was no necrosis, infiltrations or bile stasis.

## DISCUSSION

Recognition of early rejection of liver allograft remains difficult for two reasons. First, it is not known which one of many liver functions may be mostly affected by the rejection process. Thus, none of the biochemical liver tests may prove useful. Secondly, temporary liver ischemia during preservation and transplantation is followed by more or less pronounced insufficiency of that organ. It is not possible to distinguish between the two forms of insufficiency: due to rejection, and ischemia. The immediate posttransplantation period is characterized by increased serum transaminases and lactic dehydrogenase activity, and high hematocrit. Alkaline phosphatase activity and serum bilirubin level remain normal. Biochemical changes due to liver ischemia at the time of transplantation subside usually within 2—3 days, unless there was prolonged ischemia for more than 1·5 hours. In that case impaired liver function due to ischemia lasts longer and may be superimposed on hepatocyte insufficiency due to rejection.

Liver allograft in recipients with no immunosuppression is rejected within 7 days. The rejection process is characterized by increased serum bilirubin level and high alkaline phosphatase, transaminase and lactic dehydrogenase activity. Histologically two typical changes can be found: mononuclear infiltrates around portal areas and central veins, and necrosis around central veins (7, 8). In this type of rejection, not modified by immunosuppressive therapy, necrosis of hepatocytes with high serum transaminase activity predominates.

The clinical picture of rejection remains different in recipients treated with pharmacological (imuran, steroids) or biological (ALG) immunosuppressive agents, or combination of both. In this situation the rejection process is prolonged and not easily detectable with usually available means. Early rejection of the liver may be classified clinically into 3 types (8). The first type-nonicteric rejection — is characterized by normal serum bilirubin concentration, transitory bilirubinuria, and decreased fecal urobilinogen. There might be some usually transitory increase in alkaline phosphatase and transaminase activity. The second type-rejection crisis — may be recognized by sudden increase of serum bilirubin concentration within 24—48 hours to rather high values. This may be preceded by rapid rise of alkaline phosphatase activity. No major changes in serum transaminases activity are observed. The third tape of rejection — slow rejection — is characterized by steady increase in serum bilirubin level, alkaline phosphatase activity and slight fluctuations of transaminases activity. This 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 (3, 5, 7, 8). In the other liver segments or even lobes become necrotic, and serum transaminases activity rises to high values (1, 8). Immunological factors are responsible for liver tissue necrosis, what has been proved histologically and with immunofluorescent techniques (8).

The results of experimental studies indicate that the process of liver allograft rejection in dog, pig and monkey is similar to that in man. Thus experimental observations may prove helpful in evaluation of human liver transplants (2, 3, 4, 8). The first stage of our liver transplantation programme included experimental studies on biochemical changes in liver recipient during early rejection, correlation of their intensity and survival time of recipients, and adverse effects of immunosuppressive therapy.

Our experiments revealed that the most consistent change occurring during rejection was an increase in serum bilirubin concentration. Late increase in serum bilirubin level correlated well with prolonged survival of recipients. On histology intracellular and intracanalicular bile stasis was found. Hyperbilirubinemia did not prove to be a good early index of liver rejection. It occurred at the time of advanced liver insufficiency, when any trial to control the process with immunosuppressive drugs proved to be ineffective. Dogs died within a few days. Serum transaminases activity estimation was not very helpful in detection of early rejection. Transaminases activity was high immediately after transplantation, due to ischemia during preservation and transplantation. At the time of death of animals it remained at relatively low levels. Immunosuppressive therapy may damage to some extent the liver cells also of an untransplanted liver. This has, however, never been accompanied by intracellular or intracanalicular bile stasis.

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

## CONCLUSIONS

1. Early rejection of liver allograft in dog is characterized by the increased serum bilirubin concentration, whereas the other biochemical tests remain unreliable.

2. High serum bilirubin concentration is, however, a rather late sign of liver rejection process and does not prove helpful for regulation of the immunosuppressive regimen.

3. Better understanding of the mechanism of impaired bile production in the liver allografts is needed to find a reliable biochemical index of early rejection.

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