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Effect of homograft ischemia upon serum proteins after orthotopic liver transplantation

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There have been several reports concerning the protein metabolism of orthotopic liver homografts after their transplantation to nontreated recipients. Serial changes in plasma proteins[3-6, 9] or turnover rates of amino acid isotopes[3-5] were measured. In dogs which received livers that had not been badly damaged by ischemia, it was found that plasma concentrations of protein fractions were initially well maintained[6] with the possible exception of albumin,[9] and that the rate of protein synthesis was generally increased until shortly before death.[3-5]

These short-term observations did not suggest that grave defects of protein synthesis would constitute a major problem after this kind of operation. However, subsequent studies by Brettschneider and co-workers[1] have shown that drastic falls in serum proteins often occur after orthotopic homotransplantation of livers damaged after preservation for a number of hours, and that the presence or absence of these changes provided an important clew to the long-term prognosis of recipient animals treated with immunosuppressive agents.

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In the present study this question has, therefore, been re-examined by measuring the plasma proteins in dogs which were under immunosuppressive therapy and which received both badly and minimally injured homografts. The use of immunoelectrophoretic techniques permitted qualitative study of the way in which specific protein fractions were affected, first by ischemic injury and then by subsequent modified rejection.

METHODS

Mongrel dogs weighing 10 to 23 kilograms were used. Immunosuppressive therapy for the nonoperated control animals as well as for those subjected to transplantation was with daily azathioprine, a progressively diminishing seven-day postoperative course with prednisone beginning at 5 mg. per kilogram per day, and daily subcutaneous injections of 1.0 to 2.5 ml. horse antilymphocyte globulin (ALG). Orthotopic liver transplantation was carried out with standard techniques[10]; arterial reconstruction was with end-to-end hepatic arterial anastomosis. The methods of liver preservation have been described elsewhere[1] and will be alluded to under the different experimental groups. Postoperatively, frequent measurements were made of serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase, and bilirubin.

Total serum proteins were determined with refrectometry.*[7] Electrophoresis was done with a microzone electrophoresis cell.†[2] Immunoelectrophoresis‡ was performed with Scheidegger's[8] technique of using rabbit anticanine sera.§

Experimental groups.

Group 1—Controls. Five nonoperated animals were treated with the 3 immunosuppressive agents.

Group 2—Minor ischemic injury. Four dogs received orthotopic liver homografts which were cooled in the donor by intraportal infusion with chilled electrolyte solution and then transferred within an hour.

Group 3—Moderate ischemic injury. Six dogs received livers which had been stored extracorporeally for 8 to 24¾ hours with a combination of hypothermia, portal and hepatic arterial perfusion with diluted blood, and hyperbaric oxygenation. This preservation technique was the most effective one tested in a recent study.[1]

Group 4—Severe ischemic injury. Twelve dogs received homografts after 8½ to 24¾ hours' preservation by techniques similar to those described in Group 3. However, certain details, such as optimal perfusion rate, had been changed.[1] By the first postoperative day there was evidence by standard liver function tests of grave ischemic injury.

RESULTS

Changes in first week.

Group 1. The total serum protein and its fractions were unchanged in these five dogs during the seven-day period of study.

Group 2. The average SGOT on the first postoperative day was 119 SF units (range 70 to 175) as compared to a normal range in this laboratory of 0 to 40 units, indicating that damage to these organs was not severe.

Before transplantation, both donor and recipient animals had serum proteins which were within normal. Subsequent changes were expressed as percentage deviations from the preoperative recipient values. During the first postoperative week, these levels were maintained at 85 to 110 percent (average 100 percent) whether the grafts were subsequently rejected at an early or late time. For example, one animal died of acute rejection after seven days, despite which protein concentrations were well maintained during the first six days (Fig. 1).

During this period there was no statistically significant change in the electrophoretic pattern, although the α_2-globulin was slightly depressed in all four animals, to an average of 85 percent. The results of immunoelectrophoresis of the sera were consistent with these findings, in that ceruloplasmin, α_{2M},

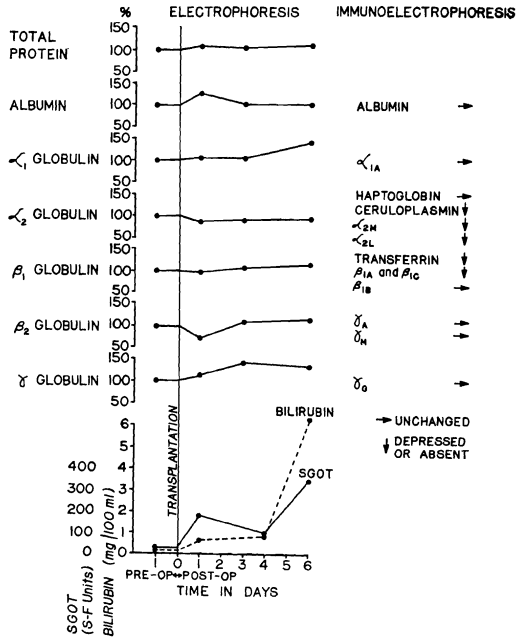

Fig. 1. Relentless rejection which occurred after orthotopic liver transplantation in a dog despite treatment with azathioprine, prednisone, and antilymphocyte globulin. Note that the protein fractions as measured with electrophoresis were relatively stable. The proteins measured with immunoelectrophoresis are listed at the same level as the electrophoretic classes in which these are included.

*T.S. Meter, American Optical Co., Buffalo, N. Y.

†Beckman Instruments, Inc., Fullerton, Calif.

‡The apparatus for electrophoresis in agar was manufactured by LKB-Produckter AB, Stockholm, Sweden. Veronal buffer was used at a pH of 8.6 and ionic strength of 0.05 in agar and 0.1 in tank.

§Hyland Division Travenol Laboratories, Inc., Los Angeles, Calif.

and alpha-2 lipoprotein (α_{2L}), which have the electrophoretic mobility of α_2-globulin, all appeared to be reduced in at least three of the four dogs. These changes occurred only after four or five days. At this time the β_{1A} precipitation band also became indistinct.

The γ-globulin had already become elevated in two dogs, which later died of rejection after seven and 21 days. The two others, which lived for 29 and 100 days respectively, had early depression of the γ-globulin. However, these alterations could not be well seen by immunoelectrophoresis.

Group 3. The average SGOT on the first postoperative day was 381 SF units (range 110 to 645). The increased ischemic injury was reflected in a depression of the total serum protein to 79 to 97 percent of the original values (average 87 percent) during the first seven days. There were variable minor declines in the concentrations of all the electrophoretic classes of proteins, including the γ-globulins, which were depressed to 79 percent. With immunoelectrophoresis the subgroups of the α_2-globulins appeared depressed in the same general way as in Group 2.

Group 4. The SGOT rise on the first day was to an average of 920 SF units (range 305 to 3250). Daily total proteins in eight dogs were depressed during the first week to an average of 83 percent (63 to 100 percent). Changes in the protein fractions were highly variable. Only one of these dogs survived for more than 13 days; the exceptional animal died after 19 days.

In these eight dogs, as well as in four others in which electrophoresis was not performed, immunoelectrophoretic studies often revealed a striking selective attenuation or even a total loss of discrete protein moieties. An example is shown in Fig. 2. On the first postoperative day precipitation bands had completely disappeared for the ceruloplasmin, α_{2M} and α_{2L} components of the α_2-globulin. In addition, the β_{1A} portion of β_1-globulin and the γ_A and γ_M portions of the β_2-globulin could not be found. Most of the precipitation bands which remained were shorter or less distinct than in the control sample.

During the ensuing days there was a gradual return of some of the missing precipitation bands, but the α_{2L} as well as β_{1A} and γ_M remained absent until the death of the animal on the sixth day. Qualitatively, similar findings were evident in all 12 experiments, although frequently the losses were less extreme than those illustrated.

Subsequent changes in dogs surviving chronically after operation. Several of the animals in Groups 2 and 3 had good hepatic function from the beginning. In others, early evidence of injury, including minor depressions in some of the serum proteins, had returned to or toward normal within a week or 10 days. In eight such dogs it was possible to ascribe subsequent changes to rejection.

The serum proteins remained relatively normal in these animals, often for weeks or

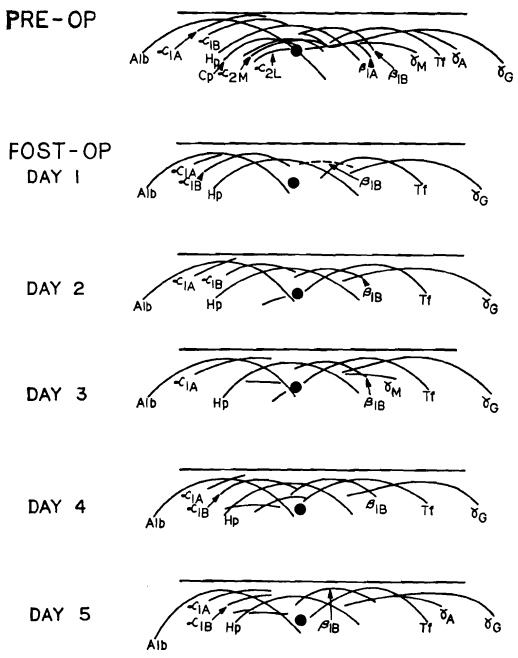

Fig. 2. Changes seen by immunoelectrophoresis in the serum of a dog which received a damaged liver homograft. Note the early postoperative disappearance of several precipitation bands including ceruloplasmin (Cp), α_{2M}, α_{2L}, β_{1A}, γ_M, and γ_A. These tended to return, but never completely to control status.

months. With the onset of fatal late rejection from three weeks to three months postoperatively there were declines in two of three dogs in total serum protein and most of its component fractions, except for the γ globulins, which rose in two instances. The serum proteins in the other five animals were little changed by rejection crises which reversed spontaneously; four of these dogs are still alive from two to four months postoperatively.

DISCUSSION

For kidneys the degree to which tissue anoxia during preservation adversely influences the long-term prognosis can be precisely investigated in the absence of an immunologic barrier, since autografts can be used for testing. With livers this approach is not feasible for obvious technical reasons, and an assessment of the long-term suitability of preserved hepatic homografts can be obtained only if these are transplanted to recipients which are provided with effective immunosuppression.

All animals in this study were treated with immunosuppression. Cooled and immediately transplanted liver homografts were used, and the results during the first week were generally consistent with previous reports of protein metabolism after orthotopic transplantation to untreated recipients. Serum protein levels and individual protein fractions were maintained at near normal levels, as would be expected with good liver function. The only detail which differed from earlier observations was that increases in α_2-globulin concentration[3-6] were not seen. These animals continued to have normal or near-normal serum proteins until fatal rejection occurred. At that time an over-all decrease in serum protein concentration developed in some dogs several days before death. There was a variability in immune globulin concentrations both early and late after operation. However, quickly developing rejection was more common in animals with elevated γ globulins.

The most important factor in early postoperative protein metabolism seemed to be the degree of ischemic injury imposed upon the homografts. When this was moderate there were significant but reversible changes in some of the protein components, particularly those of α_2 class. With more severe damage there was often complete disappearance for several days of some of the immunoelectrophoretic bands. These tended to return, but never completely to normal. All of these dogs eventually died in less than 19 days. In such experiments the after-effects of ischemia in combination with an unanalyzable further immunological injury to the homografts apparently precluded long-term survival.

SUMMARY

Serum proteins were studied with electrophoresis and immunoelectrophoresis in drug-modified recipients of orthotopic hepatic homografts. The homografts that were provided had been subjected to minor, moderate, and severe ischemic injury. In the early postoperative period serum protein and its components were well maintained in the recipients of minimally traumatized livers and depressed in the others in rough proportion to the degree of prior anoxic damage. The significance of these, as well as of subsequent changes in serum proteins, are discussed in relation to evaluation of liver preservation techniques.

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