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EXTRACORPOREAL PORCINE LIVER PERFUSION

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Five patients with hepatic coma complicating acute viral hepatitis have been treated in our department with extracorporeal porcine liver perfusion. Two of them regained consciousness soon after the second perfusion, recovered within several weeks, and were discharged from the hospital. Three others died with symproms of progressive liver insufficiency. In the following report the clinical, biochemical, and hematological data obtained in 10 perfusions are summarized.

In 1966—1969 eighty experimental extracorporeal liver perfusions were performed in pigs at the Surgical Research Laboratory in Warsaw (Nielubowicz et al., 1969; Olszewski et al., 1969, 1970a, b; Łukasiewicz et al., 1971a; 1971b;). The experiments were planned as a preparatory step for extracorporeal liver perfusion in patients with hepatic coma, and also as a supportive measure before, during and after liver transplantation. As a result of the studies the technique of isolated liver perfusion was elaborated and applied in 5 patients with acute liver insufficiency.

MATERIAL AND METHODS

The procedure was started with two intravenous drips, one into the cubital vein, the other into the large saphenous vein in the ankle region. Central venous pressure was monitored through a cannula inserted into the superior vena cava. Blood samples were collected from veins of the lower limb or forearm. Arterial pressure was monitored continuously in the radial artery. Urine was collected and hourly diuresis was measured.

Operative procedure

Cannulation of patient's blood vessels. Through a vertical 10—12 cm long incision below the inguinal ligament, the common, superficial and deep femoral artery were dissected together with the neighbouring veins. A metal cannula was inserted into the common femoral artery through

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an incision in the superficial femoral artery. The femoral artery of the patient supplied the blood to the arterial and portal systems of pig's liver. Another cannula was placed in the femoral vein.

Pig liver was removed in the operating room of the Fesearch Laboratory and transported in a sterile bag to the Perfusion Unit, where it was placed in a special container. Patient's femoral artery was connected with

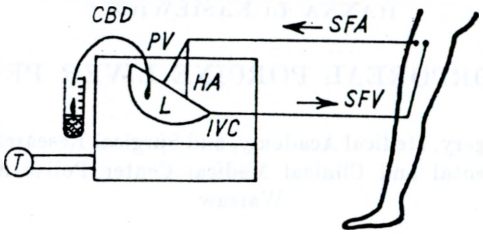

Fig. 1. Schematic drawing of the perfusion system during the extracorporeal liver perfusion in man. *SFA* — superficial femoral artery, *SVF* — superficial femoral vein, *IVC* — inferior vena cava, *HA* — hepatic artery, *PV* — portal vein, *CBD* — common bile duct, *L* — liver, *T* — thermostat.

the hepatic artery and portal vein, and the ruprahepatic portion of the inferior vena cava of the liver with the fenoral vein. The whole perfusion system was checked for the presence of air bubbles. The whole perfusion system was checked for the presence of air bubbles. The clamps were then removed and perfusion was started. The ischemia time of the liver ranged between 40 and 56 minutes. The common bile duct was cannulated and bile collected (Fig. 1).

The liver container was filled with Ringer's solution at the temperature of 38° C, so that the liver temperature could be maintained at the physiological level. Liver blood flow was measured every 30 minutes, and expressed in ml/g of liver weight/min. Perfusion of the extracorporeal liver lasted 3 or 6 hours depending on the condition of the patient and the gross appearence of the liver. After termination of the perfusion the cannulae were removed from the vessels. The femoral superficial artery was sutured or ligated close to the deep femoral artery and the femoral vein was ligated. The wound was then stitched and suction drainage was instituted. During the second perfusion, usually on the next day, the same vessels were cannulated or the other side was dissected.

Laboratory studies

The following laboratory tests were performed in all patients before the perfusion: RBC count and hemoglobin level, platelet count, serum protein content, paper electrophoresis, BUN, blood glucose, serum bilirubin, and electrolyte, blood ammonia. lactic and pyruvic acid concentra-

tion, AspAT, AlAT, LDH, GLDH and alkaline phosphatase activity. Oxygen and carbon dioxide partial pressure, as well as serum bicarbonate and blood pH, were measured. The coagulation and fibrinolysis system was studied for clotting time, prothrombin time, calcium clotting time, thrombin time, factor V and VII, prothrombin consumption test, fibrinogen concentration, whole blood fibrinolysis and euglobulin lysis time estimations. Bilirubin concentration was measured in the bile, and also in the fluid surrounding the liver in the container. Total serum protein was measured by the biuret method, electrophoretic pattern by paper electrophoresis, BUN by the method of Kowarski, blood glucose colorimetrically with orthotoluidine, electrolytes with Zeiss flame photometer, blood ammonia levels by the Jouany and Reynier diffusion method (Jouany et al., 1959), lactic acid by the method of Homolka (Homolka, 1961), and pyruvic acid by that of Nateslson (Nateslson, 1957), serum bilirubin level by the modified method of Malloy and Evelyn, aminotransferase activity by the colorimetric method of Umbreit (Umbreit et al., 1957). Paranitrophenylophosphate sodium was used for the estimation of alkaline phosphatase activity (Bergmayer, 1963). Oxygen and carbon dioxide partial pressure and blood pH were measured with Astrup's equipment. For clotting time, a high standard bovine thrombin 30—40 u./ml was used. Prothrombin time was estimated by Quick's method, calcium clotting time according to Niewiarowski (1962), thrombin time also according to Niewiarowski (1962), factor V by the method of Wolff, factor VII with that of Keller, prothrombin consumption according to Quick, fibrinogen concentration according to Quick, whole blood fibrinolysis by Halse's method, and euglobulin lysis time by the method of Niewiarowski (1962).

MATERIAL

Extracorporeal porcine liver perfusion was carried out in 5 patients with hepatic coma in acute viral hepatitis. Patients were unconscious for a period of 2 to 4 days. Three patients demonstrated typical cerebral focal symptoms. One patient was completely areflexive, including lack of reaction to light. In one clonic tremor was seen. All patients had jaundice, with an average bilirubin concentration of 22 mg %. Spontaneous breathing was maintained in most patients, only sometimes requiring mechanical assistance.

RESULTS

A. Clinical course of perfusion

Two patients with viral hepatitis recovered, and three others died. Patients who recovered were discharged from the Dept. of Infectious Diseases 2 and 5 months after the perfusion. Various untoward reactions

were observed in patients at the initial period of perfusion. At the moment of clamp release there was a rapid accumulation of blood in the extracorporeal liver. Transfusion of blood and electrolyte solutions appeared to be necessary to avoid temporary hypotension. If the transfusion was not adequate, the central venous pressure dropped rapidly, as well as the arterial pressure. The pulse rate increased, and liver blood flow diminished. The amount of transfused blood necessary to maintain normal pressure and flow was 500 to 750 ml. Postperfusion anesthetic therapy required an endotracheal tube for 6—8 hr, oxygen therapy, removal of tracheal secretion, ans dometimes, assisted ventillation.

Acid-base balance

Two patients demonstrated typical signs of respiratory alkalosis with, hyperpnoe, arterial blood pH up to 7·72, and pCO_2 30—32 mm Hp. In one patient there was evident metabolic acidosis with blood pH 7·29, pCO_2 35 mm Hg, and HCO_3 18—20 mEq/1. In two others acid-base equilibrium remained within normal limits. These two patients recovered, whereas the three others with acid-base disorders died.

Serum proteins

Before perfusion, the total serum protein concentration was 6·7 g%, dropping after a 6 hr perfusion to 4·68 g%, and remaining at that low level during the next perfusion, and for some days afterwards. The most prominent was the fall in albumin concentration, with a low albumin: globulin ratio reaching 0·55 in one patient.

Blood glucose

There was a constant tendency toward hypoglycemia. A blood glucose level of 200 mg% was maintained more or less steadily with intravenous glucose infusion. Large amounts of glucose had to be infused. During a 3 hr perfusion, patient F. J. received 1500 ml 5% of glucose solution, and 2000 ml during the second 6 hr perfusion.

Serum electrolytes

In 3 out of 10 perfusion, considerable drop in potassium levels was observed. This was caused by substantial loss of potassium to the ascitic fluid, and required intravenous replacement of that ion. No changes in sodium or chloride concentration were observed.

Aminotransferase activity

AspAT and AlAT activity increased slightly in 4 out of 10 perfusions, remaining at initial levels in all others. Experimental experience suggests that this increase in aminotransferase activity might be accounted for by the release of enzymes from the porcine liver to the patient's circulation.

Serum bilirubin

There was a substantial decrease in serum bilirubin concentration following perfusion in all the patients. The mean initial values were 19·59 mg/100 ml (28·3—12·4), decreasing to 7·77 mg/100 ml (14·5—4·93) by the end of the procedure. There was a rapid return of serum bilirubin level within a few hours of the perfusion to the initial values. The fall of bilirubin concentration and clinical improvement were not correlated

Coagulation and fibrinolysis

Slight thrombocytopenia was noted in all patients before the perfusion. Further decrease in platelet counts was observed during the perfusion, from 180,000—67,000 to 90,000—14,000/cu mm. Electron microscopic and isotope studies revealed trapping of platelets in the heterologous liver, a phenomenon which can be explained only on immunological grounds. Heparin consumption in the perfusion system was high, possibly due to platelet disintegration and release of platelet factor 4. Fibrinogen concentration fell during the perfusion to values of 120—90 mg%. Substantial shortening of euglobulin lysis time was observed.

Infusions and drugs

Each patient had multiple transfusions of blood and electrolyte solutions. The average amounts were: blood 2070 ml, dextran 400 ml, and 5% glucose 2370 ml. 3×10^6 units of penicillin was given during the perfusion. Heparin was administered according to the results of thrombin time, Antifibrinolytic drugs EACA and AMCHA were administered when euglobulin lysis time decreased below 120 min. Insulin was added to the glucose solution in a concentration 2 u./1 g of glucose. Potassium chloride was infused in cases with severe hypopotassemia in a concentration of 1 M/500 ml 5% glucose.

B. The heterologous liver

Blood flow

Mean liver blood flow was 0·54 ml/g/min. with a range of 0·125 to 0·79. Flows above 0·5 ml were considered as normal or nearly normal. Two major hemodynamic changes occurred in the course of each perfusion. ^{133}Xe elution studies revealed uneven distribution of blood flow in the liver, with high flow areas in the middle portion of the organ, the peripheral parts being relatively unperfused. There was a rapid drop in blood flow rate usually in the 3rd and 4th hour of perfusion.

Bile production

The heterologous liver produced bile at a rate of 0·08 to 1·59 ml/100 g of liver/hr, with the bilirubin concentration 124 to 410 mg%. The average bilirubin excretion with bile was 74 mg (range 6—193). Bilirubin was also eliminated with the ascitic fluid. The average amount excreted by this route was 115 mg, with the range 6—401 mg. Thus the mean total amount of excreted bilirubin was 236 mg (range 28—428), equivalent to the daily amount of bilirubin produced by a normal man.

Ascitic fluid

The amount of fluid produced was variable and ranged from 160 ml in one patient to as much as 1700 ml. The bilirubin concentration in the fluid ranged from 0·1 to 6·6 mg%, protein from 0·52 to 2·6 g%, and AspAT activity from 100 to 396 u/ml. Total protein loss to the ascitic fluid amounted to 93·5 g (range 31·2—174·2) during a 6 hr perfusion.

DISCUSSION

Extracorporeal heterologous liver perfusion for treatment of hepatic coma in man was proposed and first performed by Eiseman (1965). According to the pertinent literature 73 patients were treated with this method until 1970, and 16 out of that number recovered. The authors' experience is limited to five cases with two recoveries.

Extracorporeal liver perfusion remains at the stage of clinical therapeutic experiment, and is based on sound theoretical grounds. The liver belongs to organs indispensable for the preservation of life. Its metabolism is a complex one and not understood well by physiologists. This being so, there exists no possibility of repairing the liver in case of major degenera-

tive changes. In such a situation, the organ should be replaced by a new one. However, liver possesses some regenerative properties, and if changes in the liver tissue are reversible like in viral hepatitis or toxic injury, they may allow the patient to survive if the deficient metabolic function can be supported for some time with measures such as extracorporeal perfusion.

One of the main problems in liver perfusion is the proper selection of patients for this type of treatment. There are no objective data which would indicate that the liver function is deficient enough to discontinue conservative treatment and transfer the patient to the Dept. of Surgery for liver perfusion. According to our experience the patient in deep coma for 24—48 hr with progressive deterioration of his neurological state should be treated with perfusion as quickly as possible.

In this paper we do not discuss the immunological problems connected with the heterologous liver perfusion. The results of immunological studies have been presented elsewhere in this issue (Olszewski et al., 1973). Extracorporeal liver behaves like a xenograft. Nevertheless no major immunological reactions have been reported in the literature (Eiseman et al., 1965; Kommerall, 1969), even if the perfusion was repeated several times at short intervals.

On the whole, extracorporeal heterologous liver perfusion remains in the stage of clinical experimentation. Its value in the treatment of hepatic coma in patients with acute liver insufficiency awaits proper evaluation with the increase in clinical experience, and overcoming of some of the immune problems.

CONCLUSIONS

1. Five patients with hepatic coma complicating acute viral hepatitis were treated with extracorporeal porcine liver perfusion and two of them recovered.

2. There was no correlation between biochemical and clinical improvement.

3. Liver blood flow and coagulation disorders created major problems during the perfusion. They may be caused by the immunological process taking place in the temporary liver xenograft.

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