ANAESTH. RESUS. INTEN. THERAP.
Vol. 4, No. 3, 1976

Anaesthesia for Orthotopic Allogenic Liver Transplantation in Pigs

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Anaesthesia with a mixture of halothane, nitrous oxide and oxygen was used for allogenic liver transplantation in 24 pigs. All animals had haemodynamic disturbances due to the technique of the operation: metabolic acidosis, disturbances of bioelectric activity of the heart and electrolyte level changes. The methods of management of these disturbances are discussed. Favourable results were obtained when transfusing heparinized stored blood in place of initially used ACD blood which increased the intensity of metabolic acidosis. Details of the technique and method of anaesthesia and monitoring of animals during operation are discussed.

The operation of liver transplantation in animals is a complicated procedure causing a number of disturbances not only in the transplanted organ. The acid-base and water-electrolyte balance are disturbed leading to heart arrhythmias and conduction disturbances; other not infrequent disturbances involve the blood clotting system, respiratory function, endocrine glands and kidneys. Good surgical technique lessens the blood loss and shortens the operating time, but the procedure is inseparably connected with several episodes of relative hypovolaemia caused by: 1) sudden closing of the blood flow in the inferior vena cava, 2) sometimes inadequate work of the pump used in the liver bypassing system, 3) restoration of blood flow in the transplanted organ after removal of clamps from the portal vein and hepatic artery when the blood is inadequately supplied in the period immediately preceding the filling of the very capacious vascular bed of the liver, 4) a fall in the cardiac output when the metabolic acidosis is insufficiently controlled.

When planning this complicated surgical procedure connected with many possible risks the selection of agents and methods used for anaesthesia during the operation and in the postoperative period should be

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taken into account. Some of them, although applied successfully in patients in operations performed for hepatic failure or damage during major vascular operations or during operations on poor-risk patients, cannot be used in animals in view of physiological and pharmacodynamic differences. All agents used during anaesthesia are metabolized in the liver or affect its functions. The restricted possibilities of postoperative care should be considered also since it is of as great importance as the operation and anaesthesia.

During a survey of literature concerning liver grafting we were struck by the almost complete absence of data relating to the details of anaesthetic management. The authors of these reports focused their attention mainly on anatomical and technical details determining efficient surgical technique and they stated only, for example, that the animal had received pentobarbital (Nembutal) in a dose of 25 mg/kg of body weight. It is evident, however, that a safe management of the experimental animals during such a difficult operation also requires, besides experience, a knowledge of their anatomy and physiology and the possibility of disturbances of various systems and organs.

The purpose of the present paper is the presentation of experiences and observations collected during our endeavours to keep the experimental animals alive at least for a couple of days, since that time was necessary to obtain sufficient information about the function of the graft.

MATERIAL AND METHODS

The pigs used for the liver transplantation operation belonged to the white Polish lapear strain. Their weight ranged from 17 to 20 kg. They showed certain peculiar features of their respiration and circulation. The respiratory rate was 8 to 18/min the tidal volume was 200—250 ml. This ensured the maintenance of arterial blood pH in the range of 7.40 to 7.50 at a mean $PaCO_2$ value of 42 torr. The volume of circulating blood was about 74 ml/kg. The heart rate ranged from 60 to 80/min and the mean value of systolic arterial blood pressure (measured by the intra-arterial method in 20 non-anaesthetized pigs) was 169 torr. One mm^3 of blood contained usually 6,600,000 erythrocytes and 100 ml of blood contained usually 12 g haemoglobin. The pig liver contained up to 20% of total blood volume [1]. An anatomical peculiarity differentiating the pigs used by us from other experimental animals was the peculiar structure of their airways with a large distance from the incisors to the larynx (over 20 cm), the presence of a large sagging epiglottis and a particular position of the tracheal axis to the nasopharyngeal axis resembling an elongated letter Z. This caused not infrequently difficulties in intubation. The laryngoscope designed for humans was unsuitable for pigs.

The pigs were starved for several hours before beginning anaesthesia. One hour before the surgical pro-

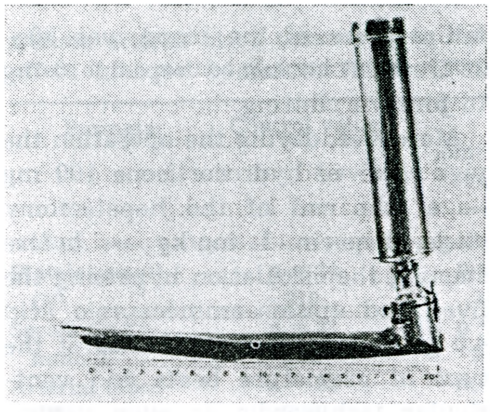

Fig. 1. Foregger laryngoscope used for endotracheal intubation in pigs.

cedure the pigs were weighed and received in premedication atropine 0.05 mg/kg and droperidol 0.125 mg/kg in intramuscular injection. The induction of anaesthesia was done by administering thiopental in 5% solution 15 mg/kg into a superficial vein of the pinna or by inhalation using a tightly-fitting rubber mask for the administration of a mixture of nitrous oxide and oxygen — 4 : 2 l/min with the addition of halothane 3—4 vol%. Then the pig was intubated using a Foregger laryngoscope with a very long blade (Fig. 1) and a rubber endotracheal tube No. 28 Rüsch type with a cuff (an introducer was used for intubation). After insertion of the endotracheal tube between the vocal chords (and removal of the introducer) it was found useful to turn it around its longitudinal axis to an angle fitting the curvature of the tube to the curvature of the trachea to avoid trauma (perforation) of the soft subglottic part of the trachea in young animals.

After intubation and filling of the cuff the tube was connected to a semiclosed to-and-fro system with an expiratory valve and the supply of the anaesthetic mixture placed in the anterior (nearer to the animal) part of CO_2 absorber filled with soda lime with a consumption indicator. The mixture of gases consisted of nitrous oxide 4 l/min oxygen 2 l/min and halothane administered periodically from a Fluotec thermoregulator vaporizer, at concentrations 0.5 to 1.0 vol%. Controlled ventilation was conducted with a Draeger Polmomat unit at respiratory rate f = 12—16/min, tidal volume VT=200 ml, inspiratory pressure IP = 12—15 cm H_2O and expiratory pressure EP = 0 cm H_2O.

Using a multichannel Mingograph 81 Elema-Schönander recorder with constant recording on paper, the following monitoring procedures were carried on: electrocardiograms in lead II, mean or momentary arterial blood pressure measured intra-arterially by inserting a polyethylene catheter into the femoral artery, central venous pressure measured by introducing a catheter through the right jugular vein into the superior vena cava to the region near the right atrium. Both pressures were transduced to the recorder through Elema EMT 33 and EMT 34 transducers.

Halothane was withdrown usually several minutes before the hepatectomy and always when the arterial blood pressure was falling or when the general condition of the animals deteriorated. At the last stage of the operation — from liver revasculari-

zation to closure of abdominal wound — analgesia with a mixture of nitrous oxide and oxygen 4 : 2 or 5 : 2 l/min was usually sufficient.

During the procedure gasometric measurements were done in arterial blood by Astrup's micromethod with a Radiometer apparatus. The determinations were performed in an adjoining room immediately after obtaining the blood samples from the artery, three times during the anaesthesia: 1) immediately after laparotomy — for establishing control values and assessing the efficiency of controlled ventilation, 2) during the hepatectomy stage, 3) after liver revascularization and connecting it to the inferior vena cave system. A fourth control determination was done always at various moments during the operation if abnormalities were found in gasometric values — for assessing the efficiency of their control. This was true particularly of metabolic disturbances connected with the hepatectomy.

The volume of fluids administered during anaesthesia was restricted as far as possible to an indispensable minimum. These fluids included: normal saline with heparin (25 mg per 500 ml of the solution) for flushing the catheters serving the pressure measurements (not more than 10—15 ml) and an 8.4% sodium bicarbonate solution for control of metabolic acidosis which was infused usually in amounts not exceeding 70—100 ml. All animals were given about 500 ml of fresh blood obtained from the donor of the liver. The temperature of the blood was 30—35°C and it contained heparin 10 mg to prevent clotting.

Moreover, during the operation the pigs received hydrocortisone 100 mg — at the end of the hepatectomy stage, heparin 1 mg/kg — before starting the circulation bypassing the liver and epislon-aminocaproic acid 4.0 g. Sometimes arrhythmias of the type of premature beats and ventricular tachycardia or even ventricular fibrillation in some cases forced us to administer calcium chloride 200—300 mg and lidocaine in repeated doses of 25 mg (Fig. 2).

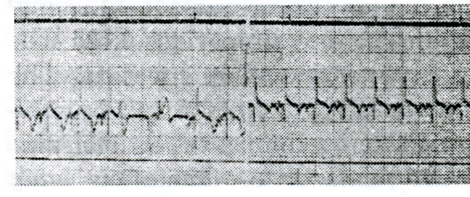

Fig. 2. Ventricular arrhythmia in the end-stage of the operation, disappearance of arrhythmia after administration of lidocaine 25 mg.

After the operation the pigs were transferred, sometimes still intubated, to a cage where they had a steady ambient temperature and where they received 40% glucose 10—20 ml.

The duration of liver transplantation was 90 to 120 min. The blood loss evaluated approximately ranged from 200 to 600 ml.

This is a report on the experiences and results obtained during operations on 24 pigs. In 12 of them the only modification of the operation was transfusion of stored blood with the addition of standard ACD solution and not with heparin.

Table I

Certain arterial blood parameters in the time period after liver vascularization — effect of the method of preservation of transfused blood

Parmeter	Control value	Value after revascularization		
		joint mean value	mean after ACD blood	mean after heparinized blood
Platelet count	n = 24	n = 24	n = 12	n = 12
G/l	210	192	176	208
Serum Ca++	n = 23	n = 23	n = 12	n = 11
mmol/l	2.02	1.90	2.00	1.87
Arterial blood	n = 22	n = 22	n = 12	n = 10
pH	7.49	7.42	7.41	7.44

RESULTS

One of the 12 pigs receiving stored blood with ACD solution survived 24 hours after the operation. The survival of other animals was below 2 hours and several pigs died even during the operation. All pigs receiving stored blood with the addition of heparin survived 24 hours and 3 survived even several weeks.

Table I and II show some biochemical and morphological values of the blood samples obtained simultaneously with gasometric samples.

DISCUSSION

The above described method of anaesthestic management during the operation of liver transplantation has been elaborated during a long period of attempts and failures.

The difficulties began with the induction of anaesthesia because the animals were usually restless, difficult to control and had significant salivation which made the insertion of the endotracheal tube even more difficult. After initial attempts of sedating the animals with intra-

Table II

Biochemical and morphological data of arterial blood in the hepatectomy stage and after revascularization

Investigation	Control value	Hepatectomy stage	Revascularization stage
Haematocrit l/l	0.38	0.39	0.37
PaO_2 KPa	19.5	25.5	25.6
$PaCO_2$ KPa	5.6	5.0	5.7
pH	7.49	7.46	7.42
Platelet count G/l	210	217	192
Serum Ca++ mmol/l	2.02	2.05	1.90
Serum glucose mmol/l	18.6	18.8	25.5

muscular promazine we found, comparing its effects with those of droperidol, that the latter was a much better sedative drug and in connection with atropine it gave a very good premedication making a smooth induction of anaesthesia possible.

Knowing the topographic difficulties connected with laryngoscopy and intubation we never tried to administer suxamethonium during the induction of anaesthesia since ventilation of pigs through a mask is difficult and uncertain at the time of apnoea.

The choice of the agent which may be used for anaesthesia for liver transplantation is the most difficult problem facing the anaesthetist. Nitrous oxide is safe and harmless, but anaesthesia obtained in this way is not sufficiently deep in the first stage of the operation and particularly during the inducation of anaesthesia. Thiopental used by us for the induction is relatively safe although it is metabolized by enzymes in the microsomes of hepatocytes. It disappears rapidly from the circulating blood and is taken up, in part, by the liver of the recipient which was, in our experiments, healthy and efficient. This barbiturate was used in operations of liver transplantation to humans [1, 5, 13]. Methohexital [5, 13] and propanidid [1, 7] were used with good results for this purpose. The detoxication of the latter seems to depend mainly on an esterase present in the liver which splits off its lateral chains.

We had similar misgivings with regard to halothane. Much has been written already about its true or untrue hepatotoxicity. It seems that opinion expressed, among others, by BUNKER *et al.*[4] and ALDRETE [1] should be accepted. According to these authors the hepatotoxicity of halothane is not greater than that of other inhalatory anaesthetic agents on condition that the anaesthesia is conducted under conditions of adequate oxygen pressure, normal blood glucose level, avoidance of hypercapnia, and low liver perfusion, and when the patient is not cachetic and has no infections. It was found, moreover, [2, 4, 10] that an overwhelming majority of documented cases of halothane hepatotoxicity was due to repeated administration of this agent and immunity reaction to it. In cases receiving halothane anaesthesia for the first time it was found, that only once in 500,000 halothane anaesthesias it was the cause of massive hepatic necrosis. These reports are encouraging and justify further administration of halothane anaesthesia in operations of liver transplantation in humans [1, 7] and animals [9, 11, 12, 13, 14].

Our experiences with the use of this drug, although based on a small number of cases, are also good but we lay stress on the necessity of its cautious administration and intermittent addition when the depth of anaesthesia decreases. In view of the intensity of metabolic and circulatory changes during hepatectomy we accepted the principle of halothane withdrawal immediately before this stage of the operation.

Controlled respiration was very easy to conduct during the above described halothane anaesthesia and muscle relaxants were not necessary. The first attempts with curare in an initial dose of 2 mg were unsuccessful because despite high doses of neostigmine after the operation the animals remained flaccid and spontaneous respiration failed to return. This observation confirms, among others, TERBLANCHE's communication [14]. It is possible that the cause of this may be a species-specific hypersensitivity to muscle relaxants.

The numerous initial failures of anaesthesia for liver transplantation in pigs have been removed in our experiments when we changed the mode of storage of blood transfused later to the recipient. Following TERBLANCHE's [14] example we began adding 10 mg heparin per 500 ml of blood in place of the previously used standard ACD solution. The 24-hour-survival rate of animals increased immediately to 100%.

Although we have as yet no convincing and unequivocal explanation of this fact we may explain it, in part at least, as an effect of the avoidance of further intensification of metabolic acidosis caused by the operation which is difficult to control despite the administration of alkalinizing agents. Metabolic acidosis is an inseparable accompaniment of liver transplantation as has been reported in Polish literature by ZAWADZKI, [15] and it leads to well known unfavourable effects, mainly involving the cardiovascular system (arterial pressure fall, decreased peripheral perfusion and myocardial block). Additional administration of citrate ions may cause an overburdening of the freshly transplanted liver and it increases even more the usually present fall in the serum ionized calcium level which may be another cause of arrhythmia and conduction disturbances in neuromuscular plates. These disturbances can be avoided when heparinized blood is transfused.

The administration of an additional dose of heparin besides the routinely used 1 mg/kg dose before beginning the blood flow bypassing the liver seems to be not without significance for perfusion of anastomosed vessels and for peripheral perfusion. Under conditions of hypotension, a slowed down blood flow in the peripheral blood vessels of venous stasis due to metabolic acidosis the administration of heparin may prevent the development of platelet aggregates which begin the process of intravascular clotting. These suppositions are confirmed, to some extent, by the results of the biochemical and gasometric determinations. A small rise in ionized calcium level towards the end of the operation in place of an expected fall may be explained as a result of prophylactic or therapeutic administration of several hundreds of milligrams of calcium chloride. In reading the results of blood pH determinations it should be kept in mind that they are obtained after infusion of sodium bicarbonate solution.

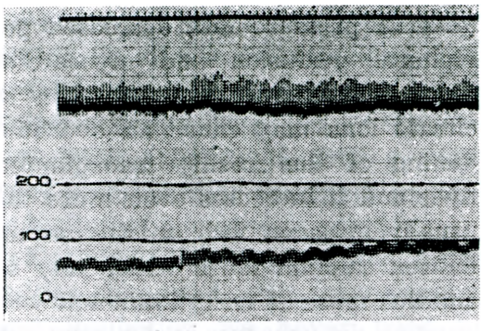

Fig. 3. Treatment of metabolic acidosis — a rise in arterial blood pressure during transfusion of 8.4% $NaHCO_3$ solution.

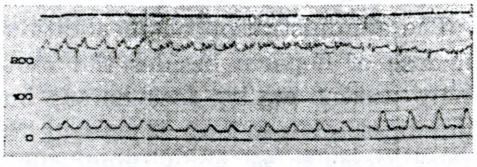

Fig. 4. Treatment of metabolic acidosis — normalization of QRS complexes and return of P wave to its normal position through Q wave during transfusion of 8.4% $NaHCO_3$ solution.

Fig. 3 and 4 show the control of metabolic acidosis by infusion of 8.4% sodium bicarbonate.

The problem of transfusion is connected with strict restriction of fluid intake during anaesthesia introduced by us. It was found that additional administration of e.g. 500 ml of 5% glucose, 0.9% NaCl or dextran 60,000 caused a worsening of the general condition of the animals, the development of postoperative respiratory failure and frequetly it contributed to the death of the animal. On autopsy oedematous fluid was found in these animals in the lumen of pulmonary alveoli. These complications could have been avoided by restricting the volume of fluid to the amount described in the part on the

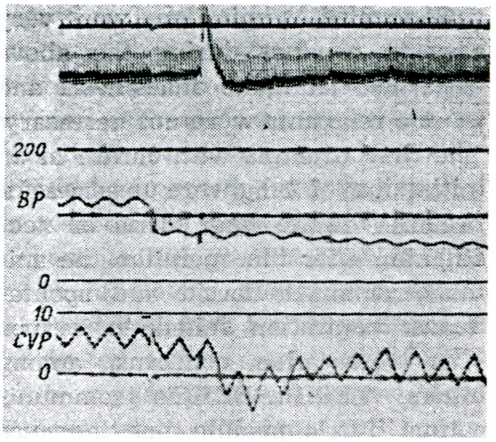

Fig. 5. Haemodynamic disturbances — arterial pressure fall and central venous pressure fall after starting liver bypass.

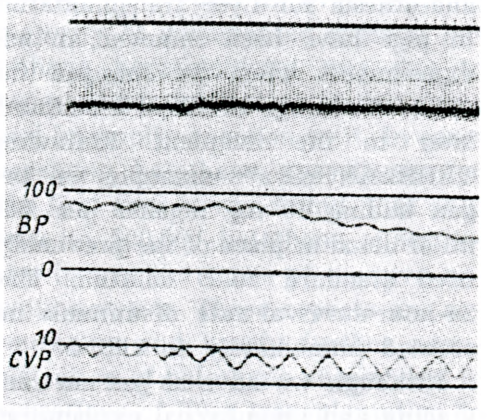

Fig. 6. Haemodynamic disturbances — arterial blood pressure fall and central venous pressure fall after starting perfusion of liver transplant (revascularization).

method of anaesthesia, we resigned even from the administration of 3.4% or 5% solutions of sodium bicarbonate in order to reduce the volume of fluids and we used more concentrated solutions.

Haemodynamic disturbances connected with the technique of the operation and reduced venous return to the heart or with the restoration of

blood flow through the transplanted liver described in the introductory part are shown in Fig. 5 and 6. The fall in arterial blood pressure caused by starting the circulation bypassing the liver could have been compensated by increasing the rate of pump work which was a part of this circulation. The rise in arterial pressure occurring after this increase is presented in the lower curve in Fig. 7.

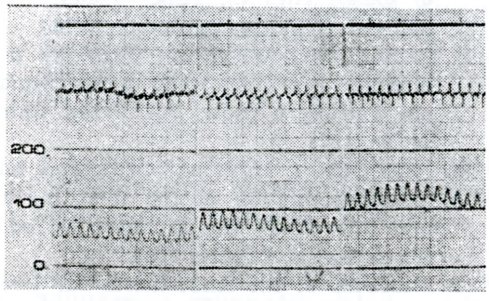

Fig. 7. Hepatectomy phase. Upper curve — reversed T wave. Lower curve — arterial pressure increase after increasing the rate of revolutions of the pump included into the liver bypass system.

It was interesting to study the changes in the bioelectrical activity of the myocardium reflected in ECG. This could not be interpreted exactly because the ECG could be monitored in only one lead II which caused that only scant data was obtained. Nevertheless, it may be assumed that the most frequent changes due to anaesthesia, disturbances of acid-base and electrolyte balance were: sinus pacemaker wandering in the initial stage of anaesthesia (in pre-hepatectomy stage) with a periodic slowing down of the heart rate, ectopic beats, probably of ventricular origin, reversal of T wave in the hepatectomy stage (Fig. 7), grade I atrioventricular block, and intraventricular block with double R deflection (before restoration of perfusion of the liver transplant). These disturbances usually not dangerous, were due to potassium depletion, calcium depletion and acidosis. They have been described by BOWES *et al.* [3], HELL [8], TERBLANCHE [14], and FARMAN *et al.* [5]. Potassium depletion was not supplemented but only calcium chloride 200—300 mg was given which, together with acidosis treatment, caused the return of normal QRS complexes and normal location of P wave (Fig. 4). Other changes of ECG included the appearance of U wave, development of ventricular ectopic beats and ventricular tachycardia which heralded usually a fatal outcome of the experiment and was refractory to pharmacological treatment.

CONCLUSIONS

1. Before starting anaesthesia in pigs the anatomical and physiological peculiarities of their respiratory and circulatory system should be studied with particular attention paid to the topography of upper airways.

2. A mixture of halothane, nitrous oxide and oxygen administered in a semiclosed system may be a good anaesthetic agent for the operation of liver transplantation in pigs.

3. The use of curare at the time of hepatectomy is not indicated in pigs, but further studies are required on this problem.

4. The blood transfused to pigs at the time of operation should be preserved with heparin and not ACD solution.

5. During anaesthesia for liver transplantation in pigs strict limitation of fluid intake is necessary.

6. Transplantation of the liver in pigs causes shifts in the acid-base and electrolyte balance which cause circulatory disturbances and require, therefore, constant monitoring and immediate compensation.

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Received February 4, 1976
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