Recognition and Prevention of Intra-operative Warm-ischaemia in Liver Transplantation

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The single most important factor determining the success of allogeneic hepatic transplantation is the use of a viable donor organ which has not suffered extensive ischaemic injury before recipient implantation (Starzl *et al.*, 1963). The intolerance of the liver to even short periods of total ischaemia is well known. Van Wyk *et al.* (1965b) showed that if the porcine liver was left in the heparinized cadaver for 30 minutes after death gross functional deterioration occurred. However, if the liver is cold-perfused immediately after donor death, and hypothermia is maintained, this period of functional integrity could be greatly extended (van Wyk and Eiseman, 1966).

Much of the difficulty in assessing hepatocellular function after death results from the differential disappearance of metabolic activity. Whereas certain hepatic functions, such as ammonia and bromsulphalein clearance, appear to be resistant to long periods of ischaemia, other indices, such as bile excretion, are relatively more sensitive, and it is probable that more subtle enzymatic pathways fail to function after only a few minutes of ischaemia (van Wyk *et al.*, 1965a). In orthotopic hepatic allotransplantation it is highly desirable that the donor liver assumes most of its metabolic functions as soon as possible after revascularization. For these reasons it would appear that any period of warm-ischaemia between donor harvesting and subsequent restoration of an effective circulation in the recipient be strenuously avoided.

The present study was initiated after a critical reappraisal of our technique for orthotopic hepatic transplantation in the pig (Peacock and Terblanche, 1967). It became clear that a potential period of warm-ischaemia existed between the time the cooled donor liver was placed in the abdominal cavity of the recipient and its final revascularization. No reference could be found in the literature regarding the behaviour of the liver temperature during this period. An experimental model was devised, which we have called a sham transplant, to mimic the conditions pertaining during orthotopic hepatic transplantation. The temperature changes of the liver were studied and methods to prevent a warm-ischaemic interval have been investigated.

MATERIAL AND METHODS

Four groups of experiments have been undertaken.

GROUP 1

An initial series of four sham transplants were performed. The animals used, the anaesthetic technique, and the general preparation were the same as reported by us previously in orthotopic hepatic allotransplantation (Peacock and Terblanche, 1967). The details of the sham transplant model are shown in Fig. 1. The portal and vena caval blood flow through the liver is bypassed by iliojugular and splenojugular bypass lines. The portal vein distal to the junction of the splenic and mesenteric veins, and the infrahepatic vena cava proximal to the entry of the renal veins, are clamped, and all ligamentous attachments of the liver divided, leaving only the hepatic artery and common bile duct intact. The suprahepatic vena cava is cross-clamped below the diaphragm but above the entrance site of the hepatic veins. The hepatic artery and common bile duct are similarly occluded. Venotomies are made in the portal vein and infrahepatic vena cava cephalad to the occluding clamps and $\frac{1}{4}$ -in (6-mm.) atrial cannulae inserted. The liver, which is now ischaemic and totally isolated, is then

perfused via the portal vein with 8 litres of modified Ringer's solution (sodium chloride 6 g./l., potassium chloride 0.4 g./l., calcium chloride (hydrated) 0.146 g./l., magnesium chloride (hydrated) 0.2 g./l., and glucose 50 g./l. Immediately before use 40 ml. of 5% sodium bicarbonate is added). The temperature of this solution is between 5 and 6° C. The effluent from the hepatic veins passes to waste via the caval cannula.

FIG. 1.—Diagram of sham transplant model.

The liver temperature is continually recorded by heat sensor needle electrodes. One is placed immediately beneath the liver capsule and a second at a depth of 3 cm. from the surface. (These are referred to as the subcapsular and core temperatures respectively.) The rectal and the oesophageal temperature of the animal are similarly recorded throughout the procedure. After cold perfusion, which lasts approximately 10 minutes, the liver is left undisturbed and ischaemic for a period ranging from 60 to 120 minutes, and the rise in liver temperature is recorded. (This mimics the period in hepatic transplantation between the time of inserting the cooled donor organ into the abdominal cavity and its final revascularization.) If survival is intended the cannulae are removed, the venotomies are closed, and the liver is recirculated. The bypass lines are then clamped and removed and all incisions closed. The completeness of vascular isolation of this preparation was confirmed by the absence of a blood-stained effluent during the ischaemic period, and by the inability to demonstrate methylene blue in the liver effluent after a large dose had been administered systemically to the animal.

GROUP 2

The behaviour of the liver temperature was also studied in porcine hepatic autotransplants and orthotopic allogeneic transplants during the intraoperative ischaemic period.

GROUP 3

The possibility that a portion of the temperature gain of the liver during this ischaemic period could be attributed to endogenous hepatic metabolic heat production was examined in a single experiment in which two pigs of the same weight and species were subjected to sham transplants under identical conditions. In one of these animals 200 mg. of potassium cyanide was added to the liver perfusion fluid to ensure hepatic metabolic paralysis, and the hepatic temperature gain of the two animals was compared.

GROUP 4

A further group of four sham transplants was then performed in which the liver was enclosed in a substance of low thermal conductivity

in an attempt to prevent excessive heat gain during the ischaemic intraoperative period. The substances used were thin sheet expanded polystyrene ($\frac{1}{8}$ in.), aluminized polyethylene terephthalate film (Melinex—Messrs. E. S. A. Robinson Ltd.), a sandwich of multiple thin fibreglass sheets between Melinex, and, finally, expanded closed cell polyethylene (Plastazote—Expanded Rubber and Plastics Ltd.). This latter substance, and the substance we now prefer, which is expanded closed cell ethyl vinyl acetate (Evazote—Expanded Rubber and Plastics Ltd.), is used in sheets of $\frac{1}{4}$ -in. (6-mm.) thickness. These are vacuum-moulded to correspond to the shape of the anterior and posterior surface of the pig's liver. Sheets are heated to 65° by immersing them in water at this temperature for two minutes. The heated sheet is then immediately applied over the mouth of the evacuated vacuum flask with a rim diameter of 9 in. (23 cm.), and the substance allowed to mould to form a shallow hemisphere. A second sheet is then moulded in a similar way but made asymmetrical by finger pressure during moulding to correspond to the shape of the posterior surface of the liver.

Sterilization of the insulator is effected by immersion in 0.5% aqueous Hibitane (chlorhexidine) solution for two hours, low temperature steam sterilization, or gamma-irradiation. The cooled donor liver is then placed between these two casings (Fig. 2), and

FIG. 2.—Two casings of expanded polyethylene enclosing donor liver—seen side on.

FIG. 3.—Completed casing with enclosed donor liver in position in abdominal cavity of recipient animal. Note the temperature probes and the aperture for access to suprahepatic vena cava, which is about to be anastomosed.

they are secured together by stapling with an office stapler. The excess is then trimmed away, and apertures are cut to allow access to the suprahepatic vena cava, portal vein, hepatic artery, and infrahepatic vena cava. Temperature probes are inserted into the liver through a small aperture cut into the anterior casing, and the liver within this enclosing insulator is placed within the abdominal cavity of the recipient and the vascular anastomoses begun (Fig. 3). The casing is cut away only when the liver has been recirculated. We are now using this routinely in pig hepatic transplantation.

RESULTS

Fig. 4 shows the mean subcapsular and core temperatures of a series of four sham transplants. With cold perfusion

the core temperature falls rapidly to a mean 8° C. The subcapsular temperature does not fall as low, presumably owing to less efficient peripheral perfusion. Once perfusion has been completed, however, there is a surprisingly rapid gain in the liver temperature while still totally ischaemic. The core temperature rose at a mean initial rate of 0.7° C. per minute, and the subcapsular temperature at a mean rate of 0.8° C. per minute. We have taken 25° C. as the temperature below which Moore (1965) states the ischaemic liver is "fairly safe." The subcapsular and core temperatures remain below this level for a mean time of only 15 and 28 minutes respectively. We

FIG. 4.—Mean hepatic core (—) and subcapsular (.....) temperature changes in a series of four non-insulated sham transplants.

have since confirmed this pattern of rapid rewarming during the ischaemic period in hepatic autotransplants and allotransplants. In our hands total revascularization of the donor liver takes approximately one hour if no difficulties are encountered. This entails a period of rapid rewarming above 25° C., totalling approximately 45 minutes in the case of the subcapsular tissues and 32 minutes in the case of the core tissues. This represents a considerable warm-ischaemic interval, which is often further prolonged when technical difficulties delay revascularization.

This rapid gain in temperature is not due to incomplete vascular isolation of the liver, as the temperature gain in the sham transplant series was similar to that observed in hepatic autotransplants and allotransplants (Fig. 5). Likewise, in the

FIG. 5.—Similar mean gain in hepatic core (—) and subcapsular (.....) temperatures between the series of four sham transplants and a series consisting of two autotransplants and two allotransplants (hepatic core (—) and subcapsular (.....) temperatures). Both series non-insulated.

preparation in which the liver was paralysed with potassium cyanide there was no significant difference in the rate of temperature gain of the liver, suggesting that endogenous metabolic heat production is not a significant factor. Thus these preliminary studies suggest that the observed heat gain of the ischaemic liver is due to conduction and radiation of heat from the surrounding warm tissues of the recipient.

Fig. 6 shows the mean core and subcapsular temperature of a series of four orthotopic hepatic allotransplants which had been insulated with an expanded polyethylene casing, as

FIG. 6.—Mean hepatic core (—) and subcapsular (.....) temperatures of the non-insulated sham transplant series as compared with the mean core (---) and subcapsular (----) temperatures of a series of four hepatic allotransplants insulated with expanded polyethylene.

compared with the mean temperature changes of the non-insulated sham transplant series. The rate of temperature gain of all parts of the liver has been reduced to a mean rate of 0.1°C . per minute by insulation. The donor liver can be maintained below 25°C . for up to 120 minutes when using this technique. Both polyethylene and ethyl vinyl acetate exhibit the same insulating properties, and both have proved superior to any other insulating material investigated.

DISCUSSION

While the intraoperative period of warm-ischaemia and its prevention by the use of an insulator has previously been described in renal transplantation (Markland and Parsons, 1963), no reference could be found in the literature to similar observations in the field of hepatic transplantation.

Any method to prevent intraoperative warm-ischaemia must of necessity be simple. The use of profound total body hypothermia of the recipient animal was discarded as adding further risks and complexities to an already complex procedure. Similarly, continuous cold perfusion of the donor liver during reimplantation was considered, but it was discarded because of the difficulties in collecting hepatic vein effluent and the increased difficulty with vascular anastomoses that would result.

The use of casings of polyethylene and ethyl vinyl acetate has proved an extremely simple way of maintaining liver hypothermia, and operative exposure has not been limited in any way. These substances have the further advantages of being cheap, easy to vacuum-mould, and strong but malleable, and they can be sterilized by low-temperature steam or preferably with gamma-irradiation. Their insulating properties result from the closed cell structure, which renders them non-porous to fluids.

Our own experience in experimental hepatic transplantation has suggested that many of the syndromes peculiar to this procedure are due wholly or partly to an ischaemically damaged

liver. These include an uncontrollable bleeding tendency when the graft is revascularized, sudden severe metabolic acidosis, hypotension and cardiac arrhythmias after graft revascularization, and early metabolic failure of the liver, especially as regards maintenance of blood glucose levels. Two further observations stress the dangers of ischaemia: van Wyk *et al.* (1965b) showed that, though livers from fresh pig cadavers were sterile, bacteria could be isolated from livers left within the cadaver for 30 minutes or longer after death despite antemortem administration of antibiotics. The intriguing suggestion that ischaemically damaged tissues are more antigenic stems from the work of Lewis and Aptekman (1952) and Foley (1953), who showed that in chemically induced tumours the liberation of antigen by the induction of ischaemic necrosis led to a heightened immunological response and rendered the animal refractory to further challenge with the same tumour.

Najarian *et al.* (1966) produced some evidence to suggest that ischaemically damaged renal allografts provided a greater antigenic stimulus than non-damaged controls, and Weir (1967) elucidated the mechanism whereby damaged hepatic cells release antigenic material. This latter hypothesis would add weight to the argument for the avoidance of ischaemic damage if considered in conjunction with the suggestion that, in the pig, the allografted liver appears to be an immunologically favoured organ, and, even in the non-immunosuppressed animal, rejection, if it occurs at all, is a weak slow process (Hunt, 1967; Calne *et al.*, 1967). Ischaemia is further implicated by the work of Swenson *et al.* (1967), who were able to demonstrate a variety of histological changes in the livers of dogs which had been subjected to varying periods of hepatic ischaemia, many of which were very similar to those previously attributed to rejection in this species.

It is now clear that many of the failures in both experimental and human hepatic allotransplantation are directly attributable to the poor quality of the liver used. To achieve the ideal of a perfect donor organ every effort must be made to avoid ischaemic injury between the time of donor death and recipient implantation. Though conditions in the experimental laboratory are such that the donor liver can be rapidly removed from a live animal and relatively rapid revascularization achieved, in human transplantation liver ischaemia may for many reasons be considerably prolonged. The inevitable period of warm ischaemia inherent in the obtaining of cadaver donor livers results in part of the acceptable ischaemic time being used up, and any device that can prevent a further period of warm-ischaemic injury before revascularization would seem worthy of study. The rapid rate of rewarming in the intraoperative ischaemic period which we have observed is a source of potential damage, and we feel that it is best avoided by the use of a liver insulator. Precisely how damaging this period is, and the degree of protection afforded by an insulator in terms of hepatocellular function, are at present under study and will furnish material for a future report.

SUMMARY

A study has been made of the temperature changes of the liver in the intraoperative period of porcine hepatic transplantation. A very rapid gain of temperature has been observed in the period between placing the cooled donor organ into the abdominal cavity of the recipient and its final revascularization. Our initial observations suggest that this is due to heat gain from the surrounding warm tissues of the recipient. We regard this as a period of potential liver damage, and it can be prevented simply by the use of a liver insulator.

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REFERENCES

- Calne, R. Y., *et al.* (1967). *Brit. med. J.*, **4**, 645.
Foley, E. J. (1953). *Cancer Res.*, **13**, 578.
Hunt, A. C. (1967). *Colston Papers*, vol. 19, "The Liver," p. 337. London.
Lewis, M. R., and Aptekman, P. M. (1952). *Cancer*, **5**, 411.
Markland, C., and Parsons, F. M. (1963). *Brit. J. Urol.*, **35**, 457.
Moore, F. D. (1965). *Surgery*, **58**, 129.
Najarian, J. S., May, J., Cochrum, K. C., Baronberg, N., and Way, L. W. (1966). *Ann. N.Y. Acad. Sci.*, **129**, 76.
Peacock, J. H., and Terblanche, J. (1967). *Colston Papers*, vol. 19, "The Liver," p. 333. London.
Starzl, T. E., Marchioro, T. L., von Kaulla, K. N., Hermann, G., Brittain, R. S., and Waddell, W. R. (1963). *Surg. Gynec. Obstet.*, **117**, 659.
Swenson, O., Grana, L., Inouye, T., and Donnellan, W. L. (1967). *Arch. Surg.*, **95**, 451.
van Wyk, J., and Eiseman, B. (1966). *Gastroenterology*, **51**, 207.
van Wyk, J., Liem, D. S., and Eiseman, B. (1965a). *Surgery*, **58**, 120.
van Wyk, J., Tait, I., and Eiseman, B. (1965b). *Surgery*, **58**, 374.
Weir, D. M. (1967). *Lancet*, **2**, 1071.

Medical Memoranda

Atrial Flutter with Block—Contraindication to use of Lignocaine

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Much interest has recently been aroused in the use of lignocaine as an antiarrhythmic agent (*Lancet*, 1967). This drug has been shown to be of value in the treatment of ventricular arrhythmias and atrial ectopic beats occurring after myocardial infarction, cardiac surgery, and direct-current cardioversion of atrial fibrillation (Hitchcock and Keown, 1959; Minuck, 1965; Spracklen *et al.*, 1968). Lignocaine has also been recommended in the treatment of digitalis-induced tachycardias (Katz and Zitnik, 1966; Greenspan *et al.*, 1966). It would therefore seem to be suitable for use in patients with a rapid arrhythmia in the face of an unclear history of digitalis administration. For this reason we wish to record a dangerous complication of the use of lignocaine—namely, the conversion of atrial flutter with 2:1 atrioventricular block to 1:1 conduction.

CASE REPORT

A 40-year-old housewife with chronic rheumatic heart disease was admitted to hospital on 5 October 1967 for treatment of a persistent tachycardia. In 1954 she had a closed mitral valvotomy for tight mitral stenosis. She was improved considerably by the operation and remained in sinus rhythm.

In April 1967 she became breathless and was started on digitalis. In August her symptoms increased and her treatment was changed to digoxin 0.25 mg. t.d.s. She was seen in the outpatient department on 28 September, when she was observed to have a regular tachycardia of 140 beats a minute. Her jugular venous pressure was markedly raised and the signs of mitral stenosis were present. An electrocardiogram showed atrial flutter with an atrial rate of 300 a minute and 2:1 atrioventricular block. The rhythm was unaffected by carotid sinus pressure. Digoxin was stopped and

FIG. 1.—Atrial flutter with 2:1 block in upper tracing converted to 1:1 conduction (lower tracing) after intravenous lignocaine.

hydrochlorothiazide with potassium supplements begun. One week later the cardiac findings were unchanged though the jugular venous pressure had fallen to normal.

The patient was admitted to hospital. Her plasma potassium at this time was 4.6 mEq/l. It was thought that the arrhythmia could still be digitalis-induced, and 100 mg. of lignocaine was given intravenously. This resulted in slowing of the atrial rate to 250 a minute with 1:1 conduction (Fig. 1). With this the patient complained of dizziness, sweated profusely, and had an unrecordable blood pressure. The previous rhythm returned spontaneously after two minutes.

Two weeks later sinus rhythm was restored with direct-current shock. Immediately after cardioversion there were frequent atrial ectopic beats. These were readily abolished by intravenous lignocaine (100 mg.) (Fig. 2). Clinical examination showed tight mitral stenosis and mild aortic incompetence. Mitral revalvotomy was successfully carried out on 26 October 1967.

FIG. 2.—Atrial ectopic beats after cardioversion abolished by intravenous lignocaine.

COMMENT

Lignocaine is generally thought to be less effective in the treatment of atrial than ventricular arrhythmias. In our case the possibility of digitalis intoxication, though rarely presenting as atrial flutter, was seriously considered; hence the decision to give intravenous lignocaine. The patient responded by developing 1:1 conduction with a ventricular rate of 250.

The mechanism of action of lignocaine in cardiac arrhythmias is not clear. Clinically it appears to have an action similar to that of phenytoin, quinidine, and antazoline. All these drugs decrease myocardial excitability and prolong conduction time. Lignocaine does not, however, seem to depress contractility of cardiac muscle in the doses recommended (Friedberg, 1966; Spracklen *et al.*, 1968). The production of 1:1 conduction in atrial flutter has been reported with quinidine, antazoline, and phenytoin (Dreifus *et al.*, 1964; Grissom *et al.*, 1967). In the above case lignocaine appears to have slowed the atrial rate without increasing the atrioventricular block. This resulted in 1:1 conduction.