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Long-duration xenogeneic extracorporeal pig liver perfusion with human blood

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Abstract Hepatic xenografts can tolerate hyperacute rejection owing to their lower susceptibility to humorally mediated injury. We investigated the possibility of long-duration xenoperfusion without immunologically controlling natural antibodies or complements. Pig livers were perfused for 9 h with human blood (Group 1) or pig blood (Group 2). Physiological conditioning and administration of prostaglandin E_1 and insulin was characteristic of our system. The portal vein and hepatic artery pressure and bile production did not significantly differ between the two groups. Despite a gradual decrease throughout the perfusion, overall oxygen consumption was significantly higher in Group 1. Liver enzymes were released at higher levels in Group 1. Histological examination revealed intact hepatic architecture in Group 2, while in Group 1 interlobular morphology was severely damaged by endothelial disruption, although hepatic sinusoidal architecture was preserved. It is concluded that, despite biochemically and histologically confirmed tissue injury, graft viability was well-maintained in xenoperfusion even without immunological manipulations.

Key words Xenoperfusion · Liver · Hyperacute rejection · Prostaglandin E_1

Introduction

In the 1960s and 1970s, extracorporeal liver perfusion (ECLP) to support failed liver functions was extensively studied [1, 5] but this procedure was abandoned for a while because of poor outcome and the advent of orthotopic liver transplantation. The current shortage of donors has shed new light on the concept of ECLP as a bridge to liver transplantation and as a diagnostic technique to assess whether patients with untreatable fulminant hepatic failure will recover from neurological dysfunction by transplantation. Porcine livers, though discordant, are considered to be the most suitable graft in terms of size, disease transmission, domestication, and anatomical similarities. However, unlike concordant xenografts, they are inevitably injured by xenogeneic hyperacute rejection mediated by preformed xenoreactive natural antibodies and complements. It is crucial to suppress this xenoreactive immune response for successful discordant xenotransplantation. Immunological manipulations for the control of hyperacute rejection may not necessarily be required in short-term xenogeneic ECLP, however, as long as the graft can function long enough to assist failed liver functions, since the liver is less susceptible to humorally mediated injury than the kidney and the heart. In this study, we explored the possibility of long-duration xenoperfusion without immunological manipulations.

Materials and methods

Ten Large White pigs from 16 to 21 kg were used as graft donors. At the procurement, the liver was completely freed from the surrounding tissues through incising the diaphragm circularly around the suprahepatic inferior vena cava (IVC), and flushed out through

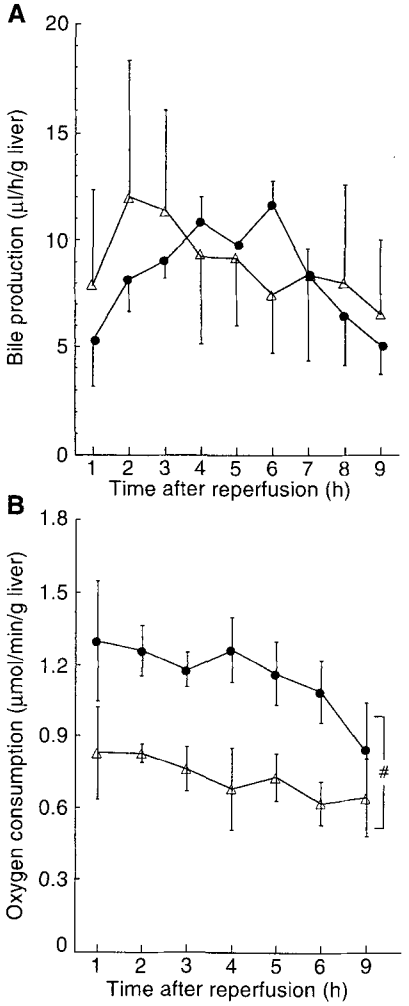

Fig. 1 **A** Bile production was not significantly different between Groups 1 (●) and 2 (Δ). **B** Oxygen consumption of Group 1 (●) was significantly higher than that of Group 2 (Δ) (# P <0.001)

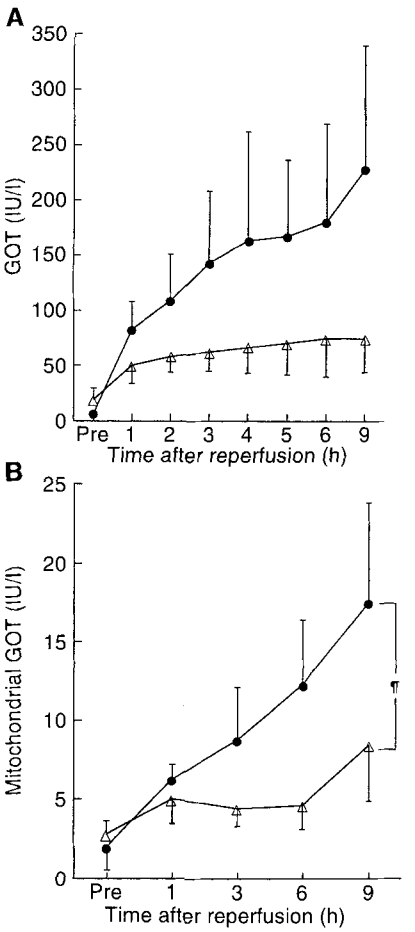

Fig. 2 A, B The changes in the release of liver enzymes in Groups 1 (●) and 2 (Δ). **A** Glutamate oxaloacetate transferase *(GOT)* release continuously increased and tended to be higher in Group 1, but not significantly so. **B** Mitochondrial GOT was significantly higher in Group 1 ($P < 0.05$)

the portal vein (PV) and the hepatic artery (HA) with 1.5 l of cold lactated Ringer's solution containing heparin. The liver was perfused via PV and HA by separate roller pumps. Total hepatic blood flow was kept constant at 1 ml/min per g liver and HA flow was adjusted to approximately 25 % of PV flow. The blood outflow was allowed through infrahepatic IVC by a hydrostatic pressure gradient of 15–20 cm H_2O. The perfusate consisted of fresh human blood or pig blood diluted with lactated Ringer's solution containing low molecular dextran, with a subsequent hematocrit value of 25–30 %. Two membrane oxygenators with heat exchanging function (Menox AL-2000, Kurare, Okayama, Japan) were used to provide a partial oxygen pressure (pO_2) of between 45 and 60 mm Hg for PV and a pO_2 of between 100 and 150 mm Hg for HA, similar to physiological levels. The liver was immersed in a 37 °C heating chamber to prevent the compression of portal vessels by the liver mass, and the temperature of the perfusate was maintained between 35.8 °C and 37.5 °C. Prostaglandin E_1 (PGE_1), supplied by Ono (Japan), and regular insulin (Humalin R, Shionogi, Tokyo, Japan) were administered continuously via PV at the rate of 25 mg/h and 1 IU/h, respectively.

In Group 1, pig livers were perfused with human blood ($n = 5$), and in Group 2, perfused with pig blood ($n = 5$). The livers were perfused for 9 h.

Graft viability and hepatocellular injury were verified by PV and HA pressures, bile production and oxygen consumption, and by the release of glutamate oxaloacetate transferase (GOT) and mitochondrial GOT (mGOT). For histological examination, liver tissues were stained with hematoxylin and eosin and analyzed by light microscopy.

Values are expressed as means ± SD. The statistical analysis was performed by two-way repeated-measure analysis of variance (ANOVA). A P-value of less than 0.05 was considered to be statistically significant.

In this animal experiment, the "Principles of laboratory animal care" (NIH publication 86-23, 1985) was followed.

Results

Cold ischemic time could be considerably shortened by our manipulation of organ procurement in both groups (24.3 ± 3.3 min in Group 1 and 23.8 ± 4.7 min in Group 2). The fluctuations of PV and HA pressures did not significantly differ between the two groups. They did not exceed 20 cm H_2O and 180 mm Hg, respectively, even in Group 2. No significant differences in bile production were found (Fig. 1 A). Oxygen consumption was significantly higher in Group 1 ($P < 0.001$, Fig. 1 B), but it tended to gradually decrease in both groups. Regardless of this fluctuation, oxygen consumption remained at a high level even at the end of perfusion (0.83 ± 0.21 μmol/ml per min in Group 1 and 0.64 ± 0.16 μmol/ml per min in Group 2). The release of GOT and mGOT continuously increased and tended to be higher in Group 1, and significant differences were found in mGOT ($P < 0.05$, Fig. 2).

Histological examination revealed that during the early phase of the perfusion, mild sinusoidal dilatation and neutrophil infiltration were observed in both groups. Thereafter, no marked hepatocellular damage was evident in Group 2, while in Group 1 severe periportal edema and extensive interlobular hemorrhage developed in addition to extravasation of fibrinous exudate. In spite of interlobular morphological deterioration, the hepatic sinusoidal architecture was comparatively preserved without massive hepatocellular necrosis.

Discussion

Xenogeneic hyperacute rejection causes endothelial disruption and loss of anticoagulant properties, thus leading to increased vascular permeability, interstitial hemorrhage, intravascular thrombosis, and rapid destruction of the graft [2]. Because of this, the xenoperfused kidney or heart would usually cease to function within minutes when unmanipulated, but the liver is specifically less susceptible to humorally mediated rejection and there is evidence that discordant hepatic xenografts may tolerate hyperacute rejection [8]. This may be related to the liver's ability to neutralize and inactivate cytotoxic antibodies by the release of soluble MHC antigens and by Kupffer cell absorption, and to the unique double blood supply of the hepatic sinusoid, which confers protective effects from ischemic damage [3, 4]. The comparatively intact architecture of the hepatic sinusoid in xenoperfused livers in this study represents this peculiar characteristic. Considering this resistance of the liver to humorally mediated injury, xenogeneic ECLP without any immunological treatment seems to be possible for a limited period.

For successful, longer-duration xenoperfusion without immunological manipulation, three problems need to be settled: deterioration of the graft viability during procurement and preservation; damage to the graft induced by ischemia reperfusion injury and extracorporeal perfusion; extracorporeal perfusion conditions far from the physiological environment, leading to inhomogeneous perfusion and hypoxia of the graft. Cold ischemic time and ischemia reperfusion injury were reduced as much as possible by reconsidering the procurement procedure. Intraportal administration of insulin can exert a beneficial influence on hepatic mitochondrial energy metabolism and has the potential to enhance the ATP-generating systems in the hepatic mitochondria [9], and PGE_1 not only ameliorates hepatic microcirculatory disturbances by vasodilatation and by inhibiting platelet aggregation [6] but also has direct cytoprotective effects on hepatocytes through stabilization of membrane microviscosity [7]. These cytoprotective properties were considered to bring about stable graft viability even in xenoperfusion. It is very difficult to create completely physiological conditions in extracorporeal circumstances, but by modifying the placement of the liver graft and regulating pO_2, and by simplified pressure gradient drainage of outflow blood, homogeneous perfusion was provided in our system.

It is concluded that, despite biochemically and histologically confirmed tissue injury, graft viability was well-maintained in xenoperfusion even without immunological manipulations. Artificial liver support devices could play an increasing role in the success of liver transplantation but no artificial livers which can completely take over all metabolic functions are in experimental or clinical use. Our newly designed ECLP could be a useful tool to provide a sufficient, stable, supporting environment for a restricted duration of less than 9 h.

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