

Appropriate Donor Size for Porcine Liver Xenotransplant

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Abstract

Objectives: Owing to an imbalance between demand and supply, which is more prominent in pediatric transplant, every year more patients lose their lives on waiting lists. In addition to the use of deceased-donor split and living-donor organs, xenotransplant could provide a solution if associated problems, such as immunologic and physiologic ones, are solved. This study sought to analyze the surgical aspects for liver xenotransplant in a porcine model.

Materials and Methods: Landrace pigs (n=22, 23 to 37 kg) underwent a laparotomy under general anesthesia. The hepatic hilum was prepared and the common bile ducts, common hepatic artery, portal vein, supra- and infrahepatic inferior vena cava were identified. The length and diameter of each vessel and bile duct and the weight of the liver were measured.

Results: Pearson tests showed a clear correlation between the increase of the pigs' weight and the livers' weight, and the length of the vessels and the bile ducts. We did not find a clear correlation between the increase of the pigs' liver weight and the diameters of the vessels and the bile duct.

Conclusions: As the first reporting, this study on

xenotransplants from the surgical point of view, we postulate that it could be possible to estimate the size of the liver and the proper length of its vessels and bile duct by weighing only the pigs. It was not feasible to match the diameter of mentioned structures by the livers' weight. However, the weight of pig's liver as well as vascular anatomy of pigs appeared to be suitable alternative for the human liver.

Key words: Xenotransplant, Liver transplant, Experimental surgery, Organ compatibility, Liver weight

Introduction

More than forty years have been passed since the first human liver transplant by Thomas E. Starzl.¹ The successful allotransplant has led to an established therapy for patients with end-stage liver diseases as a great success in the twentieth century.² However, the number of patients requiring liver transplant far outweighs the availability of suitable graft.³ Also, an increasing number of patients, being referred for liver transplant, have created a crisis in donor organ availability.^{4,5}

Despite performing many educational efforts by the public and the media during recent years, deceased-donor organ donation has not increased substantially during the past several years and it is unlikely that the supply of donor organs could achieve a level to meet demands. Moreover, using marginal deceased donors is not without risk to the patients, since the use of marginal deceased donors is associated with higher patient mortality.⁵ The insufficient number of donor organs limits the application of transplant and leads to unnecessary loss of life.⁶ Although part of this shortage also has been being compensated by different clinical solutions such as live-organ donors, split liver

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Acknowledgements: This study has received no grants and/or financial support.

M.S., H.F., M.E., A.K., S.F., M.G., N.R., and M.H. participated in the experimental operations. A.M., M.S., M.E., and H.F. participated in the design of the study and drafted the manuscript. M.G., M.H., and N.R. performed data collection. A.K. and S.F. performed the statistical analysis and revised the manuscript. J.S. was involved in drafting the manuscript and revising it critically for important intellectual content. M.S., M.E., and A.M. revised the manuscript and gave final approval of the version to be published. All authors read and approved the final manuscript.

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Experimental and Clinical Transplantation (2012) 2: 148-153

transplants, and domino transplants, these sources are still insufficient.⁷ The extreme inadequacy of available organs for liver transplant is encouraging researchers to look at other alternatives such as hepatocytes transplant, hepatocytes reactors, stem cells, and xenotransplant. Use of these inventory methods are still far from clinical use, but their feasibility has been reported.⁸⁻¹⁰

Xenotransplant means the transplant across species, which is increasingly being viewed as a potential solution to the problem of severe shortage of organs. Although xenotransplant has this potential to be an optimal solution for organ shortage, as it could provide an unlimited supply of organs,¹¹ many studies have shown that using animals for human transplant have many clinical limitations at this moment, such as hyperacute rejection, physiologic incompatibility, and the risk of transmission of infection.³ Before all the mentioned problems, one question should be answered: What are the surgical difficulties owing to size mismatch? Regarding this, from the technical point of view, the size of the liver, length, and diameter of vessels, as well as the discrepancy of the bile duct size, could be a challenge for transplant surgeons. To answer these questions, we analyzed the size of the liver, the length, and diameter of liver vessels, as well as the bile duct in different pig weight categories.

Materials and Methods

All study protocols were approved by the institution's animal welfare regulatory committee, and/or all protocols were in conformity with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health 86-23, revised in 1985. Our study was undertaken on 22 Landrace pigs, with the body weight ranging between 22.9 and 37.7 kg. All operations were performed by the same team of surgeons, who were experienced in experimental transplant surgery. All experiments were performed under general anesthesia and all the animals were killed after the experiments.

Anesthesia

After premedication by intramuscular injection of azaperone (1 to 2 mg), pigs were anesthetized with an application of standard opioid protocol including ketamine (10 mg/kg), midazolamine hydrochloride (0.25 mg/kg), and pancuronium bromide (0.08 mg/kg). Anesthesia was maintained by

intravenous infusion of fentanyl (0.05 mg/kg/h). Pigs were placed on a mechanical respirator (oxygen: 0.5 to 1.0 L/min, nitrous oxide/isoflurane: 1.5 to 2.0 L/min). Body temperature was stabilized above 36°C by applying heating blankets (Warm-Touch, Mallinckrodt Inc, Hazelwood, MO, USA).

Surgical procedure, monitoring, and evaluated parameters

After a midline abdominal incision and preparation of the hepatic hilum (Figure 1), the common bile duct, common hepatic artery, portal vein (Figure 2), and suprahepatic and infrahepatic inferior vena cava (Figure 3) were identified. During the procedure, the systemic mean arterial pressure and central venous pressure were recorded continuously via indwelling catheters and fed into the common carotid artery and internal jugular vein. The heart rate was continuously monitored by surface ECG recording (Hellige GmbH, Freiburg, Germany). Additionally, blood flow in the common hepatic artery and portal vein was monitored during the procedure by

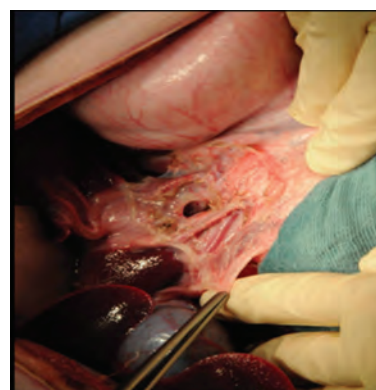


Figure 1. View of the prepared liver hilum.

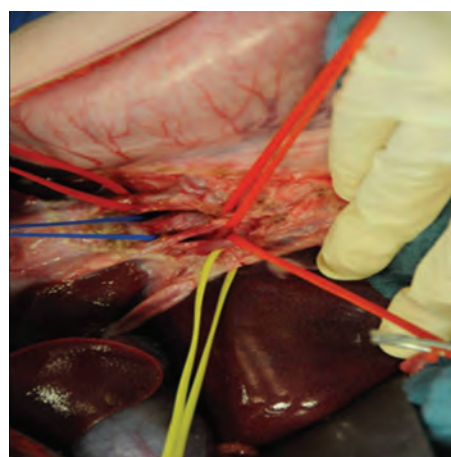


Figure 2. Preparation of the common bile duct (yellow loop), common hepatic artery (red loop), and portal vein (blue loop).

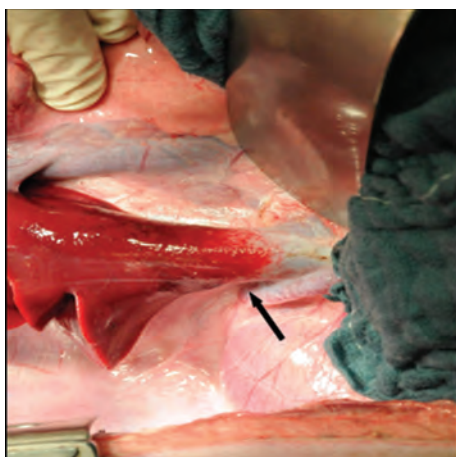


Figure 3. The prepared infrahepatic inferior vena cava (arrow).

Doppler flow meter probes (Transsonic System Inc, New York, NY, USA). After measuring the required parameters, the liver was removed and weighed. The length (L) and diameter (D) of suprahepatic and infrahepatic inferior vena cava, common hepatic artery, portal vein, and common bile duct were measured. The liver's weight also was measured after trimming off the excessive remnants of the vena cava and removing the gallbladder by cholecystectomy. The mean of measurements was calculated, and the ratio of the liver weight to the pig weight was identified.

Animal rights

The study protocol was approved by the German Committee for Animal Care, Karlsruhe, Germany. During the experiment, all the animals received human care according to institutional guidelines established for the Animal Care Facility at the University of Heidelberg.

Statistical Analyses

Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 16.0, IBM Corporation, Armonk, New York, USA). For quantitative data, medians and ranges were calculated. The quantitative data were reported as relative frequencies and percentages. In this study, the significant variables analysis was entered into a chi-square and *t* test. *P* values < .05 were considered statistically significant.

Results

Twenty-two Landrace pigs, with the mean weight of 27.5 kg (range, 22.9 to 37.7 kg) and mean of total

livers weights of 696.6 ± 91.3 g were evaluated. The mean heart rate, arterial pressure, and central venous pressure were 87 ± 8 beats/min, 62 ± 6 mm Hg, and 3.8 ± 1.3 mm Hg. Furthermore, the mean hepatic arterial flow as well as portal venous flow were 39 ± 12 , 134 ± 52 mL/100 g/min. Our study shows a clear correlation between the pig's weight and the liver's weight, which means that by increasing the pig's weight, the weight of the liver increases correspondingly (Figure 4). The mean of the length and diameter of the supra- and infrahepatic inferior vena cava, hepatic artery, portal vein, and bile duct are showed in Table 1.

Comparing the pig's weight with the length and diameter of the hepatic artery, we can see that by increasing the pig's weight, the length of the hepatic artery increases, but there is no significant change in the hepatic artery diameter according to the pig's weight (Figure 5). By looking at the relation between the pig's weight and the length and diameter of the portal vein, it is obvious that the length of the portal vein increases by increasing the pig's weight, but the

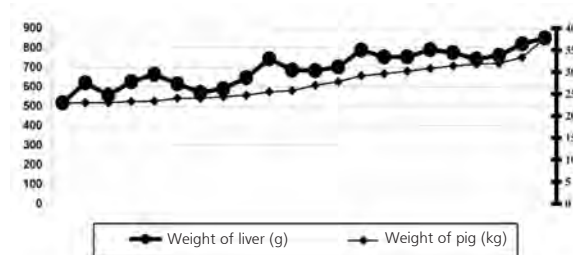


Figure 4. Correlation between the pig and liver weight.

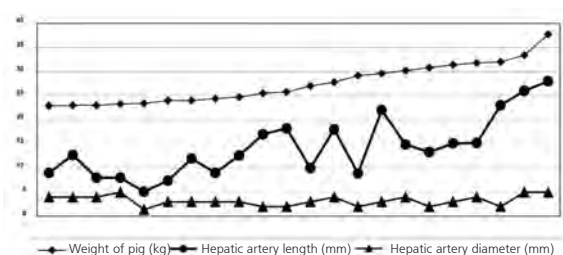


Figure 5. Relation between the length and diameter of the hepatic artery with the weight of the pigs.

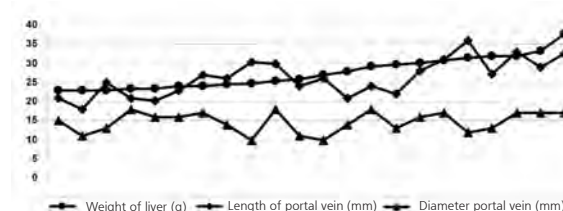


Figure 6. Relation between the length and diameter of the portal vein with the weight of the pigs.

diameter of the portal vein does not show any concordance with the pig's weight (Figure 6). Similarly, the length of the inferior vena cava shows a clear correlation with the increase of the pig's weight but the diameter does not change with increase of the pig's weight (Figure 7). The same results also were found regarding the bile ducts' length and diameter. We did not notice any significant increase in the bile duct diameter by increasing the pig's weight but the length of the bile duct increase when the pig's weight increases (Figure 8). These results showed that there is a clear correlation between the weight of the pigs and the weight of the liver, also the length of the vessels, and the bile duct (Table 2). We could not find a clear correlation between the weight of the pigs and the diameters of the vessels as well as the bile duct (Table 2).

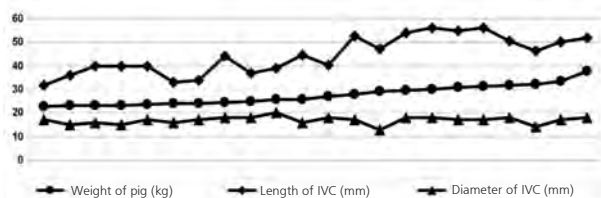


Figure 7. Relation between the length and diameter of the inferior vena cava with the weight of the pigs.

Abbreviations: IVC, inferior vena cava

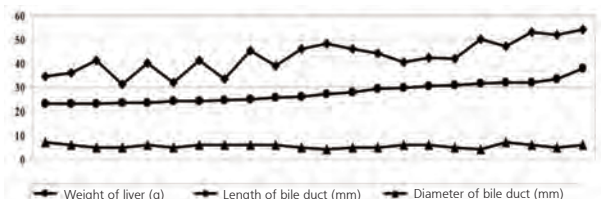


Figure 8. Relation between the length and diameter of the bile duct with the weight of the pigs.

Discussion

Xenotransplant using pig organs could resolve the shortage of suitable donor organs.^{12, 13} If pig organs could be transplanted successfully into human patients, the advantages would be great. In this regard, immediate availability, perfect metabolic condition, adequate size-matching and hepatocyte mass, and freedom from potentially pathogenic microorganisms should be sought.¹⁴ However, clinical application of xenotransplant is still far limited in large part by the immunologic barriers,^{3, 15} the risk of transmission disease,^{3, 16-19} and physiological incompatibilities.^{3, 15, 20} Furthermore, ethical and psychological issues should be considered a potential problem. Additionally, determining the approximate organ weights and dimension (size-matching) for each recipient are 2 major concerns in xenotransplant.

In xenograft rejection, the innate and acquired responses both are involved. It seems that the innate xenograft response is more difficult to control than the acquired one.⁵ Although we still have problems with acute humoral xenograft rejections, which lead to destruction of the organ within days or weeks. This can be solved with application of potent immunosuppressive drugs.^{5, 21} Liver xenografts may be relatively resistant to antibody-mediated rejection and seem to be less susceptible to hyperacute rejection than kidney or heart xenografts. Therefore, it might be a greater chance for long-term success of liver xenotransplant.^{20, 22} Another crucial questions is: Whether the pig organ is physiologically compatible with the human's environment or not, and will 1 gram of pig liver work physiologically the

Table 1. The results of the measurement of the pigs' weight, the pigs' liver weight, and the parameters of its vessels, and the bile duct (L=length, D=diameter).

Mean weight of pigs (kg)	Mean weight of liver (g)	Supra- and infrahepatic IVC (mm)				Hepatic artery (mm)		Portal vein (mm)		Bile duct (mm)	
		L	D	L	D	L	D	L	D	L	D
27.5	696.6	91.9	14.9	44.5	16.8	14.2	3.2	26.1	14.7	42.6	5.54

Abbreviations: IVC, inferior vena cava

Table 2. Pearson correlation between weight of the pigs and weight of the livers as well as vascular and bile duct parameters regarding the length (L) and the diameter (D) of the remaining structures.

Correlation (with pigs' weight)	Weight of liver	Supra- and infrahepatic IVC				Hepatic artery		Portal vein		Bile duct	
		L	D	L	D	L	D	L	D	L	D
	0.89	0.52	0.09	0.79	0.10	0.78	0.19	0.64	0.21	0.78	-0.06

Abbreviations: IVC, inferior vena cava

same as 1 gram of human's does? Evolution has demonstrated that porcine organs with the same size are just as efficient as human organs.²³ However, 1 potential problem is that some of the protein-protein reactions between species do not function well.²⁴ Additionally, the life span of pigs is less than that of humans. Therefore, the pig's organ should not be considered to last for a human lifetime.¹⁵

Treatment is further limited by the possibility of transmitting new infectious agents from the graft into the recipient.^{3, 6} A particular concern has been raised because the transfer of porcine endogenous retroviruses, as a part of pigs' genome, has shown to be acceptable with a xenograft.^{5, 25, 26} The associated risk of this infection, in principal, may be lower in xenotransplant than allotransplant, because it is possible to screen the donor for infectious agents and to eliminate it from the source of the graft.²⁰ From the ethical point of view, the use of genetically modified pigs for the routine supply of organs for xenotransplant is ethically acceptable.²⁷ Some clinicians argue that a pig containing 1 or more human genes (eg, the gene for human decay-accelerating factor) is entitled to some human legal rights.⁵ Additionally, xenotransplant may cause psychosocial problems, which might be experienced owing to the incorporation of animal organs with patients' body and fear of transmitted infections.¹¹

After solving of all these immunologic, physiologic, microbiologic problems, and ethical issues, the compatibility of the liver graft should be considered. One of the significant necessities, which should be measured, is to determine the approximate grafts' weight and dimension for each recipient.^{28, 29} There are some difficulties when the donor to the recipient's organ size are not the same, and this causes mismatch problems during the operation,³⁰ which might provide poor transplant outcome. On the other hand, the ability to estimate and select an organ which is proper in size and weight before transplant may have advantages, such as enhancement of survival and function of the xenograft,³¹ as well as a lower incidence of hyperacute rejection.³²

In this study, the mean of total liver weight was 696.6 ± 91.3 g. The percentage of whole liver weight to body weight was approximately 2.5% in pigs and 2% to 4% in humans. Hence, our data suggested that the relation of the human liver/body weight is similar to the pig liver/body weight. We cannot

determine if the pig's liver can cover completely the human liver function. In this study, our data suggest that the total liver weight of pigs might be sufficient to support the functions of the human liver in terms of the needed liver weight. Based on the report of Higashiyama and colleagues,³³ a graft of 30% of the recipients' ideal liver weight could provide adequate liver function. Furthermore, an estimated graft weight ratio $> 25\%$ (the weight of the liver graft divided by the standard liver weight of the recipient) might be acceptable.³⁴

The mean of hepatic arteries diameter was 3.2 mm. The portal veins were 14 mm or more in diameter. The vessel diameter correlates with the complication rate such vascular obstruction or stenosis in liver transplant. As reported by Inomoto and associates,³⁵ patients would be at increased risk for thrombosis after transplant if the size of these vessels were less than 2 to 3 mm in diameter. Therefore, the measured values indicate that the pig's hepatic vascular could have a sufficient diameter for anastomosis during liver xenotransplant.³⁶ In our study, the mean value of bile duct diameter was 5.54 mm. We mention that the diameter of bile ducts in pigs is well-matched to human's as measured by Horrow and associates,³⁷ the mean diameter of the human bile duct was 3.5 ± 1.2 mm.

Meanwhile, we compared the value of liver parameters against pig body weight rather than age as previously described.³⁶ According to our study, there is a clear correlation between the weight of the pig and the weight of the liver ($r = 0.89$), and also the length of the vessels and bile duct (Table 2). We did not find any significant correlation between weight of the pig and the diameter of the vessels or bile duct. Our findings might help transplant surgeons get an idea of the size and weight of the xenograft, which can be determined easily by measuring the animals' weight. The next step may be a way of finding a better size of the liver, especially greater diameter of its vessels and bile duct by genetic manipulation, or other pigs and/or other animals.

In summary, today, at the beginning of the research in the era of xenotransplant, significant barriers still remain for successful clinical xenotransplant. Recent advances in new immunosuppressive strategies, development of new pig variants, and the way to increase the donor specific tolerance can bring this treatment option closed to the clinic. Therefore, consideration of using

pig livers in an acute liver failure on a temporary basis, or as a “bridge” to support patients before transplant is likely to begin in near future. Based on the results of this study, the weight of the pig’s liver as well as vascular anatomy appeared to be suitable alternative for the human liver.

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