Multiple-organ Harvesting for Models of Isolated Hemoperfused Organs of Slaughtered Pigs

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Summary
Single- and multiorgan transplantations for the treatment of terminal cardiac, pulmonary, hepatic and renal diseases gain therapeutic importance and transplantation research using mammalian models of isolated perfused organs resembles a major field for the improvement of transplantation techniques. We established a new method of multiple organ harvesting from slaughterhouse pigs which may be used to reduce numbers of laboratory animals. 492 organs (hearts n = 191, lungs n = 108, livers n = 48, kidneys n = 145) were harvested, perfused with autologous blood to prevent artificial perfusion effects and examined for optimal organ harvesting and preservation conditions by analysing organ specific perfusion parameters. For each organ, specific techniques in regard to dissection, cannulation and periods of ischemia have to be applied to guarantee appropriate organ perfusion.
In summary, our data indicate that porcine organs obtained by multiple organ harvesting from commercial slaughterhouse animals can be efficiently used for isolated and perfused organ models. The harvesting process did not disturb the commercial use of the animals. Thus, multiple or single organ harvesting may be a useful method to reduce the use of laboratory animals.

Zusammenfassung: Multiorganentnahme von Schlachthausorganen für Modelle isoliert-perfundierter Schweineorgansysteme
Einzel- und Multiorgantransplantationen zur Behandlung terminaler Herz- Kreislauf-, Lungen-, Leber- oder Nierenerkrankungen erhalten zunehmend klinische Bedeutung und Modelle isoliert-perfundierter Säugetierorgane stellen ein wesentliches Feld zur Verbesserung der Transplantationstechniken dar.
Es wurde eine neue Methode der Multiorganentnahme bei konventionellen Schlachthaustieren entwickelt, die über den Gebrauch von Schlachthaustierorganen zu einer Reduktion von Versuchstierzahlen führen kann. 492 Organe (Herzen n = 191, Lungen n = 108, Lebern n = 48, Nieren n = 145) wurden entnommen, mit autologem Blut perfundiert und auf eine optimale Organentnahme und Konservierung untersucht. Für jedes Organ wurden spezielle Protokolle in Bezug auf Entnahme, Kanülierung und Ischämiezeiten zur Gewährleistung optimaler Organperfusionen entwickelt.
Die vorliegenden Daten demonstrieren die Möglichkeit einer effizienten Nutzung verschiedener Schlachthausorgane für isoliert-perfundierte Organe. Der Entnahmeprozess stört nicht die kommerzielle Nutzung der Tiere. Die vorliegende Methode der Einzel- oder Multiorganentnahme kann gezielt zur Reduzierung von Versuchstierzahlen benutzt werden

Keywords: transplantation, organ harvesting, slaughterhouse, preservation, isolated perfused, pig

1 Introduction

Organ transplantation resembles the ultimate treatment possibility of many respiratory, cardiac, hepatic and renal diseases and belongs to the socio-economic most expensive therapeutic pathways of modern medicine (Gridelli and Remuzzi, 2000; Platt, 1998). Although transplantation has been routinely established for organs such as the heart, lung, liver and kidney (Metcalfe and Nicholson, 2000; Schafer and Sorrell, 1999; Steen et al., 2001), the therapeutic value of the technique is still limited by a multitude of factors (Suthanthiran and Strom, 1994). Amongst them, the dysfunction of the graft organ is a major complication which depends upon factors such as surgical techniques, preservation procedures, ineffective immune-suppressive therapy and further donor/host characteristics (Suthanthiran and Strom, 1994).

Apart from the initial focus on surgical techniques, research in transplantation medicine has now become multidisciplinary and many studies are performed by the use of animal models of isolated and perfused organs (Carl et al., 2000; Clark et al., 1999; Hoerstrup et al., 2000). For this purpose, usually large numbers of small laboratory animals or laboratory-specific bovine or porcine organs are used.

Numerous problems have prevented the use of organs from slaughterhouse animals so far. The integration of organ harvesting into the slaughtering process as well as problems during perfusion have not been solved sufficiently. Therefore, perfusion of small laboratory animals has become more popular

(Ghofrani et al., 2001; Lockinger et al., 2001; Sibelius et al., 2000) although these species differ significantly in organ architecture and geometry if compared to human conditions. Furthermore, the number of animals used for investigations could be reduced by the use of organs from slaughterhouse animals as they are easily available.

Basic aspects for the successful perfusion of isolated organs from slaughtered pigs are organ harvesting and preservation. The characteristics of optimised harvesting and preservation procedures can be obtained from data about transplantation of human organs (Alfani et al., 1996; Toledo-Pereyra, 1984) but have to be modified with regard to the special situation of harvesting under slaughterhouse conditions. The present study was carried out to define first guidelines for multiple organ harvesting within the environment of commercial abattoirs. High quality organs were required for the perfusion models and simultaneously official standards in regard to the aspects of meat production had to be followed. Special modifications of the slaughtering process which are necessary to keep warm ischemia time as short as possible were identified and applied in accordance to official laws to avoid any disturbance of the meat production.

2 Animals, materials and methods

2.1 Animals

Along with a special agreement of the official meat inspection veterinarians and the slaughter company 492 organs and approximately 1500 litres of autologous donor blood were harvested in a commercial abattoir (Plumrose Inc., Eberswalde/Britz, Germany). Landrace pigs either male or female, six months old, weighing between 90 and 115 kg were used as donors.

2.2 Organ harvesting

To bypass the problems of time shortage during work at the conveyor belt, the pigs were slaughtered one by one in the sanitary isolation room. Due to different conditions of organ harvesting in the slaughterhouse, warm ischemia time (WIT) was defined as follows: start of desanguination is the beginning of WIT. The period of WIT ends when the infusion of preservation solution starts.

Before any access to the organs was possible, electrical stunning and desanguination took place. A five-minute splashing in hot water (70°C) is usually performed as next step during regular slaughtering but can be omitted. This step was omitted for the harvesting of hearts and lungs and shortened to 2 min for the harvesting of the other organs. Although the hot water treatment may be undertaken for organs other than heart and lung, it should be omitted if possible. Hot water aspiration during the slaughtering process can be deleterious for the isolated perfused hearts and lungs. Because hair removal and surface cleaning are incomplete without the hot water-technique the whole skin had to be re-moved totally according to hygienic laws (meat inspection rules and food production quality standards). This change of the slaughtering process shortened warm ischemia time for up to seven minutes.

The animals were then placed in dorsal recumbency for access to the thorax and abdomen and consecutive organ harvesting.

2.3 Preservation

All harvested organs were placed on ice for surface cooling and cannulation. They were perfused with different cold (4°C) preservation solutions (Tab. 1, 2) and stored at 4°C in an insulated box for transportation.

2.4 Perfusion

After harvesting procedures were finished, the organs were transported from the abattoir to the laboratory. Here, different organ-specific set-ups were used for the perfusion of the organs which based on a principle of two different circuits (Fig. 1). The system consisted of a dialysate and a blood circulation with a joint flow of blood and dialysate through dialysis modules (F7 or F5 Fresenius) and was described previously (von Baeyer et al., 1997). Different roller pumps were used to maintain flows at standardised pressures (e.g. blood flow could be adjusted from 0 to 500 ml/min). Apart from the lung model where air was supplied through a respirator, bubble oxygenators were used to enrich the dialysate with oxygen. An 8 channel A/D transducer was used to record parameters such as perfusion pressure, blood flow, temperature in dialysate and blood,

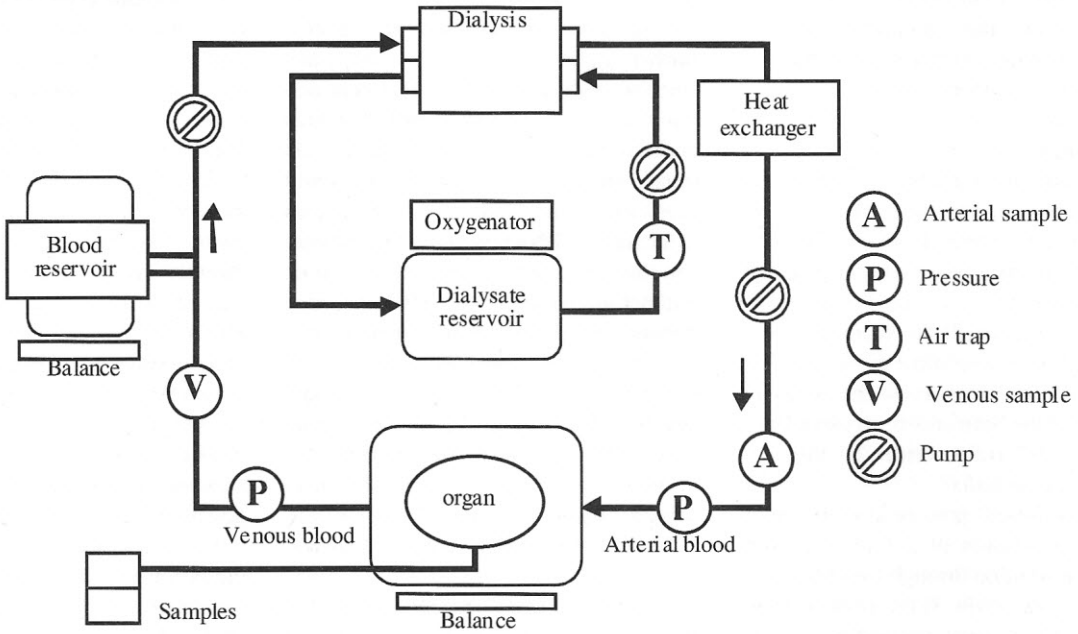
Tab. 1: Preservation solutions

Heart	modified Krebs-Henseleit solution
Lungs	Baeyer 2
Liver	Lactated Ringers solution
Kidney	HTK-Bretschneider (Custodiol®), Baeyer 2

Tab. 2: Composition of preservation solutions

	HTK		Baeyer 2	
Ions	Na^+ K^+ $Mg2^+$ Cl^- SO_4^{2-}	15 mmol/L 9 mmol/L 4 mmol/L 50 mmol/L -	Na^+ K^+ $Mg2^+$ Cl^- SO_4^{2-}	16 mmol/L 150 mmol/L 10 mmol/L 10 mmol/L 85 mmol/L
Impermeants	Mannitol	30 mmol/L	PEG Saccharose	4000 40 mmol/L
Buffers	Histidine Histidine-Cl	180 mmol/L 18 mmol/L	HPO_4^{2-}	3 mmol/L -
Additives	Tryptophan α-Ketoglutarate	2 mmol/L 1 mmol/L		- -
Osmolality		310 mosmol/L		314 mosmol/L
pH (8° C)		7.3		7.4

Fig. 1: Perfusion circuit for isolated hemoperfused heart



organ temperature or pH values in the dialysate.

3 Results

The duration of procedures of organ harvesting, handling and preservation in the slaughterhouse was optimised within n = 492 organs (hearts n = 191, lungs n = 108, livers n = 48, kidneys n = 145) to establish a standardised methodology of slaughterhouse organ harvesting. From the electrical stunning with 250V to the termination of preservation solution infusion, practicable times of 12 min for the heart isolation, 16.25 min for lung isolation, 26.50 min for liver isolation, and 27.50 min for kidney were recorded (Tab. 3) and followed by optimal functional parameters of organs in the perfusion set ups .

For perfusion, autologous blood can be collected and anticoagulation should be performed with sodium citrate (3.2%, 15 ml/l blood) and 10.000 U/l heparin. Approximately 3 litres of blood may be obtained from one pig of 90 to 115 kg weight.

For each organ, specific harvesting protocols were developed, which lead to a maximum protection for the organs.

After transport and connection to the perfusion systems, a period of warm rinsing with perfusion of 500 ml of preservation solution has to be performed prior to hemoperfusion.

3.1 Heart

The porcine slaughterhouse hearts need to be harvested and cannulated within 10 minutes of warm ischemia time, otherwise the pacemaking structures are usually irreversibly injured and the hearts do not function and beat again under perfusion. Therefore all great vessels have to be cut and opened inside the thorax directly after pericardiatomia. Retrograde infusion of 500 ml of cold cardioplegic solution (128 mMol NaCl, 2.3 mMol KCL, 25 mMol $NaHCO_3$, 0.6 mMol $MgSO_4$, 1.25 mMol $CaCl_2$, 1.3 (K)

Tab. 3: Periods of organ harvesting procedures

Harvesting of	Heart	Lungs	Liver	Kidney
Duration s	**s**	**s**	**s**	**s**
Electrical stunning (250V/15s)	15	15	15	15
Fixing	45	45	45	45
Desanguination (beginning WIT)	90	90	90	90
a) dorsal recumbency	60	60	60	
opening of thorax	90	90		
opening of abdomen			60	
organ harvesting and weighing	70	90	330	
hot water splash (70°C)			90	90
b) hanging position				90
opening of abdomen				60
removal of intestines				90
organ harvesting and weighing				130
cannulation	45	180	300	270
cold flush (end of WIT)				45
infusion of preservation solution	300	400	600	720
followed by cold storage				
duration in total s	715	970	1590	1645
min	12.00	16.25	26.50	27.50
warm ischemia time (WIT)	355	510	930	820
WIT (min)	6.00	8.50	15.50	13.75

mMol KH_2PO_4, 11.2 mMol glucose, 10 IE insulin, 30 mMol buadione monoxime) through the cannulated coronary arteries provides sufficient protection for the period of cold storage.

3.2 Lungs

If heart and lungs are both acquired they may be harvested together by an en-bloc technique. The organs have to be separated extracorporally which prolongs warm ischemia time for the heart for about one minute. If the heart has not to be saved for consecutive perfusion, it is more ef-ficient to harvest the lungs by opening of the heart and direct cannulation through the right ventricle into the *Truncus pulmonalis*.

An optimised preservation is maintained by infusion of 2 litres cold preservation solution through the cannulated *Truncus pulmonalis*. If the *Truncus* is cut off already the right and left pulmonary arteries have to be cannulated separately with smaller catheters.

3.3 Liver

Harvesting the liver follows standardised techniques from transplantation procedures (Adham et al., 1997). As *in situ* cold flush through the portal vein is not possible, it has to be performed extracorporally after organ dissection. Also, infusion of preservation solution through the cannulated liver artery is performed extracorporally with approximately two litres of cold lactated ringer solution (Schon et al., 1993).

3.4 Kidneys

Both kidneys are harvested by en-bloc technique either from the dorsal recumbent or hanging animal. After dissection of the peritoneum, a 10 cm section of the abdominal aorta and inferior *vena cava* is removed with both kidneys. Then the renal arteries and ureters are explored and dissected. The extracorporal cannulation of the renal artery is followed by infusion of 500 ml of cold preservation solution, e. g. HTK-Bretschneider (Custodiol®) preservation solution (Histidin-Tryptophan-Ketoglutarate) or Baeyer2 preservation solution (Tab. 1). The second kidney may be examined parallel or serve as control.

4 Discussion

Transplantation medicine has gained further importance over the past years since revolutionary surgical and immunologic procedures were establish which enabled the use of a variety of human organs such as heart, liver, kidneys and even lungs for routine transplantation (Metcalfe and Nicholson, 2000; Schafer and Sorrell, 1999; Steen et al., 2001; Suthanthiran and Strom, 1994). As these organs were shown to successfully function in their new host environment, the question of organ availability arose and new techniques including multiple organ harvesting from heart-beating and non-heart-beating donors were developed to bypass organ shortage (Alfani et al., 1996; Platt, 1998; Toledo-Pereyra, 1984).

Parallel to clinical progress, numerous experimental approaches focus on the use of animal models in transplantation research (Hiratsuka et al., 1998; Schotman et al., 1998; Vertrees et al., 2000; Watanabe et al., 2000). As organs of small laboratory animals display profound differences in comparison with human organs, bovine or porcine models resemble the most useful approach to human conditions with regard to organ size, function and geometry (Nielsen and Maaske, 1966).

Various problems have hindered the use of slaughterhouse animal organs so far. Contrary to the limitations of slaughterhouse-harvesting of organs for experimental studies, organ harvesting from laboratory animals displays the important advantage of *in situ* cooling, *in situ* preservation by infusion of cold preservation solutions and dissection in a bloodless field. In the situation of a commercial slaughterhouse where animals are primarily used for commercial meat production, these steps are not possible. Drug-free explantation of the organs has to precede any application of preservation or other solutions to agree with official meat production laws.

Due to the commercial slaughtering procedures, the definition of warm ischemia time is different to laboratory conditions: There is no fixed end of blood flow through the organ-supplying artery during the slaughtering process such as the clamping of an artery under laboratory conditions. In the slaughtering process, after electrical stunning of the animal has been performed, the desanguination continues with decreasing blood volumes and a still beating heart. We defined the beginning of WIT as the beginning of desanguination despite the slowly decreasing blood circulation. Even though surface cooling starts directly after organ harvesting, the parenchyma temperature only decreases efficiently by infusion of cold preservation solution. Therefore, we determined the start of the infusion as the end of WIT. It is most important to keep the different periods as short as possible. The amounts of preservation solutions as given may be varied.

The amount of autologous blood for the perfusion set-up ranges from 600 ml for heart and kidney (Dittrich et al., 2000; Modersohn et al., 2001) to 3000 ml for liver perfusions. Therefore, if the blood used for the perfusion should originate from the identical animal, the number of organs harvested from one pig may be limited as only three litres can usually be obtained from an animal. In this case, heterologous blood may be used.

Harvested slaughterhouse kidneys show a high viability during perfusion with autologous blood and are characterised by steady oxygen-consumption and tubular and glomerular function parameters. Nevertheless, they are in the state of acute renal failure with polyuria if compared to normal conditions measured in healthy animals under basal conditions. Human organs harvested for transplantation show the same signs of renal failure (Ciocca et al., 1994).

As the renal function usually improves within a few days, the acute failure has been regarded as a transient phenomena although renal compensation to acute ischemia has not yet been completely clarified.

The standardised and optimised organ harvesting and preservation procedures described here provide a sufficient organ function although the organs may show signs of ischemia and reperfusion injuries. However, this phenomenon can be investigated in numerous other organ preparations and resembles the limita-

tions of every experimental set-up. Nevertheless, the organs exhibit enough viability to be used for studies of pharmacology, pathophysiology or xenotransplantation.

In summary, we have set up criteria for the harvesting of multiple organs of slaughterhouse animals. The present data indicates that porcine slaughterhouse organs can be efficiently used for isolated and perfused organ models. Special modifications of the slaughtering process were developed in agreement with international recognised laws for meat production. The harvesting process did not disturb the commercial use of the animals and therefore, multiple or single organ harvesting from slaughterhouse animals may be a useful method to reduce the use of laboratory animals.

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