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Published in:
Basic & Clinical Pharmacology & Toxicology Online

DOI:
10.1111/bcpt.12077

Publication date:
2013

Document version
Early version, also known as pre-print

BCPT Basic & Clinical Pharmacology & Toxicology

Basic & Clinical Pharmacology & Toxicology, 2013, **113**, 75–86 Doi: 10.1111/bcpt.12077

MiniReview

Nephrectomized and Hepatectomized Animal Models as Tools in Preclinical Pharmacokinetics

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(Received 10 December 2012; Accepted 3 April 2013)

Abstract: Early understanding of the pharmacokinetics and metabolic patterns of new drug candidates is essential for selection of optimal candidates to move further in to the drug development process. *In vitro* methodologies can be used to investigate metabolic patterns, but in general, they lack several aspects of the whole-body physiology. In contrast, the complexity of intact animals does not necessarily allow individual processes to be identified. Animal models lacking a major excretion organ can be used to investigate these individual metabolic processes. Animal models of nephrectomy and hepatectomy have considerable potential as tools in preclinical pharmacokinetics to assess organs of importance for drug clearance and thereby knowledge of potential metabolic processes to manipulate to improve pharmacokinetic properties of the molecules. Detailed knowledge of anatomy and surgical techniques is crucial to successfully establish the models, and a well-balanced anaesthesia and adequate monitoring of the animals are also of major importance. An obvious drawback of animal models lacking an organ is the disruption of normal homoeostasis and the induction of dramatic and ultimately mortal systemic changes in the animals. Refining of the surgical techniques and the post-operative supportive care of the animals can increase the value of these models by minimizing the systemic changes induced, and thorough validation of nephrectomy and hepatectomy models is needed before use of such models as a tool in preclinical pharmacokinetics. The present MiniReview discusses pros and cons of the available techniques associated with establishing nephrectomy and hepatectomy models.

Knowledge of pharmacokinetics and metabolism patterns is essential in the selection of new drug candidates. In the past, many drug candidates have been discontinued from further development due to unfavourable pharmacokinetics in man [1]. Thus, early insight into metabolism and clearance pathways may be able to improve drug design, including which organs and receptors that contribute to the clearance process. Catabolic patterns play an important role in potential drug candidate modification because these affect drug clearance and can be used to obtain a suitable plasma half-life. Thus, being a key factor in determination of dosage regimen, the drug half-life ultimately influences important issues such as patient compliance, for example, when dosage intervals are short [2].

Many different *in vitro* methodologies can be used to predict *in vivo* clearance, but in general, *in vitro* methods lack several aspects of the complex whole-body clearance. Insufficiencies in the prediction of *in vivo* metabolic clearance based on *in vitro* methods often include lack of account for extrahepatic metabolism, active transporter mechanism in primary clearance organs and adaptive physiology or time- or dose-dependent kinetics [2].

On the other hand, pharmacokinetic studies in intact animals sometimes give rise to highly complex elimination patterns that do not necessarily allow individual processes to be identified. In such cases, complete or partial removal of a major excretion organ such as liver or kidneys can be helpful to shed light on the specific clearance mechanisms of the drug in question. Indeed, nephrectomy and hepatectomy have been used as models in pharmacokinetic studies to investigate clearance mechanism of many different drugs and potential drug candidates [3–11]. For example, the use of a hepatectomized rat model has clearly demonstrated that the elimination of pentobarbital proceeds primarily via hepatic metabolism [11].

Nephrectomized rats have been used to estimate clearance ratios of various drugs, demonstrating the importance of renal function in their elimination [5]. Animal models of nephrectomy and hepatectomy have also been used to study the metabolism of endogenous substances to improve the general biological insight. Thus, for example, the metabolism of parathyroid hormone has been studied in both nephrectomized rats and hepatectomized rats [12]. Removal of an organ can shed some light on which organs partake in synthesis of endogenous substances, for instance a hepatectomy model in pigs has

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Table 1.
Studies using nephrectomy models including species, survival time after establishment, study aim and anaesthetic agents used.

Species	Survival	Aim	Anaesthetic and analgesic agents	Investigators
Rat	3–4 days	Effect of aluminium hydroxide gel on the survival time and on the blood levels of sulphates, phosphates, calcium, urea and cholesterol after bilateral nephrectomy.	Diethyl ether	Rosenkranz [68]
	45–48 hr[1]	Study of parathyroid hormone metabolism	Not reported	Segre *et al.* [12]
	Not reported	Study of renal and extrarenal elimination rate of varies drugs	Pentobarbital	Morávek *et al.* [5]
	Not reported	Protein metabolism in renal failure model	Diethyl ether	Holeček *et al.* [69]
	Not reported	Pharmacokinetics of romiplostim	Induction: pentobarbital Maintenance: methoxyflurane	Wang *et al.* [4]
Mouse	Not reported	Determine the role of the kidney and acute renal failure in influencing serum cytokines and pulmonary homoeostasis	Avertin (2,2,2-tribromoethanol)	Hoke *et al.* [13]
	Not reported	Serial determination of glomerular filtration rate	Ketamine and xylazine	Qi *et al.* [15]
	Not reported	Study of anaesthetics protection against liver and intestine injury after acute kidney injury	Pentobarbital	Kim *et al.* [20]
Pig	Not reported	Extrarenal clearance of iohexol, inulin, and ^{51}Cr-EDTA	Pre-medication: azaperone Induction: thiopental Maintenance: thiopental or ketamine and midazolam	Frennby *et al.* [70]
	Not reported	Biliary and total extrarenal clearance of inulin and iohexol	Pre-medication: azaperone Induction and maintenance: thiopental	van Westen *et al.* [16]
Sheep	Not reported	Pharmacokinetic study of ^{125}I-erythropoietin	Pentobarbital	Widness *et al.* [71]

[1]Not survival time, but rather time in experiment, that is, indicating a survival for at least this long.

been used to demonstrate up-regulation of FVIII synthesis in extrahepatic tissue [3].

Basically, the removal of an organ can give two important pieces of information regarding clearance mechanisms. First, it clearly demonstrates to which extent the organ of choice is clearing the compound under investigation; second, it shows to what extent the compound is cleared outside the organ. Both are highly relevant in the development of potential drug candidates, and moreover, this information can be used to determine dosage and dosage intervals of a given drug used in human beings with impaired organ function.

Regardless of the benefits of surgical models, the obvious downside of such models is their massively invasive nature that induces dramatic and ultimately mortal systemic changes in the animals employed. The changes induced are characteristic of organ failure, and therefore, animal models lacking an organ have commonly been used for this purpose in particular. Thus, on top of the technical challenges involved in employing surgical models in pharmacokinetic studies, the time dependency of the obtained data often complicates their interpretation. This MiniReview will focus on surgical models of nephrectomy and hepatectomy and the pros and cons of their use as preclinical tools to investigate the clearance mechanism of potential drug candidates.

Nephrectomy

Nephrectomy means surgical removal of the kidneys and is often termed 'bilateral nephrectomy' to emphasize that both kidneys are removed. Nephrectomy has been used as a model of acute renal failure [13], whereas partial nephrectomies, typically 5/6 nephrectomies, have been used to study chronic renal failure [14]. Nephrectomy models have also been used in metabolism and clearance studies [4,5,12,15,16]. Rats and mice are most commonly used for nephrectomy, but other species have also been employed (table 1). A broad range of drugs have been studied in nephrectomized rats, and the obtained data clearly demonstrate the varying importance of renal clearance (fig. 1). These experiments were performed to predict clearance of these drugs in patients with renal insufficiency [5]. The clearance of romiplostim in rats showed important dose dependencies, the kidneys playing a predominant role in the clearance of high doses but only minor at lower doses [4]. Thus, dose-dependent clearance mechanisms are important to keep in mind when using such animal models. Metabolism of endogenous parathyroid hormone has also been studied in nephrectomized rats. Segre and coworkers used ^{125}I-labelled parathyroid hormone to nicely outline the relative importance of kidneys and liver in its clearance (fig. 2) [12].

Inulin is considered the gold standard of exogenously administered markers for measuring glomerular filtration rate (GFR) [17]. Glomerular filtration rate has been studied in nephrectomized mice by the means of inulin clearance, and as expected, both GFR and inulin clearance were found to be abolished [15]. However, conflicting findings have also been reported [16]. As a pharmacokinetic model, nephrectomy can be used to assess renal and extrarenal clearance.

Anatomical challenges when doing nephrectomy. The anatomy of the kidneys rarely causes problems when performing nephrectomy. Three structures need to be ligated to remove the kidneys, the renal artery, the renal vein and the

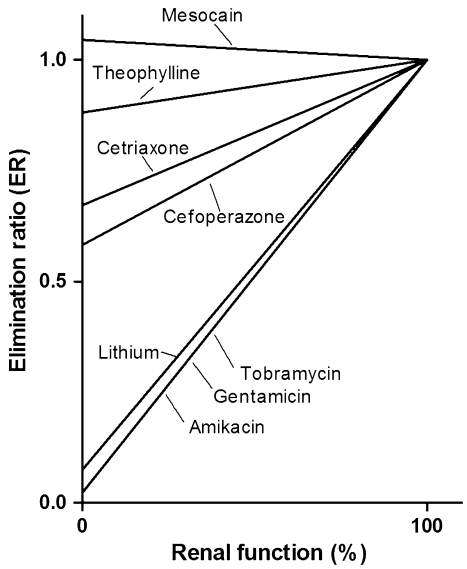

Fig. 1. Elimination ratio (ER) *versus* renal function for various drugs. Elimination rates were measured in animals with varying degree of renal function and compared with control animals with normal renal function. ER is defined as the ratio of the measured elimination rate constant during decreased renal function to that of control animals. The figure shows that lithium, amikacin, gentamicin and tobramycin are primarily cleared via the kidneys; ceftriaxone and cefoperazone are to some degree cleared via the kidneys, whereas theophylline and mesocain have very little or no renal clearance. The figure is modified from Ref. [5].

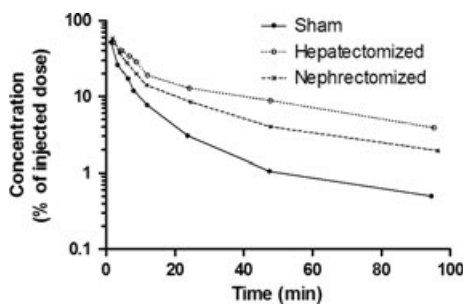

Fig. 2. Concentration *versus* time curve of ^{125}I-labelled parathyroid hormone in sham-operated, hepatectomized and nephrectomized rats. While both liver and kidney play a role in the elimination kinetics of parathyroid hormone, the liver is shown to have most impact on the course of concentration *versus* time curve. Unbroken line with filled circles: sham-operated rats, cross with broken line: nephrectomized rats and open circles with dotted line: hepatectomized rats. The figure is modified from Ref. [12].

ureter (fig. 3). It is important not to harm or interrupt the adrenal glands and their blood supply as they are in close proximity to the kidneys. Thus, special attention must be on *a. renalis rami adrenales caudales* and *v. renalis ramus adrenalis caudalis*, due to their direct connection to the blood supply of the kidneys.

Anaesthesia. A wide range of anaesthetics have been used in nephrectomy studies in the literature, unfortunately without particular attention on describing why the given anaesthetic was

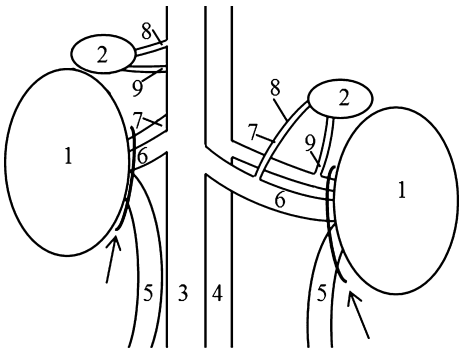

Fig. 3. Diagram of the kidneys, adrenal glands, ureters and blood vessels of interest when performing nephrectomy. The diagram is based on the anatomy of the rat with placement of ligatures needed for nephrectomy (arrows). 1: kidneys, 2: adrenal glands, 3: inferior vena cava, 4: abdominal aorta, 5: ureters, 6: renal veins, 7: renal arteries, 8: adrenal veins, 9: adrenal arteries.

chosen (table 1). Anaesthetics and analgesics that are metabolized in the liver can sometimes cause problems in nephrectomized animals. If pharmacologically active metabolites are formed and these are cleared by the kidneys, nephrectomy will result in unpredictable plasma concentrations that can affect the depth of anaesthesia. As an example, morphine gives rise to the active metabolite morphine-6-glucuronide, which is cleared via the kidneys [18]. The effect of opioid metabolites has been reviewed elsewhere [19]. As discussed below, extrarenal clearance can be influenced by chronic renal failure. However, although extrarenal clearance could potentially influence the anaesthetic and analgesic effect in animals undergoing nephrectomy, the time delay from nephrectomy establishment to observed changes in extrarenal clearance will in most cases limit its relevance in pharmacokinetic studies. It has been shown that choice of anaesthesia can influence and even protect against hepatic and intestinal injury after nephrectomy [20] underlining its importance in such models. In spite of this, renal clearance seems to play a minor role for most anaesthetics, and therefore, the range of agents used for nephrectomy studies is quite similar to that available for animals with normal renal function. However, choosing an inhalation anaesthetic like isoflurane that is primarily eliminated via the lungs may still be a good choice to avoid even the slightest risk of undesired changes in elimination of the anaesthetic agents used.

Monitoring. When doing surgery on laboratory animals, one should always consider what to monitor both intraoperatively and post-operatively. Proper monitoring is necessary to keep track of the well-being of the animal, and parameters commonly used in nephrectomy studies are listed in table 2. Blood urea nitrogen (BUN) is mainly cleared by the kidneys and is considered a good marker of renal function, particularly in cases with decreased urine flow [21]. Another important biochemical marker, plasma creatinine, is frequently used clinically to assess renal function [22]. Creatinine is generated endogenously from creatine at a constant rate and is solely excreted by the kidneys

Table 2.
Measures of monitoring typically employed in nephrectomy studies.

Species	BUN	Creatinine	Glucose	pH	Temperature
Rat	[12,68,69]	[69]	[69]	[69]	
Mouse	[13]	[13,20]			[20]
Pig		[70]			[16,70]
Sheep				[71]	

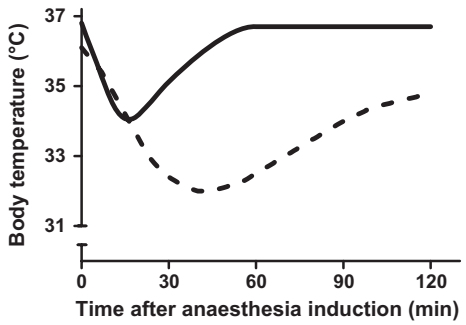

Fig. 4. Typical body temperature *versus* time curve of rats undergoing nephrectomy. The figure shows data compiled from experiments where body temperature (°C) was measured in rats undergoing nephrectomy as well as during the post-operative period. Rats were kept under continued isoflurane anaesthesia on a heating pad with (unbroken line) or without temperature control (dotted line). An obvious drop in temperature is seen as long as the abdominal cavity is opened, and the surgery is ongoing; after closure of the abdominal incision, the body temperature slowly rises again. Better body temperature control is achieved using a homoeothermic heating system.

[22], and these characteristics make creatinine an obvious marker of renal function [21]. However, in some cases, plasma creatinine and its clearance may stay within normal range even when renal function is impaired [22]. The ratio between BUN and creatinine is constant and approximately 10:1 in healthy animals, and it has therefore been suggested that the ratio in particular may be used as diagnostic biomarker in renal dysfunction [22]. Body temperature is another key parameter to monitor. Importantly, it should be maintained within the normothermic range in pharmacokinetic studies, as temperature is well known to affect metabolic turnover. Expectedly, hypothermic rats have been shown to have a lower metabolic rate than normothermic counterparts [23]. Moreover, hypothermia decreases both renal and hepatic clearance. In dogs, it has been reported that hypothermia lowers GFR as measured by inulin and creatinine clearance [24]. Also, indocyanine green clearance in rats has been found to be temperature dependent. As indocyanine green is a marker for hepatic blood flow [25], this further underlines the need for temperature monitoring and maintenance during pharmacokinetic studies. If not heated properly, keeping an animal under general anaesthesia will often result in a drop in body temperature. When performing abdominal surgery, the temperature may drop even further due to heat loss from the exposed abdomen. However, as shown in fig. 4, keeping the animal normothermic during surgery and post-operative can be challenging. The figure shows attempts on temperature control of rats undergoing nephrectomy under continued isoflurane.

Hepatectomy

Hepatectomy refers to surgical removal of the liver. The procedure results anatomically and functionally in an anhepatic state. Anatomically, an anhepatic state is a state without any hepatic tissue, whereas a functionally anhepatic state refers to the absence of functional hepatic tissue [26]. Total hepatic devascularization is another surgical methodology used to establish a functionally anhepatic state. Lempinen *et al.* [26] have made a short historical introduction to hepatectomy and hepatic devascularization. The use of an anhepatic state as a model of fulminant hepatic failure has been reviewed elsewhere [27].

Several species have been described in an anhepatic state. Laboratory animals used for anhepatic studies are primarily rats and pigs, but other species have also been used as listed in table 3. It is worth noting that there is considerable variation in survival times depending on animal species and even within species depending on the technique used. With up to 3 days of survival, pigs seem to be most resistant [28], whereas studies in other species typically report survival times of <24 hr (table 3).

As an example of the use of a hepatectomy model, a study using hepatectomized rats reported that elimination of pentobarbital proceeds primarily via hepatic metabolism (fig. 5). Importantly, however, hepatectomy itself changed the volume of distribution of pentobarbital significantly from that observed in normal rats [11]. Hepatectomized rats have also been used to investigate the clearance of ^{125}I-labelled parathyroid hormone (fig. 2). The study used hepatectomized and nephrectomized rats to show that both kidney and liver play a role in clearance of parathyroid hormone [12]. Some information is also available from human beings as the temporary anhepatic state during surgical liver transplantation has been used to estimate hepatic and extrahepatic metabolism of the analgesics [29,30] and anaesthetics [31] used. Thus, it has been reported that in human beings, both morphine [30] and fentanyl [29] are primarily metabolized by the liver. Another study in anhepatic human beings showed that extrahepatic metabolism is the predominant in propofol clearance [31]. Here, the focus will be on the use of laboratory animals undergoing hepatectomy or hepatic devascularization as pharmacokinetic models to assess hepatic and extrahepatic clearance.

Anatomical challenges when doing hepatectomy. There are two main anatomical challenges when doing total hepatectomy (fig. 6). The first is the need for portosystemic shunting. The blood supply to the intestinal tract drains into the portal vein, which then goes to the liver and branches into a second capillary system. The portal vein receives about 10–25% of the cardiac output depending on the species [32], and occlusion or diversion of the portal vein is necessary when performing hepatectomy to avoid massive haemorrhage and untimely death of the animal. Simply clamping the portal vein without portosystemic shunting will result in splanchnic congestion, leading to ischaemic injury to the splanchnic organs [33,34] and subsequently death of the animal. Thus,

Table 3.
Studies using anhepatic models species, survival time after establishment, study aim and anaesthetic agents used.

Species	Survival	Aim	Anaesthetic and analgesic agents	Investigators
Rat	27 hr	Creation of technique and determination survival time	Diethyl ether	Meehan *et al.* [49]
	Not reported	Study of pentobarbital metabolism	Not reported	Ossenberg *et al.* [11]
	Not reported	Study of parathyroid hormone metabolism	Not reported	Segre *et al.* [12]
	Hepatectomy: 5.75 ± 0.67 hr at 37.5°C 8.51 ± 0.78 hr at 35.5°C Devascularization: 5.61 ± 1.06 hr at 37.5°C 8.30 ± 0.70 hr at 35.5°C	Comparison of hepatectomy and devascularization	Not reported	Peignoux *et al.* [46]
	18 hr	Creation of a bloodless technique	Diethyl ether	Holmin *et al.* [41]
	36 hr	Creation of a one-stage technique with maintenance of gastrointestinal function	Diethyl ether	Yamaguchi *et al.* [47]
	10.63 ± 1.60 hr	Creation of a simple and easy technique	Diethyl ether	Omokawa *et al.* [50]
	20 ± 5 hr	Creation of a reproducible technique of a fulminant hepatic failure model	Diethyl ether	Rozga *et al.* [51]
	Not reported	Study of brain oedema and intracranial hypertension after hepatectomy	Methoxyflurane	Olafsson *et al.* [48]
	20 ± 5 hr	Creation of a one-stage technique with venous graft	Diethyl ether	Azoulay *et al.* [40]
	10.5 hr (range 5.5–21.5)	Creation of a one-stage microvascular technique	Halothane	Engelbrecht *et al.* [43]
	3.93 ± 0.42 hr	Creation of technique to establish model to evaluate the function of artificial liver support devices	Diethyl ether	Umehara *et al.* [44]
	Not reported	Study of extrahepatic metabolism	Diethyl ether	Ping *et al.* [9]
	Not reported	Study of extrahepatic metabolism and gene expression	Isoflurane	Gu *et al.* [7]
Pig	24.5 hr (range 15–40)	Creation of technique	Pentobarbital	Lempinen *et al.* [26]
	Hepatectomy: 16–36 hr Devascularization: 17–28 hr	Plasma amino acid patterns in acute hepatic failure	Pre-medication: ketamine Induction: ketamine Maintenance: halothane, protoxide	Mazziotti *et al.* [39]
	16.88 ± 5.38 hr	Creation of a reproducible technique of a fulminant hepatic failure model	Pre-medication: atropine, ketamine, diazepam and azaperone Induction: fentanyl, ketamine and propofol Maintenance: fentanyl and propofol	Filipponi *et al.* [56]
	46 ± 6 hr	Creation of technique using a self-made, three-way prosthesis	Induction: ketamine, azaperone, atropine and isoflurane Maintenance: sufentanil and ketamine	Sosef and Gulik [58]
	Not reported	Study of extrahepatic factor VIII expression and plasma clearance of human FVIII	Not reported	Hollestelle *et al.* [3]
	51.2 ± 18.7 hr	Creation of a pig model without extracorporeal circulation and prolonged post-operative survival	Induction: atropine, ketamine, azaperone and diazepam Maintenance: ketamine, fentanyl and midazolam	Knubben *et al.* [57]
	53 ± 5 hr 74 ± 6 hr[1]	Study of dummy device effects on haemodynamics, body temperature and survival.	Induction: atropine, ketamine, azaperone and diazepam Maintenance: ketamine, fentanyl and midazolam	Thiel *et al.* [28]
Dog	12–20 hr	Creation of one-stage technique with end-to-end anastomosis of v. cava caudalis	Thiamylal	Alican *et al.* [37]
	5 hr	Creation of a one-stage technique without alterations in extrahepatic metabolism	Induction: thiopental and succinylcholine Maintenance: halothane	Daloze *et al.* [45]
Rabbit	4 hr	Study of extrahepatic metabolism	Isoflurane	Nyberg *et al.* [10]
Sheep	Not reported	Pharmacokinetic study of ^{125}I-erythropoietin	Pentobarbital	Widness *et al.* [71]

[1]Prolonged survival time with the use of extracorporeal circuit.

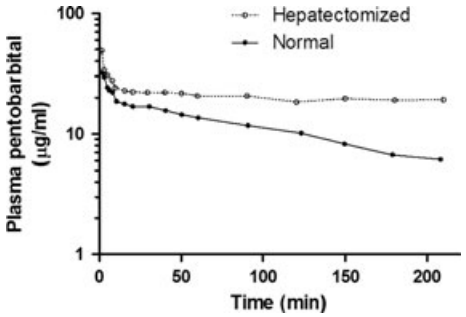

Fig. 5. Pentobarbital blood concentration *verses* time curve in normal and hepatectomized rats. The figure shows a prolonged terminal half-life of pentobarbital in the hepatectomized rat compared with the normal rat indicating that the liver is important in the clearance of pentobarbital. Open circles and dotted line represent hepatectomized rats, and unbroken line and filled circles represent normal rats. The figure is modified from Ref. [11].

survival time of a rat with clamped portal vein is approximately 50 min. [35]. Portosystemic shunts can be used to reduce the ischaemic injury caused by splanchnic congestion [34].

The second main challenge to overcome is the close connection between the *v. cava caudalis* (VCC) and the liver. Some species have a long intrahepatic part of the VCC, for example, as seen in the rat [36] and pig [26], whereas the intrahepatic part is very short in, for example, dogs [37]. Human beings have a retroperitoneal VCC [36]. Removal of all liver tissue in animals with an intrahepatic part of the VCC requires removal of the intrahepatic part as well. Moreover, it is necessary to re-establish flow in the VCC by the use of anastomosis or prosthesis, when removing a segment of a large vein such as the VCC [38,39].

Hepatectomy with one, two or three stages in the rat. In the rat, interruption of the portal flow exceeding 30 min. will result in an irreversible state of shock [33] – some even state that rats only tolerate a maximum of 20 min. of interruption of the portal vein flow [40,41].

A part of the VCC is completely intrahepatic in the rat. The caudate process of the liver is closely connected to this intrahepatic part [36]. The caudate process constitutes approximately 2–3% of the total liver mass [36,42], and its position makes total hepatectomy a surgical challenge, because a venous graft or prosthesis is needed to replace the intrahepatic part of the VCC to complete a total hepatectomy in one stage. As mentioned above, shunting of the portal blood is absolutely necessary to avoid splanchnic congestion, and removal of the intrahepatic part of VCC is needed to remove 100% of the hepatic tissue in the rat. Several different procedures have been developed to overcome these two critical steps in hepatectomy of the rat. The techniques can be grouped into one, two or three-stage techniques, respectively (table 4).

In the one-stage technique, the entire procedure is completed in one operation with the model being established right away. The drawback of one-stage techniques is that they often require complex microvascular surgical skills [43]. Two- and three-stage techniques are performed over two and three operations, respectively, with varying time intervals in between the operations. These multistage techniques have been developed to simplify the establishment of hepatectomy models. The major drawbacks of two- and three-stage techniques are that

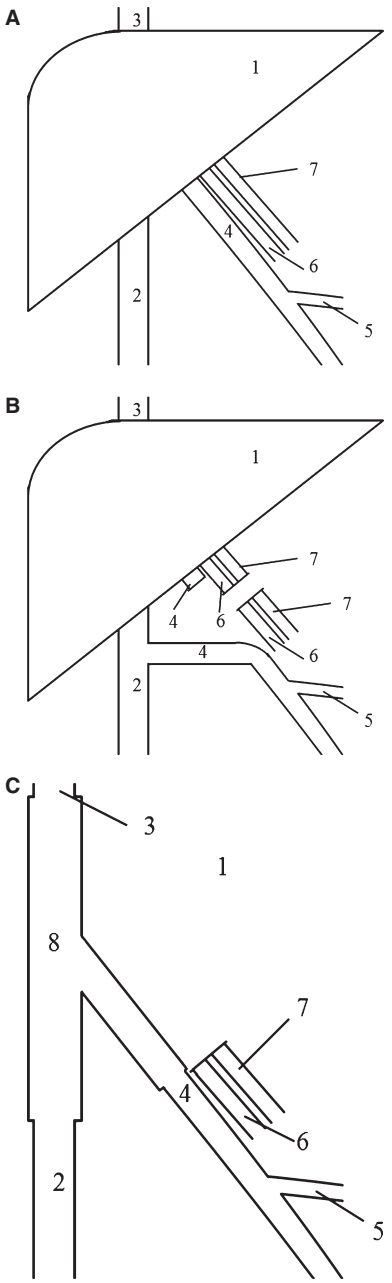

Fig. 6. Diagram of the liver and the blood vessels of interest when performing hepatic devascularization or hepatectomy. The diagram is based on the anatomy of the rat. (A) Normal anatomy of the liver. (B) Hepatic devascularization with end-to-side portacaval shunt and ligation of the bile duct and the hepatic artery. (C) One-stage hepatectomy with the use of a Y-shaped graft or prosthesis and ligation of the bile duct and the hepatic artery. 1: liver, 2: infrahepatic inferior vena cava, 3: suprahepatic inferior vena cava, 4: portal vein, 5: gastroduodenal vein, 6: hepatic artery, 7: bile duct, 8: Y-shaped graft or prosthesis.

Table 4.

Technical approaches employed to achieve an anhepatic state in the rat.

Number of stages	Type of hepatectomy/ devascularization	Use of graft or prosthesis to replace part of v. cava caudalis	% liver tissue removed from circulation	Comments	Investigators
One stage	Hepatectomy	V-shaped polyethylene prosthesis	100%		Yamaguchi *et al.* [47]
	Hepatectomy	Y-shaped vascular graft	100%		Azoulay *et al.* [40]
	Hepatectomy	Vascular graft	100%		Engelbrecht *et al.* [43]
	Devascularization	Intact v. cava caudalis	Approximately 100%	Portacaval shunt using a heparinized silicone catheter	Ping *et al.* [9]
	Devascularization	Intact v. cava caudalis	Approximately 100%	Duration of study: 1 hr clamping of v. porta without portacaval shunt	Gu *et al.* [7]
Two stages	Hepatectomy	Intact v. cava caudalis	Approximately 100% (caudate process not removed)	3 days for establishment	Segre *et al.* [12]
	Hepatectomy	Intact v. cava caudalis	Approximately 100% (leaving small amount of liver tissue undergoing ischaemic necrosis)	2 weeks for establishment	Rozga *et al.* [51]
	Hepatectomy	Collateral formation	100%	Approximately 1 month for establishment	Meehan *et al.* [49]
	Hepatectomy/ Devascularization	Intact v. cava caudalis	Hepatectomy: approximately 100% (caudate process not removed) Devascularization: approximately 100%	2 days for establishment. Two groups: hepatectomyand devascularization	Olafsson *et al.* [48]
	Hepatectomy	Splenic transposition with collateral formation	Approximately 100% (caudate process destroyed by electrocoagulation)	2 weeks for establishment	Omokawa *et al.* [50]
Three stages	Hepatectomy	Collateral formation	100%	23 days for establishment	Ossenberg *et al.* [11]
	Hepatectomy	Collateral formation	100%	5 weeks for establishment	Holmin *et al.* [41]
	Hepatectomy	Collateral formation	100%	2 weeks for establishment	Umehara *et al.* [44]
	Hepatectomy/ Devascularization	Collateral formation	Hepatectomy: 100% Devascularization: approximately 100%	22 days for establishment. Three groups: hepatectomy and two different techniques of devascularization	Peignoux *et al.* [46]

they are very time-consuming [37,44], have a high mortality rate [40,41] and often lead to formation of adhesions and collaterals [37]. Moreover, it has been speculated that the time interval between different stages allows the animal to adapt to the situation and induction of changes in the extrahepatic metabolism [45]. Some techniques are based on hepatectomy, while others are based on hepatic devascularization (table 4). The devascularization techniques have the advantage of being able to leave the intrahepatic part of VCC intact, but serious drawbacks include the potential release of enzymes and ammonia from the necrotic liver and risk of having a unintended continued blood supply to the liver [46].

One-stage hepatectomy or hepatic devascularization techniques employ a number of different strategies to avoid splanchnic congestion. Venous shunt of the splanchnic circulation can be done in different ways using, for example, Y-shaped graft [40], V-shaped polyethylene prosthesis [47], side-to-side mesentericocaval shunt [43] or silicone catheter [9], whereas others simply clamp the portal vein and accept splanchnic congestion [7]. The procedures using Y-shaped graft [40] and V-shaped polyethylene prosthesis [47] also address the problem of removal of the intrahepatic part of VCC as part of the Y-shaped graft or V-shaped prosthesis is used to replace its intrahepatic section. In contrast, techniques using end-to-side portacaval shunt, side-to-side mesentericocaval shunt or silicone catheters do not solve the issue with the intrahepatic part of the VCC, which therefore needs to be addressed separately. Side-to-side mesentericocaval shunts combined with a vascular graft for the intrahepatic part of the VCC is one solution that has been described for total hepatectomy in the rat [43]. Alternatively, the combination of a heparinized silicone catheter and hepatic artery ligation has been shown to completely block hepatic blood flow in rats resulting in hepatic devascularization [9]. Simply clamping the portal vein will inevitably cause splanchnic congestion, and consequently, this approach should only be used for experiments with a very limited time course. Clamping of the portal vein is only used for hepatic devascularization in experiments in which the intrahepatic part of the VCC is kept intact [7].

Two-stage hepatectomy or hepatic devascularization techniques require longer time to establish. Published procedures last from 2 days [48] and up to 1 month [49]. In general, two-stage techniques are performed to allow formation of collateral circulation to overcome the challenge with the intrahepatic part of the VCC [49,50] and atrophy of liver lobes prior to hepatectomy [51] or simply to ensure survival from the portacaval shunt before hepatectomy [12,48] or hepatic devascularization [48]. Three-stage hepatectomy techniques are performed to allow formation of collateral circulation in stage one, while stage two is used for establishing a portacaval shunt and stage three for performing the actual hepatectomy [41,44]. However, even though multistage techniques are easier to perform, their severe drawbacks render the one-stage model as the only valid hepatectomy model for use in preclinical pharmacokinetics. Hepatic devascularization may be an attractive alternative due to its simplicity, but total hepatectomy should be preferred when possible.

Anaesthesia. When removing the liver – the primary clearance organ of many anaesthetics and analgesics – balancing anaesthesia can be a considerable challenge. Removal of the liver raises a number of issues regarding the drug of choice, the clearance of the chosen drug in the anhepatic animal and the dosing intervals needed to keep the animal anaesthetized throughout the procedure without overdosing. Table 3 gives an overview of anaesthetic agents used in hepatectomy studies. Many older studies used diethyl ether that is now considered ethically questionable due to its irritant properties and availability of alternatives such as isoflurane, and consequently, ether is no longer used in modern animal laboratory facilities. Generally, inhalation anaesthetics are widely used in anhepatic models due to their lower degree of hepatic metabolism. Thus, as an anhepatic state only influences the effect and dosing of inhalations anaesthetics to a limited extent, the challenging task of managing injection anaesthetics at a state of decreased metabolism can be avoided. Likewise in healthy human beings, it is well known that <0.2% of an anaesthetic gas-like isoflurane is metabolized [52]. Inhalation anaesthetics are also preferred in studies with conscious anhepatic animals due to their rapid and predictable clearance by exhalation compared with the often very slow hepatic clearance of many injection anaesthetic agents. Overall, inhalation anaesthetics should be preferred over injectable anaesthetics in animal models of hepatectomy due to the improved control of the anaesthetic depth.

Monitoring. As animal welfare is a major concern when performing animal experiments today; objective measures of stress are needed to limit the discomfort of the animal to the lowest possible level. The most frequently monitored parameters in animal models of hepatectomy can be found in table 5. Some are used for continued monitoring of the animal during the experiment, while others are of particular interest when evaluating the data obtained from the anhepatic animal.

Table 5
Measures of monitoring typically employed in anhepatic animal models.

Species	Temperature	Glucose	Ammonia	Creatinine	Haemoglobin	Bilirubin
Rat	[11,41,46,50]	[9,43,44,47,50,51]	[40,44,46,48,51]	[9]	–	[9,47]
Pig	[28,56]	[28,39,56,57]	[26,28,39,56,58]	[28,56–58]	[28,39,56–58]	[28]
Dog	–	–	[45]	[45]	[45]	–
Rabbit	–	[10]	–	–	–	–
Sheep	–	–	–	–	[71]	–

Ammonia is an important blood parameter in hepatectomy. It is well known that high blood levels of ammonia cause numerous changes in the body including intracranial hypertension [48,53,54], brain oedema [48,54] and ultimately cerebral herniation, which is a common cause of death in human beings with acute liver failure [55]. Rise in brain ammonia concentration has been associated with development and progression of encephalopathy in awake hepatic devascularized rats with loss of righting reflex followed by loss of corneal reflex [54]. Blood glucose rapidly decreases after initiating an anhepatic state and is known to shorten survival time if the animal is not glucose supplemented. In agreement, Azoulay and coworkers found that the mean survival time in hepatectomized rats without glucose supplement was 6 ± 0.5 hr compared with 20 ± 5 hr with glucose supplement [40]. Glucose has been supplemented in various ways in hepatectomy models. Constant intravenous glucose infusion is most commonly employed. Here, some adjust the amount of glucose infused to blood glucose levels [10,28,40,43,47,56–58], while others infuse at a constant rate without adjustments to blood glucose levels [26,37,39,41,44–46,48,50,51]. However, less refined methods such as intraperitoneal injections of glucose [41], intragastrical injection of a single bolus of glucose [8], hourly subcutaneous injection of glucose [49] and bolus injections of glucose at blood sampling times [12] have also been used.

As mentioned in the nephrectomy section, body temperature should generally be closely monitored as it influences drug clearance *per se*. Moreover, body temperature is also important to monitor in hepatectomy and hepatic devascularization models because of its significant impact on survival time. Thus, for example, lowering the body temperature of hepatectomized rats from 37.5 to 35.5°C has been shown to increase survival time by about 50% [46].

Clearly, the choice of animal species influences the number and character of parameters it is possible to monitor. In particular, attention should be paid to the amount of blood drawn from the animal to avoid causing hypovolaemia. Obviously, the risk of causing hypovolaemia due to blood sampling is likely to decrease with increasing animal size due to the higher blood volume. Consequently, hypovolaemia is of particular concern in rodent hepatectomy models because of the risk of considerable blood loss during the vascular surgery performed. This makes haemoglobin a highly relevant parameter in studies with hepatectomy models. If replacement fluid is used, measured haemoglobin can also be used to correct for haemodilution that would otherwise compromise in particular pharmacokinetic studies.

Use and Limitations as Pharmacokinetic Models

Kidneys and liver are vital organs for maintaining homoeostasis. Removing a vital organ will cause changes to the organism, which will likely influence the data obtained and therefore compromise the validity of such results. The kidneys and the liver are highly perfused organs receiving approximately 12–22% and 18–33% of cardiac output, respectively, depending on the species [32]. Clearly, changing the blood flow to these organs will have dramatic haemodynamic consequences and may directly or indirectly influence multiple measurable parameters and should therefore be taken into account when interpreting the data obtained.

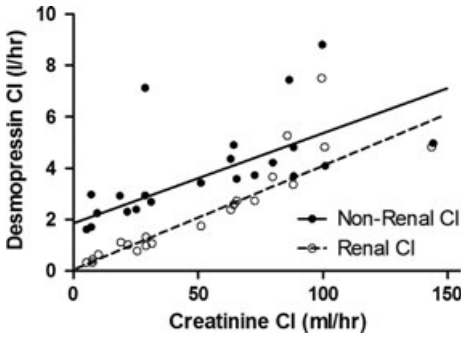

Fig. 7. Non-renal and renal clearance of desmopressin and the relationship with creatinine clearance in patients with varying degree of renal impairment. The unbroken line represents the covariate relationship based on nonlinear mixed effects modelling between non-renal clearance of desmopressin and creatinine clearance while the broken line represents corresponding covariate relationship between renal clearance of desmopressin and creatinine clearance. Filled circles are individual observations of non-renal clearance, and open circles are individual observations of renal clearance. The figure is modified from Ref. [63].

Nephrectomy. Several studies of the effect of renal failure on extrarenal clearance have been conducted, and the subject has been reviewed several times in the past covering different aspects [59–62]. Touchette and Slaughter reviewed the effect of renal failure on hepatic clearance and concluded that the specific mechanism involved in inhibition of extrarenal clearance during renal failure remains unclear, while the inhibition phenomenon itself is well-established and should be considered [59]. Recent reviews have looked more closely into the mechanism behind this inhibition. Vilay *et al.* [60] proposed that acute kidney injury affects hepatic drug metabolism and transporter function and that these two components partly explain the changes observed in extrarenal clearance as a result of acute kidney injury. Sun *et al.* [61] reviewed the effect of renal failure on uptake and efflux drug transporters and concluded that the effect of renal failure on these transporters contributes significantly to the pharmacokinetic changes observed. Pichette and Leblond [62] suggested that a decrease in extrarenal metabolism is due to reduction in cytochrome P450 during chronic renal failure. A human study of individuals with varying degrees of renal impairment clearly showed that renal clearance of desmopressin varies with renal function as measured by creatinine clearance. Likewise, non-renal clearance of desmopressin was found to vary with creatinine clearance (fig. 7) [63].

The above-mentioned changes in extrarenal clearance observed during renal failure may question the usefulness of nephrectomy models in pharmacokinetic studies. If the extrarenal clearance is affected by the model itself, then the model is of less value. Liver perfusion studies have indicated that

effects on extrarenal clearance may be caused by components in uraemic blood [64,65]. It is worth noting that the change in extrarenal clearance can be either increased [64] or decreased [65,66] clearance depending on the drug. However, all the above-mentioned changes in extrarenal clearance have been observed during progression of renal failure or chronic renal failure that do not necessarily resemble the situation in pharmacokinetic studies, where data are typically collected directly following the surgical procedure. Thus, short-term pharmacokinetic studies using nephrectomized laboratory animals may therefore still be of great value, provided that the experiments are completed before development of uraemia and resulting changes in extrarenal clearance.

An example of the successful use of a nephrectomy model in studies of clearance mechanisms is provided by Moravek and coworkers, who examined renal and non-renal clearance of eight different drugs [5]. Although they clearly provided interesting renal and non-renal clearance data on the drugs tested using a large number of animals (>20 nephrectomized rats per drug), some validation issues still need to be addressed. Polyfructosane-S was used as a marker of glomerular filtration rate [5]. However, as no model compound of non-renal clearance was included, the questions of model-induced changes in non-renal and renal clearance could not be properly addressed.

Hepatectomy. Hepatectomy is known to cause considerable haemodynamic changes including, for example, decreased mean arterial pressure [56–58], increased central venous pressure [57], increased heart rate [48,57,58], decreased oxygen saturation [57] and increased arterial blood pH [48]. Hence, if haemodynamic homoeostasis is essential for an intended study, hepatectomy will most likely be of little or no use due to the marked changes induced by the model *per se*.

It has been reported that a portacaval shunt in normal rats can cause changes in renal function [67]. Importantly, these changes were observed 30 days after the portacaval shunt was established, and the procedure did not include hepatectomy. Regardless, the results suggest that the use of multiple stage hepatectomy techniques may result in altered renal function and drug clearance and thus adversely affect pharmacokinetic studies. In agreement, Daloze *et al.* [45] suggested that only one-stage hepatectomy techniques should be used to avoid potential changes in extrahepatic metabolism.

Additional drawbacks of one-stage hepatectomy models include the requirement for extensive surgical training (for some models even microsurgical training) and the fact that the animals do not survive total hepatectomy for longer periods of time. As discussed above, the choice of species influences these disadvantages as, for example, performing the surgical techniques becomes increasingly difficult with decreasing size of animal, and survival time is influenced by animal species. While different species have varying survival time (table 3), the observed intraspecies variation in survival time can to a large extent be explained by the difference in the technique employed.

However, even with these serious limitations in mind, hepatectomy still remains a valuable method to evaluate hepatic and extrahepatic metabolism in preclinical drug development in cases where liver perfusion models and other *ex vivo* methods cannot be used.

An example of the successful application of a hepatectomy model is the investigation of clearance mechanisms of plasma-derived human FVIII in pigs [3]. This study showed that even though the liver does play a role in the clearance of FVIII, 90% of the used FVIII dose was cleared within the first 24 hr in hepatectomized pigs [3]. Unfortunately, this study used a very low n-value (n = 2) and displayed an increased interindividual variation in hepatectomized compared with control animals. The experiment therefore also underlines the challenges of conducting hepatectomy studies in pigs in general.

Conclusion

Animal models of nephrectomy and hepatectomy have considerable potential in preclinical pharmacokinetic studies if used with caution. It is essential to consider the length of the study, that is, time from establishment of model till completion of the data collection, because animal models of nephrectomy and hepatectomy have a markedly reduced lifespan. Moreover, the inclusion of proper sham-operated control groups is imperative in such studies because many pathophysiological changes potentially affecting the experimental outcome have been described in the literature including marked haemodynamic changes. As all relevant changes to the organism have most likely not been described at this point, great caution should be employed when interpreting data. The models should be thoroughly validated before use. While the literature describes many different techniques to establish an animal model of hepatectomy, only very few of these techniques have been properly validated. Thus, attempts of validation have been carried out in some models [9], but further validation is required when deviating from these specific techniques.

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