FABAD J. Pharm. Sci., 28, 39-49, 2003

SCIENTIFIC REVIEWS

Perfused Liver Preparation and its Applications

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Summary : *Unlike most organs of the body, the liver has a dual blood supply. Under physiological conditions, one third of its blood is supplied by the hepatic artery with the remainder coming from the portal vein. The liver has prompted numerous investigators not only to define the anatomical and functional relationship between these blood vessels but also to study various hepatic functions. The hepatic functions have been studied using various experimental models including isolated organelles, isolated and cultured hepatocytes, liver slices and perfused liver. In comparison to the in vitro models, as the structural and functional integrity of the organ is retained in the perfused liver system, this experimental model is widely used for physiological, pharmacological and pharmacokinetic investigations. In this review the perfusion system, perfusion medium and its characteristics, perfusate flow rate and pressure, the liver donors, anaesthetic agents, surgical procedures and viability assessment of the perfused liver will be described, and finally its applications will be briefly presented.*

Received : 25.2.2003
Revised : 3.4.2003
Accepted : 7.4.2003

Perfüze Karaciğer Preparatı ve Uygulamaları

Özet : *Vücuttaki diğer organlardan farklı olarak karaciğer çift kan kaynağına sahiptir. Normal fizyolojik koşullar altında kanın üçte biri hepatik arter tarafından geri kalanı ise portal ven tarafından sağlanır. Karaciğer sadece bu iki kan damarları arasındaki anatomik ve fizyolojik ilişkiyi değil değişik hepatik fonksiyonları da çalışmak için çok sayıda araştırıcıyı cezbetmiştir. Karaciğer fonksiyonları, izole organeller, izole ve kültür hepatositleri, karaciğer dilimleri ve perfüze karaciğerinde dahil olduğu değişik deneysel modeller kullanılarak çalışılmıştır. In vitro modellerle karşılaştırıldığında, perfüze karaciğer sisteminde organın yapısal ve fonksiyonel bütünlüğü korunduğu için, bu deneysel model fizyolojik, farmakolojik ve farmakokinetik araştırmalarda yaygın olarak kullanılmıştır. Bu derlemede perfüzyon sistemi, perfüzyon ortamı ve özellikleri, perfüzat akış hızı ve basıncı, karaciğer vericileri, anestezik ajanlar, cerrahi teknikler ve perfüze karaciğerin canlılık tayini için kullanılan parametreler tanımlanacak ve son olarakta uygulama alanları kısaca sunulacaktır.*

Introduction

The liver is the largest internal organ of the body. It is suspended below the diaphragm and perfused by both the hepatic artery (HA) and portal vein (PV). Under physiological conditions 25-35% of its blood is supplied by the HA and with the reminder coming from the PV. The hepatic function has been studied in various experimental models at varying levels of structural organization. Apart from the in vivo models, studies have been performed in the isolated perfused liver, liver slices, isolated and cultured hepatocytes, and isolated organelles. Of these models, the isolated perfused liver preparation is possibly the most commonly used[1-3].

The liver perfusion was first described by Claude Bernard[4] for the conversion of glucose to glycogen. Blood-perfused isolated rat livers were used by Miller and colleagues[5] at the University of Rochester to demonstrate the role of liver in plasma protein synthesis. However, it was not widely accepted by biochemists until its utility was demonstrated by Krebs[6] and Lardy[7]. During the last 40 years, the liver perfused through the portal vein (PV) has been widely used as an experimental model for physiological, pharmacological and pharmacokinetic investigations. In comparison to the other in vitro models (e.g. isolated hepatocytes, liver slices), the hepatic architecture, hepatocyte polarity (sinusoidal versus canalicular domains), zonal heterogeneities of certa-

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in metabolic substrates and enzymes, bile flow and vascular integrity are preserved in the perfused liver. In addition, the perfused liver system avoids certain complications that can arise in vivo, such as influence of other organs, plasma constituents and neural-hormonal effects[8]. The perfused liver model, particularly in the single-pass model, also permits easy manipulation of experimental conditions (such as perfusate flow, oxygen content, binding and temperature etc.) and continuous monitoring/sampling of the perfusate. The single-pass mode also minimises the accumulation of potentially interacting metabolites. Perfusion solely through the PV with an oxygen-rich medium is sufficient to maintain the viability of liver for most studies[9,10]. However, this model is considered unphysiological as it excludes the possible contribution that arises from input via the HA. Based on this argument, various investigators have used liver preparation perfused simultaneously via the PV and HA[8,11-27].

Various aspects of this method including the perfusion system, perfusion medium, liver donors, anaesthesia, surgical techniques, viability assessments of the preparation and its applications will be discussed in the following sections of this review.

Perfusion System

The basic perfusion system has three parts in series: a reservoir, a pump and an oxygenator. Peristaltic or pulsating pumps are generally used. Although the peristaltic pump works well, the pulsating pump minimises haemolysis of the erythrocytes containing perfusates. Two types of oxygenator have been used: a thin film or membrane oxygenator and a pressurized membrane oxygenator (e.g. Hamilton lung). Gores et al.[1] considered the Hamilton lung to be the most efficient and effective method for oxygenating the perfusate. Alternatively, an hollow fibre oxygenator can be used for the oxygenation of the perfusate[16]. The entire apparatus is enclosed in a temperature controlled cabinet (37°C) where the perfusion studies are performed (Figure 1).

Perfusion Medium

There are two major considerations in choosing a particular perfusate: (a) the appropriate buffer, energy source, substrate and oncotic composition; and (b) the oxygen-carrying capacity[1]. Although a num-

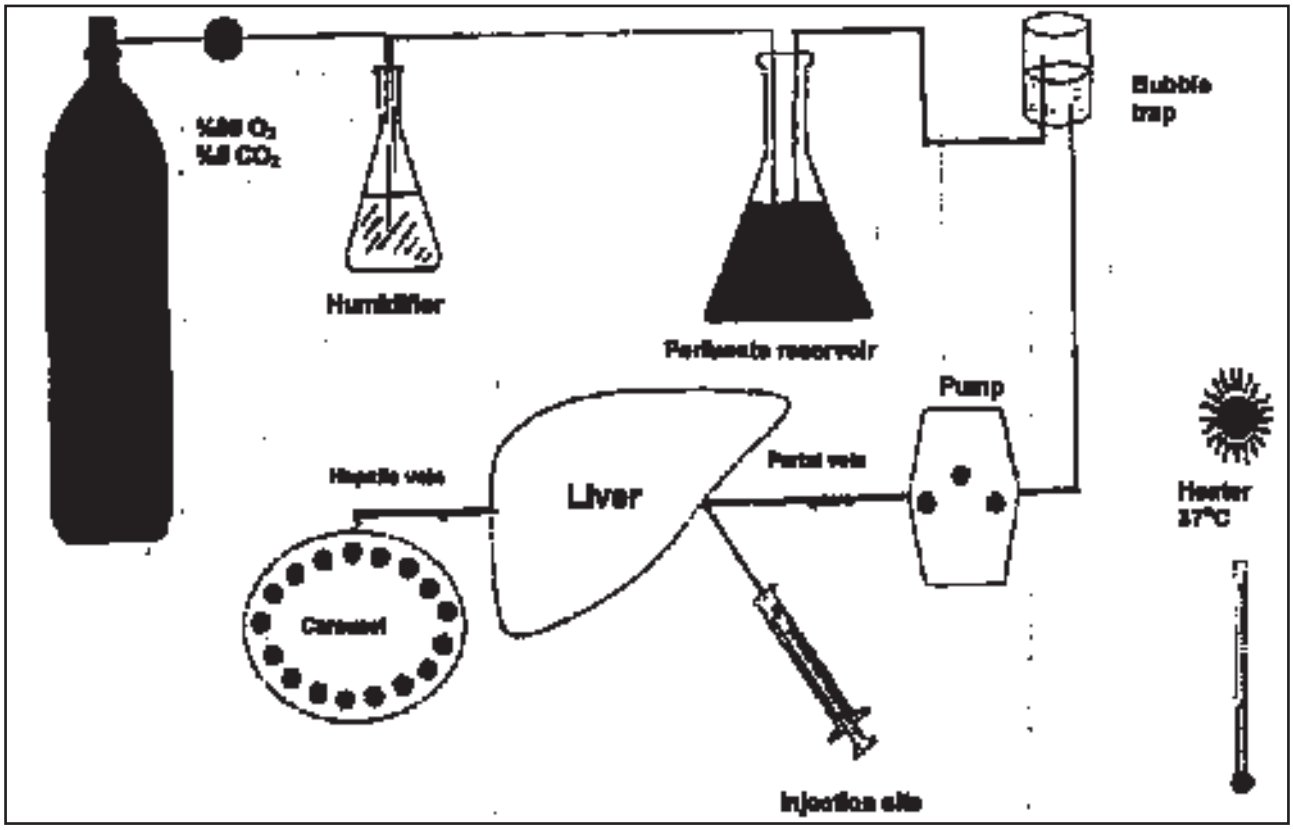

Figure 1. Flow chart assembly of the perfusion apparatus.

ber of perfusates may be used depending upon experimental circumstances, the Krebs-Henseleit bicarbonate buffer is chosen most frequently as it is easy to prepare and it easily equilibrates with a 95% O_2:5% CO_2 atmosphere, a property that aids in maintaining a stable pH. Glucose (3g/L) is usually added as an energy source[1,3,10], and sodium taurocholate (6 mg/L) to maintain bile flow (Table 1). A

Table 1. Composition of Krebs Henseleit bicarbonate buffer.

Chemical	mM	Amount (g/L)
NaCl	118	6.92
$NaHCO_3$	24.9	2.09
$CaCl_2.6H_2O$	2.5	0.56
KCl	4.7	0.35
$MgSO_4.7H_2O$	1.2	0.29
KH_2PO_4	1.2	0.16
Glucose	16.7	3.00
Sodium taurocholate	0.01	0.006

variety of substances including albumin, dextran, gelatine hydrolysates, polyvinylpyrolidone and sucrose have been introduced and employed to maintain osmotic pressure of the perfusion medium. Although albumin is probably the most commonly used substrate for maintaining osmotic pressure, there are number of disadvantages in its use including a potential toxic effect to the preparation[9,28], influence on the perfusate pressure, effect on oxygen solubility and interaction with Ca^{++} in the perfusate[9]. Of these oncotic agents, sucrose has also been used to minimize tissue oedema in the isolated rat liver preparation[29]. Additionally, more specific substrates may be necessary for studies on particular aspects of hepatic function. After preparation, the perfusate is filtered through a 0.22 µm membrane filter and oxygenated with 95% O_2:5% CO_2 for at least 30 minutes prior to adjustment of pH to 7.4 using either 1N sodium hydroxide or 1N hydrochloric acid. Oxygenation of the perfusion medium should be maintained throughout the perfusion experiment.

Oxygen-carrying capacity of the perfusate may be increased by addition of erythrocytes or compounds (e.g. per fluorocarbons) with a high binding capacity for oxygen[1,3,9]. The perfusate containing erythrocytes has the advantages of similarity to physiological conditions and delivery of oxygen according to the needs of the organ. The hematocrit value of 20% is reported to be an ideal combination of blood and perfusate for an optimum oxygen-carrying capacity at physiologic perfusion pressures[30]. However, time-consuming preparation of erythrocytes, adverse effect of haemolysis on organ function, and also binding or uptake of particular substances may limit their use[3]. As an alternative to the erythrocytes, per fluorocarbon compounds can be used as a 10% solution in Krebs Henseleit bicarbonate buffer. This preparation has high binding capacity for oxygen (40-50 ml O_2 per 100 ml) in comparison to that of blood (about 20 ml O_2 per 100 ml). However, a high PO_2 is required to adequately deliver oxygen which is not readily dissociated from fluorocarbon. Further, they are relatively expensive and release of inorganic fluoride may affect metabolic processes[3].

Mischinger et al.[28] have investigated the quality and stability of livers by comparing different perfusates. From the results of their study, they concluded that the simple Krebs Henseleit bicarbonate buffer seems to be the optimal perfusate, and addition of erythrocytes, glucose, albumin, and bile acids is unnecessary and potentially damaging to the organ.

Perfusate Flow Rate and Pressure

Using a standard perfusion medium and a suitable organ, the physical characteristics of the system, the pressure and the flow rate may be defined. This is of interest as a means of assessing the perfusion, but some metabolic and physiologic functions are dependent upon these parameters[9].

It is generally accepted that constancy of perfusion pressure and flow rate during an experiment is a prerequisite to approximate physiological conditions and to maintain uniformity between experiments[1,9]. Perfusion at lower pressures may result in non-homogeneous perfusate distribution within the liver. Use of high perfusion pressure, on the other hand, results in enlargement of sinusoidal fenestrations[31] and also barotrauma of liver. Kestens and

Lambotte[32] have suggested that portal pressure should not be higher than 15 mmHg and arterial pressure not higher than 100 mmHg. Even though use of perfusates without erythrocytes may necessitate higher flow rates (1-5 ml/min g liver)[1,3], possible endothelial injury should be born in mind.

Liver Donors

Many different species have been used as liver donors for perfusion studies including the monkey, calf, sheep, dog, cat, rabbit, pig, guinea pig, hamster, rat, mouse. Of these, the rat is most commonly used due to its convenient size and because it is relatively inexpensive to purchase and maintain; also a large body of reference data in the isolated perfused rat liver is available for comparison. Rat has also been the rodent of choice in most in vitro and in vivo studies, particularly in the drug development studies. Further, it is a convenient species for studying the effects of experimentally induced liver disease (e.g. alcoholic liver injury) and drug-induced alterations in hepatic function[1,3]. Recently, slaughterhouse organs were suggested as source organs to avoid use of high numbers of laboratory animals[33].

Anaesthesia

Part of the operative procedure is the induction and maintenance of anaesthesia. Choice of the anaesthetic agent depends on the nature of the experiment. Various anaesthetic agents including urethane, diethyl ether, barbiturates, halothane, 50% CO_2 have been used for the induction of anaesthesia. Of these agents, urethane (1g/kg, intra peritoneal) is often selected because it does not alter the bile flow. Although the use of diethyl ether, methoxyflurane and sodium pentobarbital anaesthesia in isolated liver perfusion studies does not appear to influence the overall rate and extent of lidocaine clearance, these agents should be used with caution in drug metabolism studies[34]. Pentobarbitone gives satisfactory anaesthesia when injected in doses of 50-60 mg/kg. At this dose, a rat will remain asleep for the following hour at least. Although barbiturates decrease oxygen uptake, these anaesthetics agents are washed out during surgery (within 1-2 min) and are used routinely with the open perfusion method[35]. Halothane is administered as an inhalation anaesthetic. It produces minimal mucous secretion and causes less depression than does pentobarbitone. Following the administration of an anaesthetic agent, depth of anaesthesia is usually assessed by testing the withdrawal response to toe pinch and the blink reflex. Surgical procedure should be started when there is no reaction to these tests[20].

Surgical Procedures

Surgical procedures used for the perfusion of the liver can be divided into two groups:

1. Monovascular perfusion
   1.1 Perfusion through the portal vein (Anterograde perfusion)
   1.2. Reverse perfusion (Retrograde perfusion)
2. Bivascular (Dual) perfusion

1. Monovascular perfusion

1.1. Perfusion through the portal vein (Anterograde perfusion): This procedure involves the perfusion of the liver via the portal vein. In this method, the abdomen is opened through a midline incision from tail to neck and then two mid-transverse incisions are made to right and left of the midline to expose the abdominal contents. Bleeding is minimized by clamping the major vessels on the abdominal wall. The abdominal contents are displaced to the animal's left to expose the liver, PV, right kidney, abdominal vena cava, and bile duct.

The bile duct is cannulated using PE10 tubing and then tied securely in place. The thin strands of connective tissue between the right lobe of the liver and vena cava above the right kidney are cut, and a loose ligature is passed around the abdominal vena cava. Two loose ligatures are placed around the PV. Heparin sodium (1 ml; 1000 U/ml) is injected into the online perfusate distal to the pump and then the PV is cannulated using 16GA catheter. Air in the catheter is removed by the backflow of the rat's blood. The perfusion is then started. The thorax is opened

by a longitudinal incision, and two transverse incisions are made. The outflow perfusate is collected via tubing inserted into the thoracic vena cava through the right atrium. All the loose ligatures are then tied securely, and the exposed liver moistened with saline covered with a piece of parafilm, to minimise evaporation. The preparation is then placed in the perfusion chamber maintained at a temperature of 37±2°C (Figure 2). All operative procedures are completed within 10-15 min. Interruption of the perfusate flow to the liver is less than 10 sec[16].

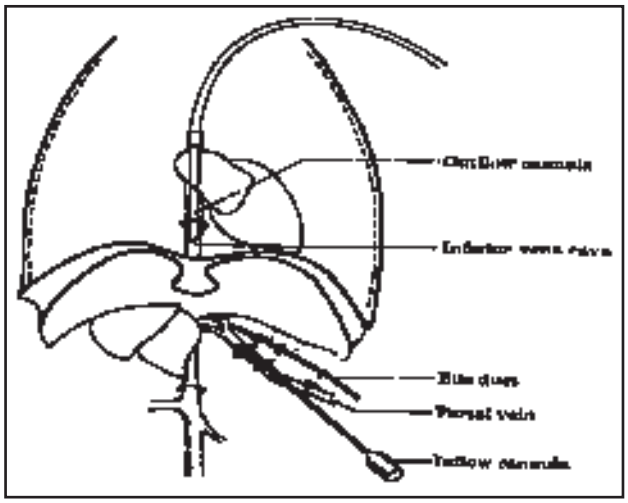

Figure 2. Surgical procedure for the perfusion through the portal vein (anterograde perfusion)[9].

1.2. Reverse (retrograde) perfusion: The surgical procedure is the same as the portal vein perfusion. The only difference is the site of perfusate administration (thoracic vena cava) and outflow perfusate collection (portal vein)[9] (Figure 3).

2. Bivascular (Dual) perfusion

This method involves the cannulation of both PV and HA. The HA, together with the splenic and left gastric arteries, arises from the celiac artery (axis), a branch of the aorta that arises just beneath the diaphragm. Before joining the middle part of the PV on the left side, the HA gives rise to a small gastroduodenal artery following the corresponding vein and then becomes the HA proper. As it lies parallel and behind the PV, if care is not taken, the HA can

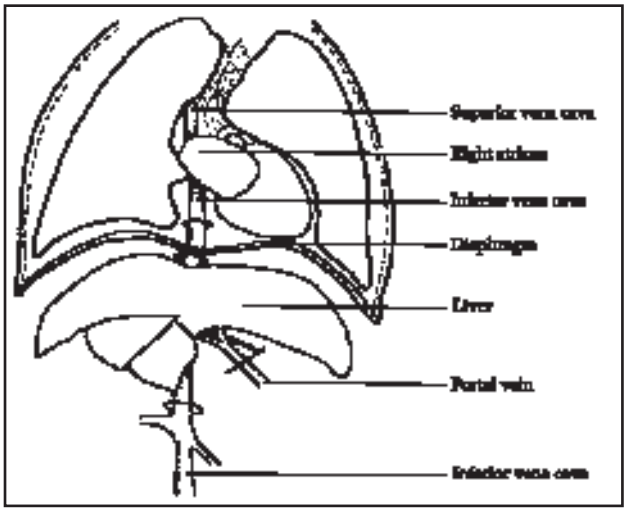

Figure 3. Surgical procedure for the reverse (retrograde) perfusion[9] .

easily be included in the ligatures placed around PV[9,36,37]. Unlike the PV, the HA proper cannot be cannulated directly due to its small size and strong adhesion to the PV. Therefore, an indirect approach is usually employed for the cannulation of the HA. The major discrimination between the methods detailed in the literature for the dual perfused rat liver preparation is the level of cannulation. In the majority of techniques, the rat liver is perfused by cannulation of the celiac artery[11-20] or aorta[21-23]. Although cannulation of the HA via the gastroduodenal artery is a more direct method, in pharmacokinetic studies this method is restricted to larger animals such as the rabbit[8,24], cat and dog[25-27], mainly due to the larger size of the vessel. However, the rat has been used successfully as an animal model for the study of the regional delivery of anti-neoplastic agents, such as mitomycin and 5-fluorouracil[38-40], via the gastroduodenal artery. Sahin and Rowland[16] compared all these methods used for the cannulation of the HA, and reported that aortic cannulation is the most troublesome due not only to the longer operation time but also the lowest success rate (about 40%) in comparison with the other methods used. Although very similar results were reported irrespective of the cannulation method for the HA, authors favoured the cannulation of HA via the celiac artery due to the highest rate of success (about 70%). A failure rate about 30-40% was due

to anatomical variation, leaking and cannulation problems[12,16].

Surgical details of the HA cannulation via the gastroduodenal artery, the aorta and the celiac artery are described in the following section[16].

a. Cannulation through the gastroduodenal artery: The abdominal contents are displaced to the animal's right after placing loose ligatures around the PV, ensuring exclusion of the HA. The common hepatic artery is ligated so as to prevent backflow of hepatic arterial perfusion into the aorta. Two loose ligatures are placed around the gastroduodenal artery (GDA). The GDA is blocked momentarily and then cut between the ligatures across half its diameter. It is then cannulated using PE10 tubing with the bevelled tip advanced to the juncture of the GDA and hepatic arteries. The loose ligatures are tightened gently ensuring that the tubing is not blocked, and perfusion then started. Abdominal contents are then displaced to the animal's left and the portal and thoracic vena cava are cannulated as described in the perfusion through the portal vein method. Finally, all the loose ligatures are tied securely and the surgical board placed in the perfusion chamber after the moistened liver is covered with a piece of parafilm. All operative procedures are completed within 20-30 min without interruption of the perfusate flow to the liver.

b. Cannulation through the aorta: Cannulation of the bile duct and preparation of the PV for the cannulation are the same as described in the anterograde perfusion. The abdominal contents are displaced to the animal's right. The thin strands of connective tissues between the liver lobes and the stomach are cut, taking care not to damage the liver. The lower oesophagus is cut between ligatures and then gently displaced downwards to expose the aorta and celiac artery. The superior mesenteric artery, left and right renal arteries and ileolumbar arteries are ligated. The left gastric and splenic arteries, branches of the celiac artery (axis) and GDA are also ligated. Two loose ligatures are placed around the aorta, one just above the celiac artery and the other at the level of the left renal artery. After all these procedures, only the hepatic artery is left patent. The abdominal contents are displaced to the animal's left and then the PV and vena cava are cannulated, as described in the anterograde perfusion method. The aorta is tied below the level of the left renal artery and above the celiac artery, then cannulated with a 18GA catheter and the second perfusion started. All loose ligatures are secured and the HA inflow is connected to a mercury manometer via a side arm anterior to the cannula. The perfusate from a common reservoir is delivered into the PV and HA. Throughout the experiment, the liver is kept moist with saline and covered with a piece of parafilm. Completion of all operative procedures takes around 40-50 min.

c. Cannulation through the celiac artery: After the abdominal contents of the rat are exposed, as described previously for perfusion of the PV, these are then displaced to the animal's left. The bile duct is first cannulated and secured into place. Loose ligatures are then placed around the abdominal vena cava and also around the PV, ensuring exclusion of the HA. At this point, the abdominal contents are deflected to the animal's right. Connective tissues between the liver and stomach are cut. Two loose ligatures are passed around the oesophagus, tied securely and then cut between the ligatures. The stomach is then gently moved downwards, to expose the aorta and celiac axis (CA). The CA, located from the ventral surface of the aorta at the level of the crus of the diaphragm, has a short trunk which divides into three branches, the left gastric, splenic (lineal) and hepatic arteries. The left gastric and splenic arteries are tied very close to their junctions to the CA. Only the HA is left patent after ligation of the GDA. Two loose ligatures are placed around the aorta, one above and one below the level of the CA. Heparin sodium (1 ml; 1000 U/ml) is injected into the online perfusate distal to the pump. After cannulation of the PV with a 16GA catheter, the cannula is immediately connected to the tubing and the perfusion started. Exsanguination of the liver is facilitated by inserting a polyethylene tubing into the thoracic vena cava via the heart. The HA is cannulated indirectly through the CA using a 18GA or 20GA catheter. The

second perfusion is then started and the arterial cannula is fixed in place using a tissue adhesive. At the end of surgery, all loose ligatures are tied securely and then the HA cannula is connected to a mercury manometer, by a side arm anterior to the arterial cannula, to monitor the perfusion pressure continuously. All operative procedures are completed within 20-30 min without interruption of flow to the liver. The exposed liver is kept moist with saline and covered with a piece of parafilm, to reduce dehydration (Figure 4).

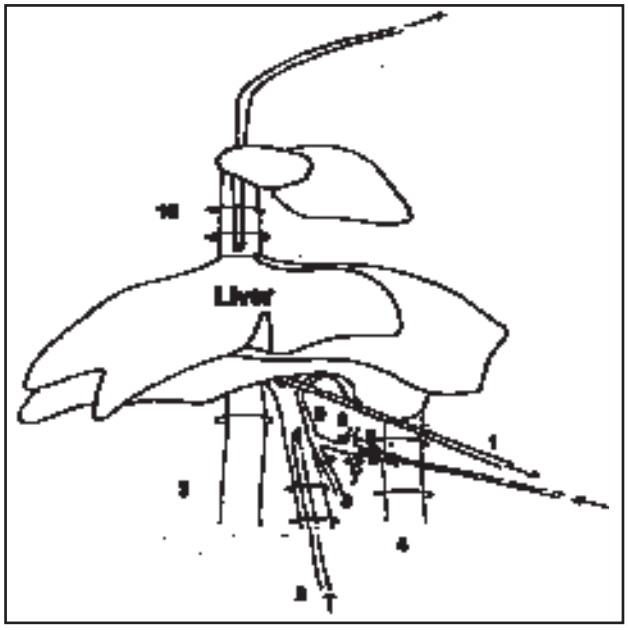

Figure 4. Surgical procedure for the dual perfusion of the rat liver[16]. Numbers represent 1.bile duct,2. portal vein, 3. abdominal vena cava, 4.aorta, 5.coeliac artery, 6.left gastric artery, 7. splenic artery, 8. gastroduodenal artery, 9. hepatic artery proper, 10. thoracic vena cava.

Methods of perfusion

Depending on the aim of the perfusion, the liver can be removed from the animal after the cannulation procedure and perfusion is conducted in an enclosed organ chamber (isolated liver), or can be left in the carcass (in situ liver). Both methods have its advantages and disadvantages. In the case of in situ perfusion: (i) the operative technique is simpler and less liable to error, (ii) tricky stage of transferring the liver from its original position to the organ bath is omitted, (iii) the preparation requires only minimum attention and may continue to function for the desired length of time, and (iv) the liver is not exposed to mechanical damage as it is not handled. On the other hand, in the isolated technique (i) the liver may be totally enclosed within an organ chamber, thus loss of fluid by evaporation and of gases by diffusion are avoided, (ii) sampling of the liver lobes during the perfusion is simplified, (iii) freeze clamping of rapid sampling of liver is more easily achieved, and (iv) some surface measuring techniques are dependent on the accurate placing and fixation of the liver during the perfusion which limit them to the isolated technique[9].

Following the surgical preparation, the perfusion medium can be circulated (recirculation) or can be delivered in a single-pass mode. Each one has its advantages and disadvantages. Once through perfusion requires a large volume of perfusion medium, since the duration of the experiment is limited by the size of this volume. No equilibrium is established since the medium is constantly changing. If the medium is delivered from a well-mixed reservoir, this in itself is no disadvantage since the composition of the medium is constant. Metabolic changes in the medium must be established by determining arterio-venous concentration difference. As a corollary to the determination of uptake by arterio-venous difference, the flow rate of the medium through the organ must be accurately known. In the case of the recirculation perfusion, the volume of the medium required is much smaller and does not influence the duration of perfusion, an equilibrium is established between the medium and the tissue, uptake or production rates may be determined by serial analysis of the mixed medium in a reservoir. However, accumulation of products or exhaustion of substrates and other essential factors may influence the course of reactions in the perfused organs[9].

Viability Assessment

The isolated liver is a dying organ with a life span of few hours only[1]. As the liver has multiple functions, each of which may deteriorate separately, it is

difficult to evaluate total hepatic function using a single test.

Most investigators use a variety of tests, performed either during or after the experiment, for the assessment of functional capacity of the liver. Some viability tests (e.g. the gross appearance of the liver, perfusate flow, pressure, pH, potassium concentration, oxygen consumption and bile flow) provide information for the assessment of the liver viability during the perfusion procedure, whereas others (e.g. structural integrity of the liver, measurement of enzyme activity in the effluent perfusate, measurement of specific hepatic functions) need to be performed after the experiment[1,3]. However, Ross[9] speculated that a number of the tests that have been used to assess the viability of the liver, such as that of enzyme leakage, glucose and ions, are insensitive since they either occur as a late event resulting from extensive disruption of the cell membranes, or they do not necessarily reflect the metabolic function of the liver. The tests used for measurement of the liver viability are summarized below[1,3,10,34,41,42]:

Gross appearance: The liver should be uniform in colour with no white spots (due to air emboli) or dark blotches (due to non homogeneous perfusion, trapped blood, or gross damage) on the surface. It should not appear swollen and the perfusate should flow uniformly out of the vena cava cannula.

Bile flow: Baseline bile flow after the cannulation should be around 1 ml/min/g liver and will decrease gradually by 15% if taurocholate is infused. In the absence of bile acid addition, bile flow may decrease by 30-40% over a 2-hour perfusion period.

Perfusion pressure: Inflow perfusion pressure should remain relatively constant, increasing not more than 1 cm of water per hour. For a given flow rate an increase in perfusion pressure suggests increased hepatic resistance and is an indicator of decreased viability.

Oxygen consumption: Maximal oxygen consumption in isolated perfused liver ranges from 1.4 to 2.5 mmol/min/g liver depending on the substrate addition. In a viable liver, oxygen consumption should remain constant.

Potassium concentration: A rapid increase in perfusate potassium concentration suggests decreased viability of liver cells as it is a predominantly intracellular ion.

Hepatic enzymes: The concentrations of hepatic enzymes (lactate dehydrogenase, transaminases, alkaline phosphatase) leaking into the perfusate are frequently monitored as measures of liver viability. Any significant increase in aminotransferase activity during the perfusion indicates liver cell necrosis. As the biliary tree is a major contributor to serum alkaline phosphatase, its elevations reflect biliary obstruction. Serum transaminase levels are sensitive indicators of hepatic damage. Hypoxia may result in the release of lysosomal enzymes (e.g. acid phosphate)[3,42].

Integrity of liver cells and organelles by electron microscopy: Swollen and distended sinusoids, absence of microvilli on the hepatocyte surface, cytoplasmic vacuolization and mitochondrial swelling are indicative of hypoxic injury. However, these procedures require specialized expertise and are not performed routinely.

Other measures of hepatic viability include assessment of the pyridine nucleotide oxidation-reduction status of the cytosol or mitochondria, measurement of tissue levels of ADP/ATP, and measurement of metabolic performance such as gluconeogenesis or ketogenesis from lactate and fatty acids or ureagenesis from ammonia. In addition hepatic clearance, transcellular transport, and metabolism may provide useful measures of liver viability. Dry to wet liver ratio should not be lower than 0.28 after 2 h of perfusion[1,3,10,34,43].

General Application of Perfused Liver Preparation

The main advantage of the perfused liver preparation is the ability to allow examination of metabolic

activity, physiological status, and/or pharmacological response of liver in the absence of extrahepatic influences.

The perfused liver is widely employed for investigation of hepatic disposition (extraction, excretion, binding, metabolism, permeability) of compounds. The data obtained in such experiments may be used to predict substrate disposition in vivo or to extrapolate disposition from experimental animals to humans. Pharmacokinetic modelling of data obtained from the liver perfusion studies may also be used to explore mechanisms underlying substrate disposition, determine rate limiting steps, and identify sites of drug interaction within the hepatobiliary systems. Furthermore, distributional space of the liver (vascular, extravascular, cellular) can be determined in the perfused rat liver by means of the indicator dilution method[13,20, 43-45].

Hepatocytes located in different parts of the hepatic acinus, the functional unit of the liver, maintain different levels of activity for different functions. The perfused liver preparation can be used to study this zonal heterogeneity. Anterograde and retrograde perfusion approaches are useful to determine the heterogeneous distributions of drug metabolising enzymes and to study zonal differences in the hepatic extraction of compounds[46-48].

A variety of hepatic metabolic functions (e.g. gluconeogenesis, lipogenesis, ureagenesis, ketogenesis and conjugation reactions) can be studied in the perfused liver preparation. Also hepatic synthetic functions such as albumin, lipoproteins, coagulation factors, and conversion of glutathione to glutathione disulfide can be evaluated in the perfused liver preparation[49-51].

Hepatotoxicity (e.g.drug-induced, alcohol-induced) can be studied in the perfused liver preparation. It is also well suited for studying transcellular transport and biliary excretion. Extensive studies of bile formation have been performed in the perfused liver preparation including effects on flow, pressure changes, choleretic and cholestatic agents[52-55].

Perfused liver preparation can be used for preparations of isolated liver cell suspensions, liver slices, and liver fractions enriched in specific organelles. Also it may be used in combination with other experimental models such as in situ perfused intestine-liver preparation[56].

It can be used to investigate pharmacologic/disease state alterations in cirrhosis, fatty liver, hypoxia. Additionally, liver regeneration, functions of cell surface and intracellular receptors can be studied in the perfused liver preparation[57,58].

Conclusions

The hepatic functions can be studied with a spectrum of liver preparations ranging from the intact organ in vivo, through perfusion systems, liver slices, isolated hepatocytes, homogenates and membrane fractions to purified enzymes. Among these methods, the use of the isolated liver preparation is attractive because of the intact architecture of the organ, and possibilities of controlling the experimental conditions. As indicated in the text, the range of applications for the perfused liver are broad, and may serve as important means of validation of results obtained in systems such as isolated or cultured cells which represent a greater departure from normal hepatic physiology.

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