Assessment and preservation of liver function in hepatic ischemia and reperfusion
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Chapter 2

Liver ischemia and reperfusion in the rat: a plea for standardization.

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Introduction

A period of liver ischemia occurs during a number of surgical procedures concerning the liver, especially during interruption of hepatic vascular flow during extensive liver resections for liver trauma or intrahepatic lesions \(^1,2\) and during storage of the liver for transplantation \(^3\). Furthermore, hypoxic injury can be the consequence of hypotensive periods and various shock syndromes \(^4\) and even has been suggested as a contributory factor in alcoholic liver disease \(^5,6\). When the blood flow to the liver is restored, reperfusion of the liver paradoxically leads to aggravation of liver damage initiated during the ischemic period.

The pathophysiological mechanisms underlying hepatic ischemia and reperfusion (I/R) injury have been the focus of a large number of animal studies. Most of these studies have been performed in rats and mice. Methodological differences have a large impact on the results obtained with these models, make comparison of results difficult and can potentially lead to wrong conclusions. The aim of this review is to highlight the different rat models for liver I/R injury and the different anesthetic procedures that are used. Ultimately, criteria are proposed to achieve a standardized model for the study of I/R phenomena in the rat liver.

To achieve this goal, the world literature was searched via Medline. Keywords included a combination of ‘rat’, ‘liver’, ‘ischemia’, reperfusion and ‘vivo’. Afterwards, all abstracts were examined and after shifting, a total of 392 references were retrieved. From these references, 105 were randomly selected and carefully examined for:

1 Hepatic liver I/R model;
2 Use of heparin during clamping;
3 Rat type and strain;
4 Fasting;
5 Anesthetic methods;
6 Temperature control.
Hepatic I/R model in the rat

In literature, 9 different I/R models are described which can be categorized in six partial liver ischemia and three total liver ischemia models.

Total liver ischemia models

In total liver ischemia models, the liver is made ischemic by occlusion of the afferent vessels to the liver or by decreasing blood supply to the liver by inducing hypotension. The latter method was encountered only once in our review of literature and will therefore not be discussed in detail. Clamping of the afferent vessels to the liver was first described by Pringle in 1908 as a method to control blood loss during major liver trauma. The Pringle maneuver is often used in total liver ischemia models in rats since it is easy to perform in rats and it well represents the clinical situation. However, clamping of the portal vein causes obstruction of efferent blood flow in the splanchnic circulation. Although most humans are able to tolerate clamping of the portal vein up to several hours due to an extensive collateral circulation, the collateral circulation of a rat is much less developed leading to splanchnic congestion within 20 minutes. To allow investigation of ischemia times longer than 20 min, the splanchnic circulation must be decompressed during ischemia by applying a porto-systemic shunt. In rats, the construction of a porto-systemic shunt requires advanced microsurgical skills and this procedure cannot be performed very easily. Secondly, blood clotting inside the shunt occurs frequently since the blood flow through the shunt is low. To prevent clotting, anti-coagulants are frequently used, which can interfere with I/R injury (see paragraph “The use of heparin during clamping”). Another, more elaborate possibility is to use a pump-driven extracorporeal shunt, which, reportedly, prevents mortality in comparison with rats who received only a passive porto-systemic shunt. Also, portal-systemic decompression during ischemia prevents the inhibition of hepatic regeneration caused by reperfusion of pooled portal blood. In our opinion, the use of a porto-systemic shunt should be avoided whenever possible, in view of the aforementioned drawbacks.

In 13 out of the 105 examined references, the Pringle maneuver was used without a porto-systemic shunt, making it the second most common used model in rats. In general, the used ischemic times are shorter than in other models of liver I/R injury, owing to the drawbacks related to splanchnic congestion.
When the Pringle maneuver is combined with a veno-venous shunt (n=11), several different types of shunts are used, ranging from different spleno-systemic shunts\textsuperscript{11,12}, multiple types of porto-systemic shunts\textsuperscript{13-20}, to a pump driven porto-systemic shunt\textsuperscript{9}. In most cases, heparin was needed to prevent blood clotting except when a (short) porto-caval shunt was used.

Selective occlusion of the portal vein to induce liver I/R injury was used in 5 of the examined articles, all by the group of Ogawa et al.\textsuperscript{21-25}. In this model, the liver is still supplied with oxygen rich blood from the hepatic artery and therefore, the liver will not be rendered totally ischemic or anoxic but merely hypoxic to a limited extent. This model has not been copied by others until now. In one article, the hepatic artery was selectively occluded in combination with a porto-systemic shunt in order to investigate the influence of acute liver failure on neuroactive amino acids and glutamate (NMDA) receptors in frontal cortex\textsuperscript{26}.

*Partial liver ischemia models*

In these models, the afferent blood supply to one or more lobes of the liver are occluded, with a maximum of 70% of total liver volume. The remaining 30% of the liver is still perfused and is able to adequately drain the blood from the portal vein. Therefore the common feature in these models is the lack of splanchnic congestion, which obviates the use of a veno-venous bypass procedure\textsuperscript{27,28}. This is considered to be a major advantage over total liver ischemia models. On the other hand, during reperfusion, when the clamp is released, both ischemic and non-ischemic liver tissue is perfused, which in most cases does not resemble the clinical situation and could complicate the interpretation of results. To overcome this drawback, some authors occluded or removed the non-ischemic parts of the liver before initiation of reperfusion, rendering only the ischemic lobes to be reperfused. Reportedly, the blood flow to the previously ischemic left lobe is only 50% of normal while that of the non-occluded right lobe is 134%\textsuperscript{29}. This preferential shunting of blood to the non-occluded tissues has been advocated to be the reason for failure of therapy with ATP-MgCl\textsubscript{2}, since occlusion of the non-ischemic part of the liver during reperfusion did show beneficial effects of ATP-MgCl\textsubscript{2}\textsuperscript{29}. Preferential shunting also causes a slower rate of recovery of high energy phosphates and energy charge to normal levels in previously occluded liver lobes\textsuperscript{30}. Perhaps, the aforementioned observations were biased by the limited period of reperfusion (60 min) in those particular studies. On the other hand, others have shown that longer reperfusion times in animals undergoing resection of the non-ischemic hepatic lobes at the time of reperfusion do not reveal differences in pulmonary and hepatic levels of epithelial cell-derived
neutrophil attractant-78 (ENA-78) or in the development of lung and liver injury, as compared with animals undergoing hepatic I/R alone due to preferential shunting to non-occluded lobes.

Due to an extensive collateral circulation, the Pringle maneuver can be applied in most patients without the need for porto-systemic bypass surgery. One could argue that in humans, the possibility of shunting of blood to the collateral circulation exists, resembling shunting to the non-occluded lobes in rats, thereby reducing the blood flow to the previously occluded liver lobes as well. Therefore, in our opinion, the partial ischemia model resembles the clinical situation most.

**Occlusion of the median and left lateral liver lobes:**
For induction of partial liver ischemia, the afferent vessels to the median and left lateral liver lobes are occluded most frequently, rendering about 70% of liver volume ischemic (54 out of 105 publications). The afferent vessels to these lobes can easily be exposed by evertting the median and left lateral liver lobes outside of the abdominal cavity by lateral pressure on the thorax without actually touching the liver. In 3 reports, the afferent vessels to the right hepatic lobes were ligated before reperfusion was initiated, whereas in 5 cases the right liver lobes were resected.

**Occlusion of the median, left and caudate liver lobes:**
To our knowledge this model has been used only twice. The rationale for using this particular model has not been described in the articles. This model has not been copied by others until now.

**Occlusion of the left lateral liver lobe:**
Selective occlusion of the vessels to the left lateral liver lobe has been used 11 times and is most often used for studying local phenomena by intravital microscopy or NMR spectroscopy.
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The left lateral liver lobe comprises about 35% of the liver and can easily be made accessible for intravitral microscopy or other imaging devices.

Occlusion of the right lateral liver lobe:
This model was used 6 times by 4 different research groups, rendering 15% of the liver ischemic. In two cases, the afferent vessels to the median and left lateral lobes were ligated or removed after ischemia before reperfusion to avoid preferential shunting. In both cases the caudate lobe was continuously perfused.

Occlusion of the right lateral and caudate liver lobes:
Selective occlusion of the afferent vessels to the right lateral and caudate liver lobes comprises about 30% of liver volume and has only been published twice. In one article, the median and left lateral lobes were removed after ischemia to prevent preferential shunting.

Occlusion of the median and right lateral liver lobes:
Occlusion of the median and right lateral liver lobes, rendering 65% of the liver ischemic, has been performed only once by Branum et al., but the rationale for using this particular model has not been explained in the article.

The use of heparin during clamping
Inducing ischemia by clamping the afferent vessels to the liver for a prolonged period brings about the risk of intravascular coagulation and hence, hampered blood flow during reperfusion. To prevent blood clotting, heparin can be administered before induction of ischemia. However, for several reasons, the use of heparin should be avoided. In the clinical setting, during major liver resections when the Pringle maneuver is used, the administration of heparin is avoided since this may cause excessive hemorrhage endangering the life of the patient. Furthermore, heparin can interfere in liver I/R injury by decreasing cytokine-induced, neutrophil chemo-attractant (CINC) or by inhibition of complement activation. Heparin is also a competitive ligand for the hyaluronic acid (HA) receptor on sinusoidal endothelial cells and therefore has been suggested to decrease HA uptake. Still, in 19 out of 105 articles (in which heparin was not a subject of interest), heparin was used during ischemia to reduce blood clotting and to ensure adequate reperfusion. Although reasoning seems valid, the beneficial effect of heparin for maintenance of liver blood flow during reperfusion has not been confirmed.
Rat gender and strain

The gender and strain of the rat can potentially influence liver I/R injury. Consensus seems to exist amongst authors about the use of the male gender in rats, since in all but three studies, male rats were used. Less consensus exists about the used rat strains. The Wistar rat has been used in 47 studies (44.7%), the Spraque Dawley in 46 (43.8%), the Fisher rat in 8 (7.6%), the Lewis rat in 2 (1.9%) and the Holtzman rat only once. A large body of evidence exists about the influence of the used rat strain on the results obtained with the study. In fact, even the same strain obtained from a different vendor can produce marked differences in outcome \(^{117,118}\). Infarct volume reportedly varies with rat strain and vendor in models of cerebral ischemia \(^{119-121}\), myocardial ischemia \(^{122}\), unilateral kidney ischemia \(^{123}\), epigastric flap ischemia \(^{124}\) and testicular toxicity \(^{125}\). Based on the aforementioned responses to ischemia and on the potential differences in liver enzyme \(^{126}\), differences in the outcome of liver I/R injury between rat strains are likely, although to our knowledge, this has not yet been described. As genetic differences between rat strains may underlie the observed differences in the outcome of liver I/R injury, investigating and comparing DNA profiles of the rat strains may prove useful for unraveling the pathophysiology of the development of liver I/R injury \(^{127,128}\).

Fasting

Fasting can have a large effect on the outcome of an experiment. Frederiks and co-workers showed that the administration of ATP-MgCl\(_2\) or adenosine-MgCl\(_2\) reduced the volume density of necrotic areas in the liver of a fasted rat from about 15% to almost zero. However, the extent of necrosis was not reduced when ischemia was induced in the liver of a fed rat which showed a more massive necrosis (about 30%) \(^{33}\). Although several authors described that livers from fed rats sustained more injury from liver I/R than fasted rats \(^{33,129}\), other authors describe the opposite \(^{130,131}\).

Le Couteur et al. showed that the livers of fed rats are resistant to hypoxia-reoxygenation injury and that aging does not increase the susceptibility of the liver to injury in the fasted state \(^{132}\). These differences in susceptibility to I/R injury might be related to a decrease in GSH content \(^{133}\), a decrease in heat shock protein mRNA \(^{134}\) or a marked augmentation of lipid peroxidation \(^{135}\) after fasting. Furthermore, an increase in fasting times tends to increase the susceptibility of the liver for I/R injury \(^{136,137}\). Also in the transplantation setting there is still much
controversy about whether fasting increases resistance to ischemic injury after cold-ischemia or not. In 40 out 105 articles reviewed, the nutritional status was not mentioned. In 20 cases rats were not fasted prior to the experiment. Fasted rats were fasted for overnight (15 times), for 24 hours (13 times), for 12 hours (11 times), for 18 hours (1 time) or for 15 hours (1 time). In 4 articles, the fasted or non-fasted state was a topic of interest and therefore both conditions were applied. In conclusion, the impact of fasting on warm and cold ischemia remains a topic of debate. In the clinical setting, fasting prior to surgery is still applied whenever possible and therefore, we feel that during liver I/R experiments fasting is appropriate.

Anesthetic methods

Introduction
The anesthetic management of experimental animals deserves a good deal of attention, not only on ethical, legal and moral grounds, but also because anesthetics can interfere with results when not carefully chosen. For the study of liver I/R injury, anesthetics which are neither toxic to the liver nor metabolized by the liver are preferable for obvious reasons. Furthermore, the effects on depression of the cardiovascular and respiratory system differ highly between anesthetic agents and could lead to undesirable effects on outcome. A major hazard is the development of respiratory acidosis during the experiment, since acidosis leads to unpredictable difficulties in interpretation of results. To tackle this problem, the respiration can be facilitated by placing a cannula in the trachea although this does not always suffice. In any case, active ventilation of the animal is preferred. Furthermore, the use of particular anesthetics can influence bile secretion, a widely used parameter for hepatocellular function. Administration of anesthetics can be performed by injection or inhalation. Both methods have their advantages and disadvantages. The advantages and disadvantages of the most commonly used anesthetics in rats, administered via both routes, will be discussed briefly. For further reading, the books by L.W. Hall and K.W. Clarke (1991) and P.A. Flecknell (1996) are highly recommended.
Injectable anesthesia

Ketamine:
Ketamine has been used in 14 out of 105 references almost always in combination with other agents like xylazine, atropine, pentobarbital or inhalation anesthetics. Ketamine can be administered by intramuscular, intraperitoneal and intravenous routes. Although often used, ketamine has several major drawbacks, which render it less favorable for the use in small rodents. The degree of analgesia produced is very variable and in small rodents, severe respiratory depression is produced following administration of the high dose needed to produce surgical anesthesia. Salivary secretion is increased and airway obstruction is a hazard. Therefore ketamine should preferably be combined with atropine to reduce excessive saliva secretion. Ketamine produces little, if any, muscle relaxation. Generally, there is an increase in skeletal muscle tonus, even when used in combination with xylazine, which could hamper the surgical procedure. Ketamine has a stimulatory effect on the cardiovascular system and the minimal arterial blood pressure is seldom less than the preoperative level. Thus, the arterial blood pressure is generally maintained during ketamine-induced anesthesia. However, the use of ketamine in combination with xylazine results almost invariably in hypotension. Furthermore, in our experience (not yet reported), the high dosage of the acidic compound ketamine needed for adequate surgical anesthesia results in an acidosis which cannot be corrected by hyperventilation.

Pentobarbital:
Pentobarbital has been used 40 times out of 105, often in combination with ketamine (5 times) or the volatile anesthetic ether (4 times). Although it is cheap and one of the most widely used laboratory animal anesthetics, there are several better alternatives available. Surgical anesthesia is only achieved when dosages are administered close to those that cause respiratory failure. At these dosages, pentobarbital causes severe cardiovascular and respiratory depression. Prolonged recovery includes vocalization, involuntary paddling, thrashing and muscle fasciculation. Complete recovery following pentobarbital anesthesia may take hours to days. Furthermore, pentobarbital is metabolized primarily by the liver and may cause further injury to an already damaged liver. Therefore, in experiments involving liver ischemia and reperfusion injury, pentobarbital should be avoided if possible.
Chloral hydrate:
Application of chloral hydrate was found 7 times out of 105 publications\textsuperscript{90,91,146}, of which 4 times in combination with atropine\textsuperscript{92-95}. It has been used exclusively by Vollmar et al. Chloral hydrate produces stable, light anesthesia with minimal effects on the cardiovascular system. However, the large dosages required for surgical anesthesia can produce severe respiratory depression, hence explaining the use of a tracheotomy in these animal experiments. Furthermore, intraperitoneal administration to rats has been associated with a high incidence of post-anesthetic ileus and the response to chloral hydrate has a considerable strain variation. In conclusion, this compound can be replaced by more effective anesthetics if surgical procedures are to be undertaken.

Urethane:
Urethane has been used 3 times by Peralta et al.\textsuperscript{101-103}. Urethane produces long periods of stable anesthesia with minimal depression of the cardiovascular and respiratory systems. However, the cardiovascular stability is in part due to the sustained sympathetic nervous system activity, associated with high circulating levels of adrenaline and noradrenaline. Furthermore, urethane is both mutagenic and carcinogenic and is regarded as a potential hazard to laboratory investigators. It is advised not to allow animals to recover from urethane anesthesia, which makes urethane not suitable for use in survival experiments.

Hypron (Fentanyl/Fluanisone):
Hypron has been used only twice in combination with diazepam by Chavez-Cartaya et al.\textsuperscript{47,78} and will not be discussed here.

\textit{Inhalation anesthetics}

Ether:
Ether was the most frequently applied inhalation anesthetic. It has been used 34 times, alone\textsuperscript{10-13,21-25,33,34,38,49,52,57,58,62,63,66,68,74,77,79,80,84,88,89,99,105} or in combination with pentobarbital (4 times)\textsuperscript{17,18,46,141} or metofane\textsuperscript{100}. Ether was one of the earliest inhalation anesthetics available and it has the justified reputation of being a safe anesthetic agent. It is easy to vaporize in simple apparatus and it is difficult to kill an animal with an overdose of ether. It is usually associated with reasonable good muscle relaxation, lesser depression of respiration than other inhalation agents and good postoperative analgesia. However, it has some considerable drawbacks. Induction of
anesthesia with ether is slow and unpleasant since it is highly irritating to the mucosa and can cause coughing, profuse bronchial and salivary secretions and occasional laryngospasm. For this reason, the use of ether for anesthetic purposes is prohibited in The Netherlands. Furthermore, ether is highly inflammable and forms explosive mixtures with both oxygen and air. Therefore, despite its popularity as an anesthetic, the use of ether is not recommended since better and less irritating agents are readily available.

Halothane:
Halothane has been used in 3 liver I/R studies by different authors 26,98,104. Halothane is relatively cheap and easy to vaporize. Induction and recovery are rapid and free from agitation during the recovery period. Halothane is not irritating to the mucosa of the respiratory tract and can produce bronchodilatation. However, halothane has a depressant effect on the cardiovascular system and moderate hypotension can be produced at surgical levels of anesthesia. Halothane is metabolized for 20-25% by the liver and kidneys and hepatotoxicity has been described 152 which makes halothane unsuitable for liver I/R injury experiments.

Methoxyflurane:
Methoxyflurane has been used in 4 liver I/R studies, alone 54,55 or in combination with ketamine 32,50. It is non-irritant, non-inflammable and non-explosive in air or oxygen. It has potent analgesic effects and has some post-operative analgesic action. High-output renal failure has been reported following prolonged administration of high concentrations, which resulted in withdrawal of the compound from medical practice and as of the year 2000, methoxyflurane is no longer available in the United States. Up to 50% of methoxyflurane is metabolized by the liver and kidneys, which renders it less suitable for liver I/R experiments.

Isoflurane:
Isoflurane has been used twice in liver I/R studies in combination with nitrous oxide 9,144. It is non-irritant, non-inflammable and non-explosive in air or oxygen. It has very rapid induction and recovery from anesthesia and the depth of anesthesia can be adjusted rapidly. It produces excellent muscle relaxation, which is helpful during operation. Isoflurane is almost completely eliminated through the respiratory tract and only 0.17% is metabolized by the liver and kidneys, which makes it ideal for liver I/R experiments although mild protective properties of isoflurane on liver I/R injury have been reported 153-155. Isoflurane like halothane requires special vaporizers and
equipment, which are expensive. For the moment, isoflurane seems to be the most favourable anesthetic in liver I/R experiments.

Enflurane:
Enflurane has been used only once and will not be discussed here.

Temperature control

In animal experiments involving rats, temperature control has not been a topic of much interest until now. In 74 out of 105 articles, temperature has not been recorded during the experiment at all or has not been mentioned in the article, assuming it has not been measured. In four articles, the temperature was controlled between 36°C and 38°C\(^{112-114,142}\), while in 10 articles the temperature was controlled between 36°C and 37°C\(^{11,90,92-95,101-103,146}\). Three times, the temperature was controlled between 36.5°C and 37.5°C\(^{7,73,97}\), once between 35°C and 36°C\(^{35}\), and also once between 37°C and 37.5°C\(^{72}\). Seven times, temperature was controlled at exactly 37°C\(^{26,45,68,89,91,98,141}\). Although in the majority of cases the temperature was not monitored at all, changes in body temperature of 2°C are also not acceptable since a rise in body temperature from 36°C to 38°C leads to a 4-fold increase in liver damage\(^{156}\). Acclaimed pharmacological protection during I/R could often be the result of a drop in body temperature, which is a common side-effect of many drugs, instead of a protective effect of the drug itself. De Haan et al. showed this potential hazard in a review in the field of spinal cord ischemia\(^{157}\), as did Duncker et al. by showing that temperature modulated the protection by adenosine during coronary artery occlusion\(^{158}\). Therefore, rigorous control of body temperature during investigation of liver I/R injury is mandatory.
Discussion

The aim of this review was to highlight the different rat models for liver I/R injury and the different anesthetic procedures that are used, with emphasis on rat model, use of heparin during clamping, rat type and strain, fasting, anesthesia and temperature control. The world literature was searched via Medline using a combination of keywords and eventually 105 references were randomly selected and carefully examined.

In total, 9 different liver I/R models were found, with or without (extracorporeal) shunts or use of heparin. Temperature was controlled in only ¼ of cases but maintained at 37°C only 7 times although temperature control at exactly 37°C proved to be highly important \(^{156}\). Anesthesia was induced and maintained with numerous compounds and combinations. More literature is becoming available about the influence of the used anesthetic on the outcome of the experiment and the impact it has on several metabolic and hemodynamic properties \(^{159-161}\).

In order to achieve a unified animal model, consensus has to be reached about the most suitable animal model in liver I/R research. In our opinion, a suitable model is a partial liver ischemia model, preferably by occlusion of the median and left lateral liver lobes (most widely used, well documented and easily applicable, no need for bypass surgery or use of heparin) with stringent body temperature control at exactly 37°C. Assuming that in humans a collateral circulation is present which could serve as a post-ischemic shunt, ligation or removal of the non-ischemic lobes pre-reperfusion seems not necessary. Not mentioned in this overview are the used ischemia and reperfusion times that differ among researchers. In the proposed partial liver I/R model, ischemia times range from 15 min to 120 min. Although every researcher has reasons for using a particular ischemia time, consensus should be a goal in this area too. The most commonly used ischemia time is 60 min, which provides enough liver injury for conducting experiments and for performing interventional studies. Reperfusion times range between 0 min and 1 month. These differences in reperfusion times are often determined by the specific research questions raised in the various studies and consensus is less easily reached.

The most suitable rat gender is male (almost used exclusively) and the strain should be Wistar or Spraque Dawley (both used most often). The influence of fasting on liver I/R injury is still controversial but since fasting is common in the clinical setting it would be appropriate to do the same in animal experiments. The most common used fasting period is overnight, which resembles the clinical situation. As an anesthetic drug, isoflurane seems to be the most favourable in liver I/R experiments at the moment. Ideally, isoflurane should be used during ventilation of
the rat to prevent the development of respiratory acidosis \[147\]. At least, when a ventilation apparatus is not available, insertion of a short cannula into the trachea, either by tracheal intubation or tracheotomy, should be performed to facilitate spontaneous respiration of the animal. If other anesthetics are used, facilitation of respiration is still advisable.

This article attempts to reach more consensus in the field of liver I/R research. As the methodology of animal experimentation has a large impact on the results, inconsistent animal studies hamper clear comparison of results and may lead to wrong conclusions about the pathophysiology of liver I/R injury and about the effectiveness of treatments. This review underscores the confusion within the currently available literature on experimental methods used in rat liver I/R research. A plea for standardization of this methodology is made along with the following proposal for a standard animal model to study liver I/R phenomena:

Standard rat liver I/R model;
1 Wister or Spraque Dawley rat;
2 Male gender;
3 Fasting overnight;
4 Isoflurane as anesthetic drug (both induction and maintenance of anesthesia);
5 Facilitation of respiration by insertion of a short tracheal cannula or (preferably) by ventilation;
6 Maintenance of body temperature at 37°C;
7 No use of systemic or local heparin;
8 Induction of partial liver ischemia by clamping of the median and left lateral liver lobes (70% of total liver volume);
9 Duration of ischemia: 60 minutes.
References


49. Kooij A, Schiller HJ, Schijns M, Van NC, Frederiks WM. Conversion of xanthine dehydrogenase into xanthine oxidase in rat liver and plasma at the onset of reperfusion after ischemia [see comments]. Hepatology 1994; 19:1488-1495.


52. Frederiks WM, Bosch KS. The proportion of xanthine oxidase activity of total xanthine oxidoreductase activity in situ remains constant in rat liver under various (patho)physiological conditions. Hepatology 1996; 24:1179-1184.


