

Hepatic ischaemia–reperfusion injury from bench to bedside

R. Bahde and H.-U. Spiegel

Surgical Research, Department of General and Visceral Surgery, Muenster University Hospital, Waldeyer Strasse 1, D-48149 Muenster, Germany

Correspondence to: Professor H.-U. Spiegel (e-mail: spiegelh@uni-muenster.de)

Background: Vascular occlusion to prevent haemorrhage during liver resection causes ischaemia–reperfusion (IR) injury. Insights into the mechanisms of IR injury gathered from experimental models have contributed to the development of therapeutic approaches, some of which have already been tested in randomized clinical trials.

Methods: The review was based on a PubMed search using the terms ‘ischemia AND hepatectomy’, ‘ischemia AND liver’, ‘hepatectomy AND drug treatment’, ‘liver AND intermittent clamping’ and ‘liver AND ischemic preconditioning’; only randomized controlled trials (RCTs) were included.

Results: Twelve RCTs reported on ischaemic preconditioning and intermittent clamping. Both strategies seem to confer protection and allow extension of ischaemia time. Fourteen RCTs evaluating pharmacological interventions, including antioxidants, anti-inflammatory drugs, vasodilators, pharmacological preconditioning and glucose infusion, were identified.

Conclusion: Several strategies to prevent hepatic IR have been developed, but few have been incorporated into clinical practice. Although some pharmacological strategies showed promising results with improved clinical outcome there is not sufficient evidence to recommend them.

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Introduction

Massive haemorrhage remains a major pitfall of liver surgery. The volume of blood lost during liver surgery significantly affects morbidity and mortality, and perioperative blood transfusions are associated with higher rates of postoperative complications and tumour recurrence^{1–4}. Devascularization techniques, haemostatic procedures, intraoperative ultrasonography and low central venous pressure anaesthesia have helped reduce intraoperative blood loss^{5–9}. Despite the availability of novel transection devices, vascular control is often required in complex hepatic resection procedures to avoid excessive bleeding¹⁰. Several techniques, such as inflow occlusion by portal triad clamping (PTC; Pringle manoeuvre) and hemi-hepatic vascular clamping or inflow and outflow occlusion (total hepatic vascular exclusion, selective hepatic vascular occlusion), have been suggested¹¹. Japanese and European surveys have revealed that 25 and 19 per cent of surgeons respectively apply the Pringle manoeuvre routinely; vascular clamping is never applied by only 7 and 10 per cent respectively^{12,13}. Routine vascular clamping

to improve perioperative outcome after liver resection remains controversial¹⁴.

Vascular occlusion causes ischaemic injury to the liver, and is exacerbated by reperfusion. Experimental studies have increased current understanding of the mechanisms of ischaemia–reperfusion (IR) injury. Several strategies to protect the liver from IR injury have been developed in animal models and their efficacy subsequently confirmed in clinical studies. As a result, surgical procedures such as ischaemic preconditioning (IP) and intermittent clamping (IC) have found their way into clinical practice, whereas clinical evaluation of pharmacological approaches remains limited. This review outlines the various strategies for reducing normothermic hepatic IR injury in experimental and clinical studies (*Fig. 1*).

Methods

In a PubMed analysis using the limits ‘randomized controlled trials’ (RCTs) and ‘english’, and the keywords ‘ischemia AND hepatectomy’, ‘ischemia AND liver’, ‘hepatectomy AND drug treatment’, ‘liver AND intermittent clamping’ and ‘liver AND ischemic preconditioning’, 144

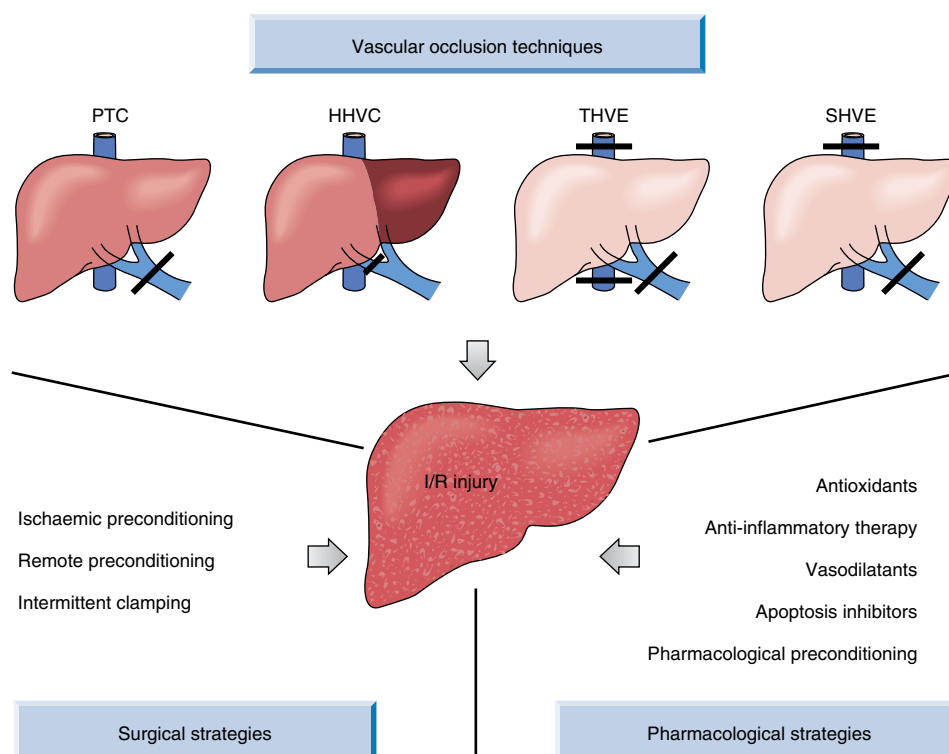


Fig. 1 Synopsis of surgical and pharmacological strategies for hepatic ischaemia–reperfusion (IR) injury induced by portal triad clamping (PTC), hemihepatic vascular clamping (HHVC), total hepatic vascular exclusion (THVE) or selective hepatic vascular exclusion (SHVE)

papers published up to 2008 were identified. RCTs investigating pharmacological or surgical interventions were included, whereas transplant studies, studies applying the *in situ* cooling technique and those comparing vascular occlusion or liver resection techniques were excluded. To avoid duplication among overlapping studies from the same group, the first published study was retained and the others excluded. One article was identified by cross-checking references. A total of 26 RCTs were included, 12 investigating surgical therapeutic and 14 investigating pharmacological approaches (Fig. 2).

Ischaemia–reperfusion injury

Ischaemia is defined as the interruption of oxygen and substrate supply with complete block of removal of metabolites¹⁵. Ischaemia causes a drop in cellular adenosine 5'-triphosphate (ATP) concentration, failure of the ATP-dependent Na^+/K^+ pump, cell oedema and an increase in cytosolic calcium concentration, all of which contribute to cell damage. Reperfusion of the liver enhances cell injury by oxidative stress and inflammatory reactions. The initial phase of IR injury (within 2 h after reperfusion)

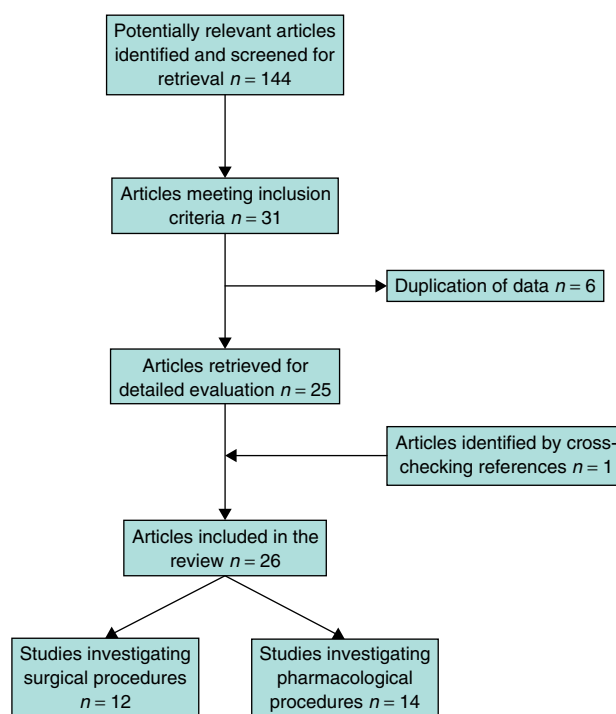


Fig. 2 Articles identified during the review process

involves the release of reactive oxygen species (ROS) and proinflammatory mediators, such as tumour necrosis factor (TNF) α , interleukin (IL) 6, IL-1 and eicosanoids, by Kupffer cells¹⁶. ROS may lead to lipid peroxidation, mitochondrial permeability transition, activation of signal transduction pathways (for example by activating nuclear factor (NF) κ B and activator protein (AP) 1), caspase activation, necrosis and apoptosis of hepatocytes and endothelial cells^{17,18}. The late phase (6–48 h after reperfusion) is characterized by neutrophil-mediated inflammatory responses¹⁹. Cytokines, chemokines and complement factors recruit neutrophils into the liver, which damage hepatocytes through ROS or protease release¹⁹. Endothelin (ET) 1-mediated vasoconstriction and increased platelet aggregation induce microcirculatory disturbances, aggravating liver damage^{20,21}.

Steatotic livers are more susceptible to hepatic IR injury than non-steatotic ones²². Overexpression of the mitochondrial uncoupling protein 2 in fatty livers may contribute to decreased cellular ATP levels²³. Furthermore, downregulation of peroxisome proliferator-activated receptor α , an important regulator of the hepatic inflammatory response to IR, and overexpression of adiponectin, a fat cell-secreted hormone with antidiabetic and anti-inflammatory activities, in fatty livers after IR may play a role²⁴. Cirrhotic livers are also more prone to hepatic IR injury, because capillarization, sinusoidal endothelial cell defenestration, development of a continuous basement membrane, deposition of collagen fibres in Disse's space, intrahepatic shunts and fibrous septa impair exchanges between blood and hepatocytes²⁵. Increased ET-1 concentrations and increased susceptibility to hepatocyte apoptosis via the Fas pathway in cirrhotic livers may worsen the injury^{26–28}.

The influence of neoadjuvant chemotherapy on ischaemic tolerance is largely unknown. Chemotherapeutic agents such as irinotecan or oxaliplatin can lead to steatosis/steatohepatitis or sinusoidal obstruction, and aggravate liver damage²⁹.

Experimental studies

Surgical strategies

IP consists of a short episode of IR to induce resistance to a subsequent longer episode of ischaemia. IP can reduce microcirculatory disturbances, leucocyte adhesion, Kupffer cell activation, release of proinflammatory cytokines, oxidative injury and apoptosis³⁰. Several mediators, including adenosine, nitric oxide (NO), heat-shock proteins (HSPs) and TNF- α , have been proposed^{31–33}. An early phase of protection occurs immediately after IP

and a later phase after 12–24 h, which is associated with the induction of cytoprotective genes (such as those encoding HSPs)³⁰. Experimental studies have shown that IP is a time-dependent phenomenon^{31,34,35}. An ischaemic period of 3 min followed by 5 min of reperfusion does not prevent IR injury, but a 5-min–10-min sequence does³⁴. At least 5–10 min of IP is necessary to induce NO generation via adenosine, whereas the upper limit for the window (15 min) is defined by the concentration of xanthine capable of removing the generated NO, leading to the formation of peroxynitrite^{31,35}.

Remote preconditioning (RP) consists of a short period of ischaemia in an organ distant from the target tissue to induce tolerance to subsequent IR³⁶. The principle of RP was discovered in myocardial tissue in 1993. Although still not fully understood, possible mediators include opioids, NO, adenosine, bradykinin and adenosine, and a neurogenic pathway^{36,37}. Recently, RP has been shown to reduce hepatic IR injury in a rabbit model of hind limb RP before hepatic IR³⁸.

IC comprises vascular inflow occlusion with short periods of reperfusion in between. The liver tolerates IC better than continuous ischaemia^{39–41}. Experimentally, intermittent ischaemia is associated with lower systemic endotoxin, TNF and IL-6 levels, and reduced oxidative stress^{41–43}. IC and IP in part may activate similar pathways, or IC may work in the same way as repeated preconditioning^{44,45}. For example, IC and IP both maintain hepatic microcirculation, decrease Kupffer cell activation, and reduce apoptosis with the prevention of cytochrome C release and caspase-3 activation^{45,46}. Furthermore, the intervening periods of reperfusion in IC could allow washout of harmful factors and allow hepatocytes to recover^{45,46}. In animal experiments, IP was superior for periods of ischaemia up to 75 min, whereas IC conveyed better protection for prolonged ischaemia times^{45–47}. In older rats, liver resection using IC preserved hepatocyte and liver function better than resection with IP⁴⁸. Both, IP and IC, might reduce IR injury in steatotic and cirrhotic livers^{49,50}.

Pharmacological strategies

Antioxidant therapy aims at supporting endogenous antioxidants and inhibiting ROS generation. Overexpression or administration of antioxidant enzymes (superoxide dismutase (SOD) and catalase) and of antioxidants such as vitamin E, vitamin C, glutathione, *N*-acetylcysteine, α -lipoic acid and melatonin protect against experimental IR injury^{51–58}. Inhibition of reduced nicotinamide adenine dinucleotide oxidase with apocynin or of xanthine oxidase with allopurinol, eliminating Kupffer cells with gadolinium

chloride, and iron chelation with desferrioxamine all protect against hepatic IR injury in animals^{59–62}. A variety of anti-inflammatory strategies have been shown to reduce IR injury via selectin, cyclo-oxygenase 2 and protease inhibition, cytokine (including TNF- α and IL-1) and chemokine blockade, and use of immunosuppressants such as steroids, calcineurin inhibitors (tacrolimus, ciclosporin) and mammalian target of rapamycin (mTOR) inhibitors (sirolimus)^{63–70}.

Vasoactive mediators, such as ET, angiotensin II, thromboxane (TX) A₂, NO, carbon monoxide, prostaglandin (PG) E₁ and prostacyclin, may all play a role in hepatic IR injury^{71–76}. ET receptor blockade, stimulation of NO production, carbon monoxide, PGE₁ and angiotensin converting enzyme inhibitors can reduce the severity of IR injury by improving hepatic capillary perfusion^{71–74,77–79}.

Antiapoptotic strategies, including mitochondrial permeability transition inhibitors (ciclosporin A, trifluoperazine), anti-Fas or anti-Fas ligand antibodies, antiapoptotic proteins (Bcl-x(L)), caspase inhibitors and others, have been explored in basic research, but concerns over their potential carcinogenicity limit their therapeutic application^{17,80–83}.

The concept of pharmacological preconditioning is based on protective mechanisms induced by IP. In mice, treatment with low-dose TNF- α before ischaemia protected the liver against IR injury by mimicking the biological effects of IP, namely mitogen-activated protein kinase and NF κ B activation⁸⁴. Moreover, A₂ receptor stimulation (adenosine, dipyridamole), NO supplementation (L-arginine, spermine NONOate), induction of haem oxygenase 1 (for example through haemin, doxorubicin, simvastatin or isoproterenol) and administration of atrial natriuretic peptide reduced warm IR injury^{85–93}. Recently, anaesthetic preconditioning with volatile anaesthetics has shown promising results and may involve the same intracellular signalling pathways as IP^{94,95}.

Clinical studies

Six RCTs comparing IP with continuous ischaemia^{96–101} and four investigating the protective effects IC^{102–105} were identified; two studies compared IP with IC^{106,107} (Table 1). Because of its simplicity and safety, limb RP is attractive as a mechanism for reducing hepatic IR injury in the clinical setting; however, clinical data are not yet available.

Ischaemic preconditioning

Based on data obtained in rodents, Clavien and colleagues^{96,108,109} first performed a pilot study in 24 patients and then a RCT, which confirmed the protective

effect of IP in the human liver during hepatic resection. This effect in terms of decreased aminotransferase levels correlated with age (greater benefit in younger patients) and the volume of liver remaining after resection. Patients with at least 25 per cent steatosis were particularly protected in terms of postoperative serum peak aspartate aminotransferase (AST) levels⁹⁶. Clinical evidence for a benefit of IP in patients with liver cirrhosis is limited. Patients with cirrhosis subjected to less than 20 min of ischaemia had lower postoperative aminotransferase and bilirubin levels if they had IP⁹⁹.

A further RCT using Clavien's protocol confirmed that IP is better tolerated than continuous clamping in healthy liver⁹⁸. In spite of a longer duration of ischaemia, AST and alanine aminotransferase (ALT) levels in patients with IP rose less. Use of the Pringle manoeuvre following IP provided better intraoperative haemodynamic stability and anti-ischaemic effects than the Pringle manoeuvre alone, and this was associated with reduced activation of polymorphonuclear leucocytes and downregulation of potentially cytotoxic functions^{97,110,111}. IP may contribute to reduced blood loss, fewer blood transfusions and fewer postoperative complications¹⁰¹, but this observation is at variance with the results of the larger Clavien study⁹⁶ (Table 2). A meta-analysis comparing the Pringle manoeuvre with and without IP found no significant differences in mortality, morbidity, liver failure, blood loss or haemodynamic changes¹¹². Furthermore, IP failed to protect against IR injury under vascular exclusion of the liver in another RCT¹⁰⁰. The discrepancy between these and previous findings could have resulted from a longer ischaemic period and absence of hepatovenous backflow.

Intermittent clamping

To reduce IR injury and extend the duration of ischaemia, IC was introduced into clinical practice by Makuuchi and colleagues¹¹³. The most prolonged period of inflow occlusion yet reported is 322 min of IC in normal livers¹¹⁴. IC may lead to increased blood loss during reperfusion and may prolong surgery^{36,102,107}. However, a recent Cochrane meta-analysis reported no increase in blood loss or duration of operation when intermittent was compared with continuous PTC¹¹². It is possible that IC confers the advantage of checking haemostasis of the cut surface during each period of reperfusion¹¹.

In a controlled clinical study, Belghiti and co-workers¹⁰² demonstrated superior parenchymal tolerance to IC with 15-min ischaemia and 5-min reperfusion cycles over continuous occlusion and the safe prolongation of total

Table 1 Surgical strategies against ischaemia–reperfusion injury evaluated in prospective randomized clinical trials

Reference	Design	IR mode (min)†	No. of patients	Ischaemia time (min)*	Markers of injury	Comments
Ischaemic preconditioning Clavien <i>et al.</i> ⁹⁶	IP	10/10	50	36(5.9)	AST↓, ALT↓, AP→, bilirubin→, PT→	Increased benefit of IP for younger patients, longer ischaemic time (up to 60 min), lower resected liver volume (< 50%), patients with steatosis
	C-PTC		50	35(6.8)		
Choukèr <i>et al.</i> ⁹⁷	IP	10/10	14	32(6.3)	AST→, ALT↓	Catecholamines↓ after reperfusion
Nuzzo <i>et al.</i> ⁹⁸	C-PTC	10/10	19	35(11)		Ischaemia time significantly↑ in IP group
	IP		21	54(19)	AST↓, ALT↓ (n.s.), bilirubin→, PT→	
Li <i>et al.</i> ⁹⁹	C-PTC	5/5	21	36(14)		Patients with cirrhosis
	IP		14	18.0(6.3)	AST↓, ALT↓, bilirubin↓, albumin↑ (n.s.), caspase-3 activity↓, apoptotic SEC↓	
Azoulay <i>et al.</i> ¹⁰⁰	C-PTC		15	17.4(2.3)		
	IP		30	44.5(9.2)	AST→, ALT→, GST→, bilirubin→, PT→	
Heizmann <i>et al.</i> ¹⁰¹	C-HVE	10/10	30	47.7(8.3)		Transfusions↓
	IP		30	34(14)	ALT→, bilirubin→	
Intermittent clamping Belghiti <i>et al.</i> ¹⁰²	C-PTC		31	33(12)		
	IC	15/5	44	46(18)	AST→, ALT→, bilirubin→, PT→, postoperative liver failure↓	
Capussotti <i>et al.</i> ¹⁰³	C-PTC		42	41(13)		Increased benefit of IC in patients with underlying liver disease (steatosis, chronic liver disease): ALT↓, bilirubin↓
	IC		17	40.4(11.7)	No significant effect on liver enzymes or coagulation	
Esaki <i>et al.</i> ¹⁰⁴	C-PTC	15/5	18	35.5(13.9)		Patients with cirrhosis
	IC30	30/5	48	48 (13–208)	AST→, ALT→, bilirubin→, PT→, amylase→	
Brooks <i>et al.</i> ¹⁰⁵	IC15	15/5	44	62 (15–150)		Total ischaemia time↓, blood loss per unit transection area→
	Under PTC					
Under PTC	IC10	10/5	6	n. r.	ALT and bilirubin: not interpretable	
	IC20	20/10	7		P _L CO ₂ and pH returned to baseline at end of 5-min reperfusion with 10/5 mode, but not with 20/10 mode	
Ischaemic preconditioning versus intermittent clamping Smyrniotis <i>et al.</i> ¹⁰⁶	Under PTC					
	IP	10/10	27	41.4(4.3)	< 40 min ischaemia: AST→, bilirubin→, PT→, caspase-3↓	
	IC	15/5	27	42.5(6.3)	> 40 min ischaemia: AST↑, bilirubin→, PT→, caspase-3↑, apoptotic activity↑	
	Under SHVE					
Petrowsky <i>et al.</i> ¹⁰⁷	IP	10/10	36	37.7(1.5)	AST→, ALT→, PT→, bilirubin→	Effect of IP decreased with increasing age in a population > 60 years
	IC	15/5	37	40.0(2.1)		
	Under PTC					IC exerted stronger protective effect in steatotic livers

*Values are mean(s.d.) or median (range). These studies compared protective surgical strategies (ischaemic preconditioning, IP; intermittent clamping, IC) with the induction of ischaemia alone (continuous portal triad clamping, C-PTC; continuous hepatic vascular exclusion C-HVE; selective hepatic vascular occlusion, SHVE). †Periods of ischaemia and subsequent reperfusion in IP or the cycles of ischaemia–reperfusion (IR) in IC. The effects of treatment occurred at some time point during the postoperative period: ↓, decrease; →, no change; ↑, increase. AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase; PT, prothrombin time; n.s., not significant; SEC, sinusoidal endothelial cells; GST, glutathione S-transferase; n.r., not reported; P_LCO₂, hepatic partial pressure of carbon dioxide.

Table 2 Clinical outcome of identified clinical trials evaluating surgical strategies

Reference	Overall blood loss	Duration of operation	Transection time	Morbidity	LOS ICU	LOS hospital
Ischaemic preconditioning						
Clavien <i>et al.</i> ⁹⁶	→	→	n.r.	→	→	→
Choukèr <i>et al.</i> ⁹⁷	→	→	→	n.r.	n.r.	n.r.
Nuzzo <i>et al.</i> ⁹⁸	n.r.	→	n.r.	→	n.r.	n.r.
Li <i>et al.</i> ⁹⁹	→	→	n.r.	→	n.r.	↓
Azoulay <i>et al.</i> ¹⁰⁰	→	→	n.r.	→	→	→
Heizmann <i>et al.</i> ¹⁰¹	↓	→	n.r.	↓	→	n.r.
Intermittent clamping						
Belghiti <i>et al.</i> ¹⁰²	→	n.r.	n.r.	→	n.r.	n.r.
	↑(during transection)					
Capussotti <i>et al.</i> ¹⁰³	→	→	n.r.	→	n.r.	n.r.
Esaki <i>et al.</i> ¹⁰⁴	→	↓	↓	→	n.r.	→
Ischaemic preconditioning versus intermittent clamping						
Smyrniotis <i>et al.</i> ¹⁰⁶	↓	↓	n.r.	→	→	→
Petrowsky <i>et al.</i> ¹⁰⁷	→	→	↓	→	→	→
	↓(during transection)					

Only results from studies reporting overall blood loss, duration of operation, transection time, morbidity, length of stay in the intensive care unit (LOS ICU) or in hospital (LOS hospital) are shown: →, no change; ↓, decrease; ↑, increase; n.r., not reported.

ischaemia time to 120 min. Esaki and co-workers¹⁰⁴ compared 15 min with 30 min of IC and noted no differences in liver function, blood loss or serum amylase. However, the longer ischaemic period was associated with better operative efficacy reflected by a shorter total ischaemia time and greater transection area per unit transection time. On the other hand, a small clinical trial comparing 10 min–5 min with 10 min–20 min clamp–release regimens showed that occlusion times greater than 20 min were associated with prolonged periods of hepatic hypercarbia; the impact of the different IC regimens on liver function was not interpretable¹⁰⁵.

Many authors have advocated the use of IC during partial hepatectomy in patients with liver cirrhosis^{10,22,102,115}. The cirrhotic liver tolerates intermittent ischaemia for up to 200 min without increased postoperative complications and mortality¹¹⁵. Belghiti *et al.*¹⁰² demonstrated that parenchymal tolerance of cirrhotic livers to IC was better than to continuous clamping but another RCT comparing these techniques showed similar blood loss during hepatectomy in cirrhosis and no differences in postoperative outcome and liver function¹⁰³. Clinical evidence for the protective effect of IC in steatotic livers is limited, but available data suggest that severe parenchymal injury in patients with at least 20 per cent steatosis occurs less frequently with IC than with continuous clamping¹⁰².

Intermittent clamping versus ischaemic preconditioning

Ischaemia time can be extended safely to 120 min using either IC or IP^{109,116,117}. IP and IC are probably equally effective in protecting against IR injury in patients without cirrhosis for short ischaemia times, but IP is associated with less blood loss and a shorter transection time¹⁰⁷. The protective effects of IP were observed only in patients younger than 60 years. IC might be preferred for patients older than 65 years¹⁰⁷. Furthermore, the protective effects conferred by IC are stronger in steatotic livers and so IC should be preferred for patients with severe steatosis and an expected prolonged ischaemia time. Another RCT comparing IP with IC for liver resection under selective hepatic vascular occlusion has suggested that IP should be preferred for ischaemic periods of less than 40 min, but IC when the ischaemia time exceeds 40 min¹⁰⁶.

Overview

IC and IP both seem to confer protection, but most studies are underpowered to prove benefit in terms of clinical outcome (Table 2). Both strategies can reduce parenchymal injury and extend ischaemia time. Some studies indicate that IC could be the preferred technique for prolonged ischaemia, pathological liver and elderly patients, whereas IP might be more favourable for short ischaemia times and young patients.

Pharmacological strategies

A total of 14 RCTs evaluating antioxidants, anti-inflammatory drugs, vasodilators, pharmacological preconditioning and glucose infusion were identified (Table 3)^{118–131}.

Antioxidants

Vitamin E (α -tocopherol) is an important radical scavenger. A placebo-controlled study in 47 patients indicated that preoperative administration of vitamin E can prevent vitamin E deficiency after IR, reduce the increase in liver enzyme levels and shorten intensive care unit stay¹²⁰. Vitamin E infusion had no impact on postoperative complications or length of hospital stay (Table 4). Two trials evaluating a multivitamin infusion containing tocopherol and ascorbate before reperfusion showed that lipid peroxidation was decreased and prothrombin time significantly improved in the treatment group. No significant differences in terms of aminotransferase levels and postoperative outcome were found^{118,132}.

There is increasing evidence that α -lipoic acid exerts antioxidant effects in biological systems through direct ROS quenching and via transition metal chelation¹³³. A clinical trial with 24 patients reported that α -lipoic acid reduced the number of apoptotic hepatocytes, lowered postischaemia AST levels and increased ATP content in liver tissue after IR, without differences in clinical outcome¹²¹.

Allopurinol might protect from the formation of free radicals through modification of the xanthine oxidase pathway. Pretreatment with allopurinol had no significant effect on IR injury, morbidity and mortality in one small clinical trial¹¹⁹. In another small trial pretreatment with mannitol, an osmotic diuretic with free radical-scavenging properties, resulted in lower postoperative blood levels of lipid peroxidation products, possibly owing to enhanced diuretic elimination. No positive impact on the postoperative clinical course was noted¹²².

Anti-inflammatory therapy

Glucocorticoids interact with NF κ B and AP-1, thereby inhibiting expression of inflammatory genes and increasing transcription of genes encoding anti-inflammatory proteins¹³⁴. Because of their antiproliferative properties, potential side-effects have been of much concern. A systematic review of single-shot high-dose methylprednisolone before elective surgery found no significant increase in the incidence of adverse effects¹³⁵. Three RCTs evaluating the effect of methylprednisolone on hepatic IR were

identified^{124–126}. Methylprednisolone administration did not improve short-term outcomes in patients undergoing liver resection with continuous inflow occlusion¹²⁴, but attenuated IR injury after intermittent inflow occlusion, and reduced postoperative complications and length of hospital stay¹²⁵ (Table 4). The protective effect of methylprednisolone on hepatic IR was confirmed by lower serum levels of aminotransferases, bilirubin and inflammatory cytokines in the third trial¹²⁶.

In two RCTs gabexate mesilate, an inhibitor of various serine proteases, including neutrophil elastase, and of neutrophil activation, protected the liver from IR injury in the setting of hepatectomy under IC^{65,123,136,137}. Based on their findings, Kim and colleagues¹²³ considered the appropriate pretreatment time to be 12 h before surgery. Postoperative levels of aminotransferases and IL-6 were lower in the treatment groups. There was a tendency towards better postoperative morbidity, especially when IC was applied for 30 min rather than 15 min.

Vasodilators

Although the preliminary evidence from experimental approaches suggests that vasodilators hold therapeutic promise, only three RCTs have been performed^{127–129}. A small-scale study including 17 patients showed that TXA2 synthetase inhibitor lowered AST levels only on the first day after surgery and TXB2 levels immediately after operation¹²⁷. Two other studies demonstrated the protective effects of PGE1 and the phosphodiesterase III inhibitor amrinone respectively in patients with cirrhosis^{128,129}. In 24 patients with cirrhosis who underwent subsegmentectomy using the Pringle manoeuvre, treatment with PGE1 led to lower plasma aminotransferase levels, but with no effect on postoperative complications or hospital stay¹²⁸. Another study demonstrated that, in contrast to PGE1, amrinone enhanced indocyanine green elimination and lactate metabolism during hepatic resection in patients with cirrhosis¹²⁹. Postoperative AST and ALT levels did not differ significantly between the groups.

Pharmacological preconditioning

Isoflurane and sevoflurane have been studied widely in the context of attenuation of myocardial IR injury^{138,139}. Pharmacological preconditioning with sevoflurane as a hepatoprotective strategy has been described in one RCT with 64 patients¹³⁰. Patients in the control group received a propofol-based intravenous regimen; in the preconditioning group propofol was replaced by sevoflurane 30 min before ischaemia. Sevoflurane lowered

Table 3 Pharmacological strategies for preventing/treating ischaemia–reperfusion injury evaluated in prospective randomized clinical trials

Reference	Treatment	No. of patients	Vascular occlusion	Ischaemia time (min)*	Markers of injury	Comments
Antioxidants						
Cerwenka <i>et al.</i> ¹¹⁸	Omnibionta®, 2 ampoules i.v. before reperfusion	T 32 C 26	C-PTC	Overall 48(24-2)	ALT↓ (n.s.), AST↓ (n.s.), bilirubin→, PT↑, lactate→	
Vriens <i>et al.</i> ¹¹⁹	Allopurinol, 10 mg/kg orally 1 day preop., 5 mg/kg i.v. before surgery and before reperfusion	T 8 C 8	C-PTC	53 (30–130) 45 (25–120)	No significant effect on liver enzymes, blood clotting factors, MDA, GSH, GSSG, vitamin C	
Bartels <i>et al.</i> ¹²⁰	Vitamin E, 1800 units i.v. 1 day preop.	T 19 C 28	C-PTC	28.6 (12–40) 33.0 (7–68)	AST↓, prevention of vitamin E deficiency	
Dünschede <i>et al.</i> ¹²¹	α-Lipoic acid, 600 mg i.v. 15 min before ischaemia	T 12 C 12	C-PTC	30	ALT↓, AST↓, ATP↑, apoptotic hepatocytes↓	
Kostopanagiotou <i>et al.</i> ¹²²	Mannitol, 20% 1.5 ml/kg i.v. for 30 min before ischaemia	T 15 C 15	C-PTC	32.5 (23–39) 33.5 (20–41)	AST→, bilirubin→, PT→, MDA↓	Urine output↑, colloid fluid infusion↑
Anti-inflammatory drugs						
Kim <i>et al.</i> ¹²³	Gabexate mesilate, 2.0 mg/kg/h i.v. 12 h preop. until day 2	T 30 C 30	IC	63(13) 65(16)	ALT↓, AST↓, IL-6↓	
Muratore <i>et al.</i> ¹²⁴	Methylprednisolone, 30 mg/kg i.v. 30 min before resection	T 25 C 28	C-PTC	41.1(15.9) 37.7(17.8)	AST→, ALT→, bilirubin→, PT→, IL-6↓ ASTand ALT↓ in chronic liver disease IL-6→ in steatotic liver	
Aldrighetti <i>et al.</i> ¹²⁵	Methylprednisolone, 500 mg i.v. before anaesthesia	T 36 C 37	IC	52.4 (20–89) 43 (20–78)	AST↓, ALT↓, bilirubin↓, PT↑, AT-III↑, platelets↑, fibrinogen↑, IL-6↓, TNF-α ↓	
Pulitanò <i>et al.</i> ¹²⁶	Methylprednisolone, 500 mg before surgery	T 21 C 22	IC	48(18) 29(17)	AST↓, ALT↓, bilirubin↓, PT↑, AT-III↑, IL-6↓, TNF-α ↓	
Vasodilators						
Shirabe <i>et al.</i> ¹²⁷	TXA2 synthetase inhibitor OKY 046, 0.2 mg/kg/h during surgery	T 9 C 8	HHVC	35(16) 40(30)	AST↓, ALT→, hepaplastin test↑, TXB2↓, PGE2→, 6-keto PGF1α →	
Sugawara <i>et al.</i> ¹²⁸	PGE1, 0.02 µg/kg/min i.v. from start of operation until 09.00 hours on day 1 postop.	T 12 C 12	C-PTC	70.2(5.8) 72.6(12.2)	AST↓, ALT↓, bilirubin↓, nitrate↑, IL-1→, IL-6↑, TNF-α →	Patients with cirrhosis
Orii <i>et al.</i> ¹²⁹	Amrinone, 4.0 µg/kg/min i.v. during surgery	15	IC	66(49)	AST→, ALT→	Patients with cirrhosis
	PGE1, 0.02 µg/kg/min i.v. during surgery	15		71(46)	Amrinone: ICG elimination↑, lactate↓, base excess↓	
	Control	15		70(38)	PGE1: ICG elimination↑ (n.s.), lactate↓ (n.s.), base excess↓ (n.s.)	
Pharmacological preconditioning						
Beck-Schimmer <i>et al.</i> ¹³⁰	Sevoflurane for 30 min (10 min 3.2 vol%, then washout) before ischaemia	T 30 C 34	C-PTC	36.03(5.62) 35.12(6.32)	AST↓, ALT↓ (especially in patients with steatosis), bilirubin→, AP→, iNOS mRNA↑	
Tang <i>et al.</i> ¹³¹	250 ml 25% glucose with 10 units insulin and 10 ml 10% K ⁺ infusion during the 24 h before operation	T 29 C 28	IC	19.21(4.54) 21.04(5.11)	AST↓, ALT↓, AP↓, hepatic tissue ATP↑, SOD↑, MDA↓	Mainly patients with cirrhosis

*Values are mean(s.d.) or median (range). The effects of treatment occurred at some time point during the postoperative period: ↓, decrease; ↑, increase; →, no change. i.v., Intravenous; T, therapy group; C, control group; C-PTC, continuous portal triad clamping; ALT, alanine aminotransferase; n.s., not significant; AST, aspartate aminotransferase; PT, prothrombin time; MDA, malondialdehyde; GSH, glutathione; GSSG, glutathione disulphide; ATP, adenosine 5'-triphosphate; IC, intermittent clamping; IL, interleukin; AT, antithrombin; TNF, tumour necrosis factor; HHVC, hemihepatic vascular control; TX, thromboxane; PG, prostaglandin; ICG, indocyanine green; AP, alkaline phosphatase; iNOS, inducible nitric oxide synthase; SOD, superoxide dismutase.

Table 4 Clinical outcome of identified clinical trials evaluating pharmacological strategies

Reference	Morbidity	LOS ICU	LOS hospital
Antioxidants			
Vriens <i>et al.</i> ¹¹⁹	→	→	→
Bartels <i>et al.</i> ¹²⁰	→	↓	→
Dünschede <i>et al.</i> ¹²¹	→	→	→
Kostopanagiotou <i>et al.</i> ¹²²	n.r.	→	→
Anti-inflammatory drugs			
Kim <i>et al.</i> ¹²³	↓ (n.s.)	n.r.	n.r.
Muratore <i>et al.</i> ¹²⁴	→	n.r.	→
Aldrighetti <i>et al.</i> ¹²⁵	↓	n.r.	↓
Pulitanò <i>et al.</i> ¹²⁶	↓ (infectious complications)	n.r.	↓
Vasodilators			
Sugawara <i>et al.</i> ¹²⁸	→	n.r.	→
Orii <i>et al.</i> ¹²⁹	→	n.r.	n.r.
Pharmacological preconditioning			
Beck-Schimmer <i>et al.</i> ¹³⁰	↓	→	↓ (n.s.)

Only results from studies reporting reporting morbidity, length of stay in the intensive care unit (LOS ICU) or in hospital (LOS hospital) are shown: →, no change; ↓, decrease; n.r., not reported; n.s., not significant.

the rate of complications and reduced IR injury, as defined by low levels of aminotransferases after liver surgery with continuous inflow occlusion. These protective effects were more pronounced in patients with liver steatosis. Patients with cirrhosis were excluded from the study.

Glucose infusion

High hepatic glycogen content is thought to protect from hepatic IR injury by supplying glucose for ATP generation. In one RCT intravenous administration of high concentrations of glucose 24 h before surgery restored hepatic tissue ATP content, increased SOD activity, lowered malondialdehyde levels and reduced the rise in liver enzymes after hepatic IR¹³¹. Most patients in this study had cirrhosis, a condition known to be accompanied by impaired glycogen metabolism. Clinical outcome was not evaluated.

Overview

Pharmacological agents may provide powerful protection and prevent postoperative complications through their systemic effects. Because of the heterogeneity of the available studies regarding underlying liver disease, extent of liver resection, duration of ischaemia, type of ischaemia (continuous or intermittent), type of drugs administered,

dosing regimens and reported outcomes it is difficult to recommend the use of any of these pharmacological strategies. Methylprednisolone and sevoflurane showed the most promising results in terms of improved clinical outcome (*Table 4*). Both approaches are easily applicable therapeutic options to protect the liver. Large multicentre trials are required to provide sufficient evidence for their recommendation.

Comments

Despite the existence of promising strategies for reducing IR injury, many questions remain. Because hepatocellular carcinoma requiring surgery is often associated with cirrhosis or damage resulting from chemotherapy, it is crucial to understand the basic pathophysiological events after IR in damaged livers. The impact of aetiology (alcohol or hepatitis virus) on IR injury as well as factors that promote tumour recurrence after IR need to be determined in clinical studies. Experiments in mice have shown that IR can promote the growth of colorectal cancer liver metastases and that selective clamping can help to protect against their accelerated growth^{140,141}. Experimental studies have also demonstrated that hepatic IR during liver resection can lead to impaired liver regeneration and that some strategies to protect against IR injury can also preserve regenerative capacity^{142,143}.

Surgical strategies can affect multiple aspects of IR injury whereas pharmacological approaches often affect only a few factors, but have a systemic effect. Taken the complexity of IR, combining drug treatments or surgical and pharmacological approaches offers the potential for optimization.

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References

- 1 Nagorney DM, van Heerden JA, Ilstrup DM, Adson MA. Primary hepatic malignancy: surgical management and determinants of survival. *Surgery* 1989; **106**: 740–748.
- 2 Agarwal N, Murphy JG, Cayten CG, Stahl WM. Blood transfusion increases the risk of infection after trauma. *Arch Surg* 1993; **128**: 171–176.
- 3 Yamamoto J, Kosuge T, Takayama T, Shimada K, Yamasaki S, Ozaki H *et al.* Perioperative blood transfusion promotes recurrence of hepatocellular carcinoma after hepatectomy. *Surgery* 1994; **115**: 303–309.

- 4 Stephenson KR, Steinberg SM, Hughes KS, Vetto JT, Sugarbaker PH, Chang AE. Perioperative blood transfusions are associated with decreased time to recurrence and decreased survival after resection of colorectal liver metastases. *Ann Surg* 1988; **208**: 679–687.
- 5 Cunningham JD, Fong Y, Shriver C, Melendez J, Marx WL, Blumgart LH. One hundred consecutive hepatic resections: blood loss, transfusion and operative technique. *Arch Surg* 1994; **129**: 1050–1056.
- 6 Strasberg SM, Drebin JA, Linehan D. Use of a bipolar vessel-sealing device for parenchymal transection during liver surgery. *J Gastrointest Surg* 2002; **6**: 569–574.
- 7 Takayama T, Makuuchi M. Intraoperative ultrasonography and other techniques for segmental resections. *Surg Oncol Clin N Am* 1996; **5**: 261–269.
- 8 Lortat-Jacob JL, Robert HG, Henry C. Un cas d'hépatectomie droite réglée. *Mem Acad Chir (Paris)* 1952; **78**: 244–251.
- 9 Tung TT. *Les Résections Majeures et Mineures du Foie*. Masson: Paris, 1979.
- 10 Man K, Fan ST, Ng IO, Lo CM, Liu CL, Wong J. Prospective evaluation of Pringle maneuver in hepatectomy for liver tumors by a randomized study. *Ann Surg* 1997; **226**: 704–711.
- 11 van Gulik TM, de Graaf W, Dinant S, Busch OR, Gouma DJ. Vascular occlusion techniques during liver resection. *Dig Surg* 2007; **24**: 274–281.
- 12 Nakajima Y, Shimamura T, Kamiyama T, Matsushita M, Sato N, Todo S. Control of intraoperative bleeding during liver resection: analysis of a questionnaire sent to 231 Japanese hospitals. *Surg Today* 2002; **32**: 48–52.
- 13 van der Bilt JD, Livestro DP, Borren A, van Hillegersberg R, Borel Rinkes IH. European survey on the application of vascular clamping in liver surgery. *Dig Surg* 2007; **24**: 423–435.
- 14 Rahbari NN, Wente WN, Schemmer P, Diener MK, Hoffmann K, Mottschall E *et al.* Systematic review and meta-analysis of the effect of portal triad clamping and outcome after hepatic resection. *Br J Surg* 2008; **95**: 424–432.
- 15 Caldwell-Kenkel JC, Currin RT, Coote A, Thurman RG, Lemasters JJ. Reperfusion injury to endothelial cells after cold storage of rat livers: protection by mildly acidic pH and lack of protection by antioxidants. *Transplant Int* 1995; **8**: 77–85.
- 16 Wanner GA, Ertel W, Müller P, Hofer Y, Leiderer R, Menger MD *et al.* Liver ischemia and reperfusion induces a systemic inflammatory response through Kupffer cell activation. *Shock* 1996; **5**: 34–40.
- 17 Selzner N, Rudiger H, Graf H, Clavien PA. Protective strategies against ischemic injury of the liver. *Gastroenterology* 2003; **125**: 917–936.
- 18 Zwacka RM, Zhang Y, Zhou W, Halldorson J, Engelhardt JF. Ischemia/reperfusion injury in the liver of BALB/c mice activates AP-1 and nuclear factor kappaB independently of IkappaB degradation. *Hepatology* 1998; **28**: 1022–1030.
- 19 Jaeschke H. Molecular mechanisms of hepatic ischemia–reperfusion injury and preconditioning. *Am J Physiol Gastrointest Liver Physiol* 2003; **284**: G15–G26.
- 20 Pannen BH, Al-Adili F, Bauer M, Clemens MG, Geiger KK. Role of endothelins and nitric oxide in hepatic reperfusion injury in the rat. *Hepatology* 1998; **27**: 755–764.
- 21 Vollmar B, Glasz J, Leiderer R, Post S, Menger MD. Hepatic microcirculatory perfusion failure is a determinant of liver dysfunction in warm ischemia–reperfusion. *Am J Pathol* 1994; **145**: 1421–1431.
- 22 Ezaki T, Seo Y, Tomoda H, Furusawa M, Kanematsu T, Sugimachi K. Partial hepatic resection under intermittent hepatic inflow occlusion in patients with chronic liver disease. *Br J Surg* 1992; **79**: 224–226.
- 23 Serviddio G, Bellanti F, Tamborra R, Rollo T, Capitanio N, Romano AD *et al.* Uncoupling protein-2 (UCP2) induces mitochondrial proton leak and increases susceptibility of non-alcoholic steatohepatitis (NASH) liver to ischaemia–reperfusion injury. *Gut* 2008; **57**: 957–965.
- 24 Massip-Salcedo M, Zaouali MA, Padriša-Altés S, Casillas-Ramirez A, Rodés J, Roselló-Catafau J *et al.* Activation of peroxisome proliferator-activated receptor-alpha inhibits the injurious effects of adiponectin in rat steatotic liver undergoing ischemia–reperfusion. *Hepatology* 2008; **47**: 461–472.
- 25 Huet PM, Giroux L, Laurens M, Crenesse D. Effect of cold ischemia–warm reperfusion on the cirrhotic rat liver. *Liver Transpl* 2008; **14**: 486–493.
- 26 Kuddus RH, Nalesnik MA, Subbotin VM, Rao AS, Gandhi CR. Enhanced synthesis and reduced metabolism of endothelin-1 (ET-1) by hepatocytes – an important mechanism of increased endogenous levels of ET-1 in liver cirrhosis. *J Hepatol* 2000; **33**: 725–732.
- 27 Tsuchiya Y, Suzuki S, Inaba K, Sakaguchi T, Baba S, Miwa M *et al.* Impact of endothelin-1 on microcirculatory disturbance after partial hepatectomy under ischemia/reperfusion in thioacetamide-induced cirrhotic rats. *J Surg Res* 2003; **111**: 100–108.
- 28 Li SQ, Liang LJ, Huang JF, Li Z. Hepatocyte apoptosis induced by hepatic ischemia–reperfusion injury in cirrhotic rats. *Hepatobiliary Pancreat Dis Int* 2003; **2**: 102–105.
- 29 Manekeller S, Sioutis M, Hirner A, Minor T. [Influence of neoadjuvant chemotherapy on liver integrity and ischemic tolerance.] *Z Gastroenterol* 2008; **46**: 17–21.
- 30 Carini R, Albano E. Recent insights on the mechanisms of liver preconditioning. *Gastroenterology* 2003; **125**: 1480–1491.
- 31 Peralta C, Hotter G, Closa D, Gelpí E, Bulbena O, Roselló-Catafau J. Protective effect of preconditioning on the injury associated to hepatic ischemia–reperfusion in the rat: role of nitric oxide and adenosine. *Hepatology* 1997; **25**: 934–937.
- 32 Redaelli CA, Tian YH, Schaffner T, Ledermann M, Baer HU, Dufour JF. Extended preservation of rat liver

- graft by induction of heme oxygenase-1. *Hepatology* 2002; **35**: 1082–1092.
- 33 Peralta C, Fernández L, Panés J, Prats N, Sans M, Piqué JM *et al.* Preconditioning protects against systemic disorders associated with hepatic ischemia–reperfusion through blockade of tumor necrosis factor-induced P-selectin up-regulation in the rat. *Hepatology* 2001; **33**: 100–113.
 - 34 Hardy KJ, McClure DN, Subwongcharoen S. Ischaemic preconditioning of the liver: a preliminary study. *Aust N Z J Surg* 1996; **66**: 707–710.
 - 35 Peralta C, Closa D, Xaus C, Gelpí E, Roselló-Catafau J, Hotter G. Hepatic preconditioning in rats is defined by a balance of adenosine and xanthine. *Hepatology* 1998; **28**: 768–773.
 - 36 Kanoria S, Jalan R, Seifalian AM, Williams R, Davidson BR. Protocols and mechanisms for remote ischemic preconditioning: a novel method for reducing ischemia reperfusion injury. *Transplantation* 2007; **84**: 445–458.
 - 37 Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P. Regional ischemic ‘preconditioning’ protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation* 1993; **87**: 893–899.
 - 38 Kanoria S, Jalan R, Davies NA, Seifalian AM, Williams R, Davidson BR. Remote ischaemic preconditioning of the hind limb reduces experimental liver warm ischaemia–reperfusion injury. *Br J Surg* 2006; **93**: 762–768.
 - 39 Isozaki H, Adam R, Gigou M, Szekely AM, Shen M, Bismuth H. Experimental study of the protective effect of intermittent hepatic pedicle clamping in the rat. *Br J Surg* 1992; **79**: 310–313.
 - 40 van Wagenveld BA, van Gulik TM, Gelderblom HC, Scheepers JJ, Bosma A, Endert E *et al.* Prolonged continuous or intermittent vascular inflow occlusion during hemihepatectomy in pigs. *Ann Surg* 1999; **229**: 376–384.
 - 41 Hewitt G, Halliday I, McCaigue M, Campbell G, Rowlands B, Diamond T. Mortality, endotoxaemia and cytokine expression after intermittent and continuous hepatic ischaemia. *Br J Surg* 1995; **82**: 1424–1426.
 - 42 Ozmen MM, Oruc MT, Besler HT, Kulah B, Safali M, Polat A *et al.* Comparison of the effects of continuous and intermittent portal triad occlusion (PTO) in rats. *Hepatogastroenterology* 2003; **50**: 2127–2132.
 - 43 Uchinami M, Muraoka R, Horiuchi T, Tabo T, Kimura N, Naito Y *et al.* Effect of intermittent hepatic pedicle clamping on free radical generation in the rat liver. *Surgery* 1998; **124**: 49–56.
 - 44 Furrer K, Deoliveira ML, Graf R, Clavien PA. Improving outcome in patients undergoing liver surgery. *Liver Int* 2007; **27**: 26–39.
 - 45 Rüdiger HA, Kang KJ, Sindram D, Riehle HM, Clavien PA. Comparison of ischemic preconditioning and intermittent and continuous occlusion in the murine liver. *Ann Surg* 2002; **235**: 400–407.
 - 46 Vajdová K, Heinrich S, Tian Y, Graf R, Clavien PA. Ischemic preconditioning and intermittent clamping improve murine hepatic microcirculation and Kupffer cell function after ischemic injury. *Liver Transpl* 2004; **10**: 520–528.
 - 47 Smyrniotis V, Kostopanagiotou G, Theodoraki K, Farantos C, Arkadopoulos N, Gamaletsos E *et al.* Ischemic preconditioning *versus* intermittent vascular inflow control during major liver resection in pigs. *World J Surg* 2005; **29**: 930–934.
 - 48 Schiesser M, Wittert A, Nieuwenhuijs VB, Morphet A, Padbury RT, Barritt JG. Intermittent ischemia but not ischemic preconditioning is effective in restoring bile flow after ischemia reperfusion injury in the livers of aged rats. *J Surg Res* 2009; **152**: 61–68.
 - 49 Saidi RF, Chang J, Brooks S, Nalbantoglu I, Adsay V, Jacobs MJ. Ischemic preconditioning and intermittent clamping increase the tolerance of fatty liver to hepatic ischemia–reperfusion injury in the rat. *Transplant Proc* 2007; **39**: 3010–3014.
 - 50 Jang JH, Kang KJ, Kang Y, Lee IS, Graf R, Clavien PA. Ischemic preconditioning and intermittent clamping confer protection against ischemic injury in the cirrhotic mouse liver. *Liver Transpl* 2008; **14**: 980–988.
 - 51 Wheeler MD, Katuna M, Smutney OM, Froh M, Dikalova A, Mason RP *et al.* Comparison of the effect of adenoviral delivery of three superoxide dismutase genes against hepatic ischemia–reperfusion injury. *Hum Gene Ther* 2001; **12**: 2167–2177.
 - 52 Yabe Y, Nishikawa M, Tamada A, Takakura Y, Hashida M. Targeted delivery and improved therapeutic potential of catalase by chemical modification: combination with superoxide dismutase derivatives. *J Pharmacol Exp Ther* 1999; **289**: 1176–1184.
 - 53 Giakoustidis D, Kontos N, Iliadis S, Papageorgiou G, Tsantilis D, Spyridis C *et al.* Severe total hepatic ischemia and reperfusion: relationship between very high alpha-tocopherol uptake and lipid peroxidation. *Free Radic Res* 2001; **35**: 103–109.
 - 54 Seo MY, Lee SM. Protective effect of low dose of ascorbic acid on hepatobiliary function in hepatic ischemia/reperfusion in rats. *J Hepatol* 2002; **36**: 72–77.
 - 55 Schauer RJ, Gerbes AL, Vonier D, Meissner H, Michl P, Leiderer R *et al.* Glutathione protects the rat liver against reperfusion injury after prolonged warm ischemia. *Ann Surg* 2004; **239**: 220–231.
 - 56 Glantzounis GK, Yang W, Koti RS, Mikhailidis DP, Seifalian AM, Davidson BR. Continuous infusion of N-acetylcysteine reduces liver warm ischaemia–reperfusion injury. *Br J Surg* 2004; **91**: 1330–1339.
 - 57 Dunschede F, Erbes K, Kircher A, Westermann S, Schad A, Riegler N *et al.* Protection from hepatic ischemia/reperfusion injury and improvement of liver regeneration by alpha-lipoic acid. *Shock* 2007; **27**: 644–651.
 - 58 Sewerynek E, Reiter RJ, Melchiorri D, Oritz GG, Lewinski A. Oxidative damage in the liver induced by ischemia–reperfusion: protection by melatonin. *Hepatogastroenterology* 1996; **43**: 898–905.

- 59 Liu PG, He SQ, Zhang YH, Wu J. Protective effects of apocynin and allopurinol on ischemia/reperfusion-induced liver injury in mice. *World J Gastroenterol* 2008; **14**: 2832–2837.
- 60 Karwinski W, Søreide O. Allopurinol improves scavenging ability of the liver after ischemia/reperfusion injury. *Liver* 1997; **17**: 139–143.
- 61 Shiratori Y, Kiriya H, Fukushi Y, Nagura T, Takada H, Hai K *et al.* Modulation of ischemia–reperfusion-induced hepatic injury by Kupffer cells. *Dig Dis Sci* 1994; **39**: 1265–1272.
- 62 Colletti LM, Remick DG, Campbell DA Jr. Desferal attenuates TNF release following hepatic ischemia/reperfusion. *J Surg Res* 1994; **57**: 447–453.
- 63 Toledo-Pereyra LH, Lopez-Nebolina F, Reuben JS, Toledo AH, Ward PA. Selectin inhibition modulates Akt/MAPK signaling and chemokine expression after liver ischemia–reperfusion. *J Invest Surg* 2004; **17**: 303–313.
- 64 Takeyoshi I, Sunose Y, Iwazaki S, Tsutsumi H, Aiba M, Kasahara M *et al.* The effect of a selective cyclooxygenase-2 inhibitor in extended liver resection with ischemia in dogs. *J Surg Res* 2001; **100**: 25–31.
- 65 Harada N, Okajima K, Kushimoto S. Gabexate mesilate, a synthetic protease inhibitor, reduces ischemia/reperfusion injury of rat liver by inhibiting leukocyte activation. *Crit Care Med* 1999; **27**: 1958–1964.
- 66 Shito M, Wakabayashi G, Ueda M, Shimazu M, Shirasugi N, Endo M *et al.* Interleukin 1 receptor blockade reduces tumor necrosis factor production, tissue injury, and mortality after hepatic ischemia–reperfusion in the rat. *Transplantation* 1997; **63**: 143–148.
- 67 Lentsch AB, Yoshidome H, Cheadle WG, Miller FN, Edwards MJ. Chemokine involvement in hepatic ischemia/reperfusion injury in mice: roles for macrophage inflammatory protein-2 and Kupffer cells. *Hepatology* 1998; **27**: 507–512.
- 68 Glanemann M, Münchow S, Schirmeier A, Al-Abadi H, Lippek F, Langrehr JM *et al.* Steroid administration before partial hepatectomy with temporary inflow occlusion does not influence cyclin D1 and Ki-67 related liver regeneration. *Langenbecks Arch Surg* 2004; **389**: 380–386.
- 69 Arias-Díaz J, Ildefonso JA, Muñoz JJ, Zapata A, Jiménez E. Both tacrolimus and sirolimus decrease Th1/Th2 ratio, and increase regulatory T lymphocytes in the liver after ischemia/reperfusion. *Lab Invest* 2009; **89**: 433–445.
- 70 Kawano K, Kim YI, Kai T, Ishii T, Tatsuma T, Morimoto A *et al.* Evidence that FK506 alleviates ischemia/reperfusion injury to the rat liver: *in vivo* demonstration for suppression of TNF- α production in response to endotoxemia. *Eur Surg Res* 1994; **26**: 108–115.
- 71 Chen MF, Chen JC, Chiu DF, Ng CJ, Shyr MH, Chen HM. Prostacyclin analogue (OP-2507) induces delayed *ex vivo* neutrophil apoptosis and attenuates reperfusion-induced hepatic microcirculatory derangement in rats. *Shock* 2001; **16**: 473–478.
- 72 Pannen BH, Köhler N, Hole B, Bauer M, Clemens MG, Geiger KK. Protective role of endogenous carbon monoxide in hepatic microcirculatory dysfunction after hemorrhagic shock. *J Clin Invest* 1998; **102**: 1220–1228.
- 73 Uhlmann D, Armann B, Gaebel G, Ludwig S, Hess J, Pietsch UC *et al.* Endothelin A receptor blockade reduces hepatic ischemia/reperfusion injury after warm ischemia in a pig model. *J Gastrointest Surg* 2003; **7**: 331–339.
- 74 Bataller R, Ginès P, Nicolás JM, Görgbig MN, Garcia-Ramallo E, Gasull X *et al.* Angiotensin II induces contraction and proliferation of human hepatic stellate cells. *Gastroenterology* 2000; **118**: 1149–1156.
- 75 Hanazaki K, Kuroda T, Kajikawa S, Amano J. Prostaglandin E1 reduces thromboxane A2 in hepatic ischemia–reperfusion. *Hepatogastroenterology* 2000; **47**: 807–811.
- 76 Uhlmann D, Scommotau S, Witzigmann H, Spiegel HU. Exogenous L-arginine protects liver microcirculation from ischemia reperfusion injury. *Eur Surg Res* 1998; **30**: 175–184.
- 77 Suematsu M, Wakabayashi Y, Ishimura Y. Gaseous monoxides: a new class of microvascular regulator in the liver. *Cardiovasc Res* 1996; **32**: 679–686.
- 78 Freise H, Palmes D, Spiegel HU. Inhibition of angiotensin-converting enzyme reduces rat liver reperfusion injury via bradykinin-2-receptor. *J Surg Res* 2006; **134**: 231–237.
- 79 Hossain MA, Izuishi K, Maeta H. Effect of short-term administration of prostaglandin E1 on viability after ischemia/reperfusion injury with extended hepatectomy in cirrhotic rat liver. *World J Surg* 2003; **27**: 1155–1160.
- 80 Lemasters JJ, Nieminen AL, Qian T, Trost LC, Elmore SP, Nishimura Y *et al.* The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. *Biochim Biophys Acta* 1998; **1366**: 177–196.
- 81 Cursio R, Gugenheim J, Ricci JE, Crenesse D, Rostagno P, Maulon L *et al.* A caspase inhibitor fully protects rats against lethal normothermic liver ischemia by inhibition of liver apoptosis. *FASEB J* 1999; **13**: 253–261.
- 82 Nakajima H, Mizuta N, Fujiwara I, Sakaguchi K, Ogata H, Magae J *et al.* Blockade of the Fas/Fas ligand interaction suppresses hepatocyte apoptosis in ischemia–reperfusion rat liver. *Apoptosis* 2008; **13**: 1013–1021.
- 83 Nagai S, Asoh S, Kobayashi Y, Shidara Y, Mori T, Suzuki M *et al.* Protection of hepatic cells from apoptosis induced by ischemia/reperfusion injury by protein therapeutics. *Hepatol Res* 2007; **37**: 133–142.
- 84 Teoh N, Leclercq I, Pena AD, Farrell G. Low-dose TNF-alpha protects against hepatic ischemia–reperfusion injury in mice: implications for preconditioning. *Hepatology* 2003; **37**: 118–128.
- 85 Howell JG, Zibari GB, Brown MF, Burney DL, Sawaya DE, Olinde JG *et al.* Both ischemic and pharmacological preconditioning decrease hepatic

- leukocyte/endothelial cell interactions. *Transplantation* 2000; **69**: 300–303.
- 86 Peralta C, Hotter G, Closa D, Prats N, Xaus C, Gelpí E *et al.* The protective role of adenosine in inducing nitric oxide synthesis in rat liver ischemia preconditioning is mediated by activation of adenosine A2 receptors. *Hepatology* 1999; **29**: 126–132.
 - 87 Peralta C, Rull R, Rimola A, Deulofeu R, Roselló-Catafau J, Gelpí E *et al.* Endogenous nitric oxide and exogenous nitric oxide supplementation in hepatic ischemia–reperfusion injury in the rat. *Transplantation* 2001; **71**: 529–536.
 - 88 Xue H, Guo H, Li YC, Hao ZM. Heme oxygenase-1 induction by hemin protects liver cells from ischemia/reperfusion injury in cirrhotic rats. *World J Gastroenterol* 2007; **13**: 5384–5390.
 - 89 Ito K, Ozasa H, Sanada K, Horikawa S. Doxorubicin preconditioning: a protection against rat hepatic ischemia–reperfusion injury. *Hepatology* 2000; **31**: 416–419.
 - 90 Rensing H, Bauer I, Kubulus D, Wolf B, Winning J, Ziegeler S *et al.* Heme oxygenase-1 gene expression in pericentral hepatocytes through beta1-adrenoceptor stimulation. *Shock* 2004; **21**: 376–387.
 - 91 Lai IR, Chang KJ, Tsai HW, Chen CF. Pharmacological preconditioning with simvastatin protects liver from ischemia–reperfusion injury by heme oxygenase-1 induction. *Transplantation* 2008; **85**: 732–738.
 - 92 Kulhanek-Heinze S, Gerbes AL, Gerwig T, Vollmar AM, Kiemer AK. Protein kinase A dependent signalling mediates anti-apoptotic effects of the atrial natriuretic peptide in ischemic livers. *J Hepatol* 2004; **41**: 414–420.
 - 93 Gerwig T, Meissner H, Bilzer M, Kiemer AK, Arnholdt H, Vollmar AM *et al.* Atrial natriuretic peptide preconditioning protects against hepatic preservation injury by attenuating necrotic and apoptotic cell death. *J Hepatol* 2003; **39**: 341–348.
 - 94 Lorsomradee S, Cromheecke S, Lorsomradee S, De Hert SG. Cardioprotection with volatile anesthetics in cardiac surgery. *Asian Cardiovasc Thorac Ann* 2008; **16**: 256–264.
 - 95 Schmidt R, Trischler E, Hoetzel A, Loop T, Humar M, Halverscheid L *et al.* Heme oxygenase-1 induction by the clinically used anesthetic isoflurane protects rat livers from ischemia/reperfusion injury. *Ann Surg* 2007; **245**: 931–942.
 - 96 Clavien PA, Selzner M, Rüdiger HA, Graf R, Kadry Z, Rousson V *et al.* A prospective randomized study in 100 consecutive patients undergoing major liver resection with *versus* without ischemic preconditioning. *Ann Surg* 2003; **238**: 843–850.
 - 97 Choukèr A, Schachtner T, Schauer R, Dugas M, Löhe F, Martignoni A *et al.* Effects of Pringle manoeuvre and ischaemic preconditioning on haemodynamic stability in patients undergoing elective hepatectomy: a randomized trial. *Br J Anaesth* 2004; **93**: 204–211.
 - 98 Nuzzo G, Giuliani F, Vellone M, De Cosmo G, Ardito F, Murario M *et al.* Pedicle clamping with ischemic preconditioning in liver resection. *Liver Transpl* 2004; **10**: S53–S57.
 - 99 Li SQ, Liang LJ, Huang JF, Li Z. Ischemic preconditioning protects liver from hepatectomy under hepatic inflow occlusion for hepatocellular carcinoma patients with cirrhosis. *World J Gastroenterol* 2004; **10**: 2580–2584.
 - 100 Azoulay D, Lucidi V, Andreani P, Maggi U, Sebah M, Ichai P *et al.* Ischemic preconditioning for major liver resection under vascular exclusion of the liver preserving the caval flow: a randomized prospective study. *J Am Coll Surg* 2006; **202**: 203–211.
 - 101 Heizmann O, Loehe F, Volk A, Schauer RJ. Ischemic preconditioning improves postoperative outcome after liver resections: a randomized controlled study. *Eur J Med Res* 2008; **13**: 79–86.
 - 102 Belghiti J, Noun R, Malafosse R, Jagot P, Sauvanet A, Pierangeli F *et al.* Continuous *versus* intermittent portal triad clamping for liver resection: a controlled study. *Ann Surg* 1999; **229**: 369–375.
 - 103 Capussotti L, Nuzzo G, Polastri R, Giulianti F, Muratore A, Giovannini I. Continuous *versus* intermittent portal triad clamping during hepatectomy in cirrhosis. Results of a prospective, randomized clinical trial. *Hepatogastroenterology* 2003; **50**: 1073–1077.
 - 104 Esaki M, Sano T, Shimada K, Sakamoto Y, Takahashi Y, Wakai K *et al.* Randomized clinical trial of hepatectomy using intermittent pedicle occlusion with ischaemic intervals of 15 *versus* 30 minutes. *Br J Surg* 2006; **93**: 944–951.
 - 105 Brooks AJ, Hammond JS, Girling K, Beckingham JJ. The effect of hepatic vascular inflow occlusion on liver tissue pH, carbon dioxide, and oxygen partial pressures: defining the optimal clamp/release regime for intermittent portal clamping. *J Surg Res* 2007; **141**: 247–251.
 - 106 Smyrniotis V, Theodoraki K, Arkadopoulos N, Fragulidis G, Condi-Pafiti A, Plemenou-Fragou M *et al.* Ischemic preconditioning *versus* intermittent vascular occlusion in liver resections performed under selective vascular exclusion: a prospective randomized study. *Am J Surg* 2006; **192**: 669–674.
 - 107 Petrowsky H, McCormack L, Trujillo M, Selzner M, Jochum W, Clavien PA. A prospective, randomized, controlled trial comparing intermittent portal triad clamping *versus* ischemic preconditioning with continuous clamping for major liver resection. *Ann Surg* 2006; **244**: 921–928.
 - 108 Yadav SS, Sindram D, Perry DK, Clavien PA. Ischemic preconditioning protects the mouse liver by inhibition of apoptosis through a caspase-dependent pathway. *Hepatology* 1999; **30**: 1223–1231.
 - 109 Clavien PA, Yadav S, Sindram D, Bentley RC. Protective effects of ischemic preconditioning for liver resection performed under inflow occlusion in humans. *Ann Surg* 2000; **232**: 155–162.
 - 110 Choukèr A, Martignoni A, Schauer R, Dugas M, Rau HG, Jauch KW *et al.* Beneficial effects of ischemic

- preconditioning in patients undergoing hepatectomy: the role of neutrophils. *Arch Surg* 2005; **140**: 129–136.
- 111 Choukèr A, Martignoni A, Schauer RJ, Dugas M, Schachtner T, Kaufmann I *et al.* Alpha-gluthathione S-transferase as an early marker of hepatic ischemia/reperfusion injury after liver resection. *World J Surg* 2005; **29**: 528–534.
 - 112 Gurusamy KS, Kumar Y, Sharma D, Davidson BR. Methods of vascular occlusion for elective liver resections. *Cochrane Database Sys Rev* 2007; (4)CD006409.
 - 113 Makuuchi M, Mori T, Gunvén P, Yamazaki S, Hasegawa H. Safety of hemihepatic vascular occlusion during resection of the liver. *Surg Gynecol Obstet* 1987; **164**: 155–158.
 - 114 Sakamoto Y, Makuuchi M, Takayama T, Minagawa M, Kita Y. Pringle's maneuver lasting 322 min. *Hepatogastroenterology* 1999; **46**: 457–458.
 - 115 Wu CC, Hwang CR, Liu TJ, P'eng FK. Effects and limitations of prolonged intermittent ischaemia for hepatic resection of the cirrhotic liver. *Br J Surg* 1996; **83**: 121–124.
 - 116 Man K, Fan ST, Ng IO, Lo CM, Liu CL, Yu WC *et al.* Tolerance of the liver to intermittent Pringle maneuver in hepatectomy for liver tumors. *Arch Surg* 1999; **134**: 533–539.
 - 117 Elias D, Desruennes E, Lasser P. Prolonged intermittent clamping of the portal triad during hepatectomy. *Br J Surg* 1991; **78**: 42–44.
 - 118 Cerwenka H, Bacher H, Werkgartner G, El-Shabrawi A, Quehenberger F, Hauser H *et al.* Antioxidant treatment during liver resection for alleviation of ischemia–reperfusion injury. *Hepatogastroenterology* 1998; **45**: 777–782.
 - 119 Vriens MR, Marinelli A, Harinck HI, Zwinderman KH, van de Velde CJ. The role of allopurinol in human liver ischemia/reperfusion injury: a prospective randomized clinical trial. *Hepatogastroenterology* 2002; **49**: 1069–1073.
 - 120 Bartels M, Biesalski HK, Engelhart K, Sendlhofer G, Rehak P, Nagel E. Pilot study on the effect of parenteral vitamin E on ischemia and reperfusion induced liver injury: a double blind, randomized, placebo-controlled trial. *Clin Nutr* 2004; **23**: 1360–1370.
 - 121 Dünschede F, Erbes K, Kircher A, Westermann S, Seifert J, Schad A *et al.* Reduction of ischemia reperfusion injury after liver resection and hepatic inflow occlusion by alpha-lipoic acid in humans. *World J Gastroenterol* 2006; **12**: 6812–6817.
 - 122 Kostopanagiotou G, Pandazi AK, Andreadou I, Markantonis SL, Niokou D, Teloudis A *et al.* Effects of mannitol in the prevention of lipid peroxidation during liver resection with hepatic vascular exclusion. *J Clin Anesth* 2006; **18**: 570–574.
 - 123 Kim YI, Chung HJ, Song KE, Hwang YJ, Lee JW, Lee YJ *et al.* Evaluation of a protease inhibitor in the prevention of ischemia and reperfusion injury in hepatectomy under intermittent Pringle maneuver. *Am J Surg* 2006; **191**: 72–76.
 - 124 Muratore A, Ribero D, Ferrero A, Bergero R, Capussotti L. Prospective randomized study of steroids in the prevention of ischaemic injury during hepatic resection with pedicle clamping. *Br J Surg* 2003; **90**: 17–22.
 - 125 Aldrighetti L, Pulitanò C, Arru M, Finazzi R, Catena M, Soldini L *et al.* Impact of preoperative steroids administration on ischemia–reperfusion injury and systemic responses in liver surgery: a prospective randomized study. *Liver Transpl* 2006; **12**: 941–949.
 - 126 Pulitanò C, Aldrighetti L, Arru M, Finazzi R, Soldini L, Catena M *et al.* Prospective randomized study of the benefits of preoperative corticosteroid administration on hepatic ischemia–reperfusion injury and cytokine response in patients undergoing hepatic resection. *HPB* 2007; **9**: 183–189.
 - 127 Shirabe K, Takenaka K, Yamamoto K, Kitamura M, Itasaka H, Matsumata T *et al.* The role of prostanoid in hepatic damage during hepatectomy. *Hepatogastroenterology* 1996; **43**: 596–601.
 - 128 Sugawara Y, Kubota K, Ogura T, Esumi H, Inoue K, Takayama T *et al.* Protective effect of prostaglandin E1 against ischemia/reperfusion-induced liver injury: results of a prospective, randomized study in cirrhotic patients undergoing subsegmentectomy. *J Hepatol* 1998; **29**: 969–976.
 - 129 Orii R, Sugawara Y, Hayashida M, Yamada Y, Chang K, Takayama T *et al.* Effects of amrinone on ischaemia–reperfusion injury in cirrhotic patients undergoing hepatectomy: a comparative study with prostaglandin E1. *Br J Anaesth* 2000; **85**: 389–395.
 - 130 Beck-Schimmer B, Breitenstein S, Urech S, De Conno E, Wittlinger M, Puhon M *et al.* A randomized controlled trial on pharmacological preconditioning in liver surgery using a volatile anesthetic. *Ann Surg* 2008; **248**: 909–918.
 - 131 Tang L, Tian F, Tao W, Cui J. Hepatocellular glycogen in alleviation of liver ischemia–reperfusion injury during partial hepatectomy. *World J Surg* 2007; **31**: 2039–2043.
 - 132 Cerwenka H, Khoschsorur G, Bacher H, Werkgartner G, El-Shabrawi A, Quehenberger F *et al.* Normothermic liver ischemia and antioxidant treatment during hepatic resections. *Free Radic Res* 1999; **30**: 463–439.
 - 133 Ghibu S, Richard C, Vergely C, Zeller M, Cottin Y, Rochette L. Antioxidant properties of an endogenous thiol: alpha-lipoic acid, useful in the prevention of cardiovascular diseases. *J Cardiovasc Pharmacol* 2009; **54**: 391–398.
 - 134 Barnes PJ. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci* 1998; **94**: 557–572.
 - 135 Sauerland S, Nagelschmidt M, Mallmann P, Neugebauer EA. Risks and benefits of preoperative high dose methylprednisolone in surgical patients: a systematic review. *Drug Saf* 2000; **23**: 449–461.

- 136 Pâques EP, Römisch J. Comparative study on the *in vitro* effectiveness of antithrombotic agents. *Thromb Res* 1991; **64**: 11–21.
- 137 Kim YI, Fujita S, Hwang YJ, Chun JM, Song KE, Chun BY. Successful intermittent application of the Pringle maneuver for 30 minutes during human hepatectomy: a clinical randomized study with use of a protease inhibitor. *Hepatogastroenterology* 2007; **54**: 2055–2060.
- 138 Müllenheim J, Ebel D, Frässdorf J, Preckel B, Thämer V, Schlack W. Isoflurane preconditions myocardium against infarction via release of free radicals. *Anesthesiology* 2002; **96**: 934–940.
- 139 Tanaka K, Ludwig LM, Kersten JR, Pagel PS, Waritier DC. Mechanisms of cardioprotection by volatile anesthetics. *Anesthesiology* 2004; **100**: 707–721.
- 140 van der Bilt JD, Kranenburg O, Nijkamp MW, Smakman N, Veenendaal LM, Te Velde EA *et al.* Ischemia/reperfusion accelerates the outgrowth of hepatic micrometastases in a highly standardized murine model. *Hepatology* 2005; **42**: 165–175.
- 141 van der Bilt JD, Kranenburg O, Verheem A, van Hillegersberg R, Borel Rinkes IH. Selective portal clamping to minimize hepatic ischaemia–reperfusion damage and avoid accelerated outgrowth of experimental colorectal liver metastases. *Br J Surg* 2006; **93**: 1015–1022.
- 142 Ypsilantis P, Lambropoulou M, Tentes I, Anagnostopoulos K, Tsigalou C, Papadopoulos N *et al.* Impaired liver regeneration following partial hepatectomy using the Pringle maneuver: protective effect of mesna. *J Gastroenterol Hepatol* 2009; **24**: 623–632.
- 143 Yamada F, Saito T, Abe T, Tsuchiya T, Sato Y, Kenjo A *et al.* Ischemic preconditioning enhances regenerative capacity of hepatocytes in long-term ischemically damaged rat livers. *J Gastroenterol Hepatol* 2007; **22**: 1971–1977.