Abstract

Ischemia-reperfusion (I/R) associated with hepatic resection and living related liver transplantation is an unsolved problem in clinical practice. Indeed, I/R induces damage and regenerative failure in clinical liver surgery. Signaling pathways regarding the pathophysiology of liver I/R and regeneration making clear distinction between situations of cold and warm ischemia, as well as liver regeneration with or without vascular occlusion, will be addressed. The different experimental models used to date to improve the postoperative outcomes in clinical liver surgery will be also described. Furthermore, the most updated therapeutic strategies, as well as the clinical and scientific controversies in the field, will be discussed. Such information may be useful to guide the design of better experimental models as well as the effective therapeutic strategies in liver surgery that can succeed in achieving its clinical application.

Keywords: liver surgery, regeneration, ischemia–reperfusion injury, warm ischemia, cold ischemia

1. Introduction

Any surgical situation involving liver hepatectomy requires subsequent regeneration in order to restore the liver/body ratio. The liver’s ability to restore tissue after loss depends on the interaction of numerous cells and a complex network of mediators [1]. In most cases, in clinical practice, liver surgery involves both ischemia-reperfusion (I/R) injury and regeneration [1]. Liver I/R injury is a pathophysiological event that occurs during surgical interventions such as liver resection or liver transplantation (LT); it controls bleeding during parenchymal dissection and has a significant effect on liver function prognosis [2–6]. I/R injury is a twostage phenomenon in which cell damage due to hypoxia and the lack of biomechanical stimulus is exacerbated upon the restoration of oxygen delivery and shear stress [7]. However, I/R injury is inevitable in liver surgery and significantly reduces the organ’s regeneration after hepatectomy [1]. Mechanisms of liver I/R injury are complex; they include mainly microcirculation failure and the related oxidative stress, a series of cellular and molecular responses, and the interaction between hepatocytes,
Liver sinusoidal endothelial cells, hepatic stellate cells, Kupffer cells, infiltrating neutrophils, macrophages, and platelets [2, 7–11].

Liver I/R injury involves great many factors and mediators. The associations between the signaling pathways are extremely complex, and at present, the events occurring between the start of reperfusion and the final outcome (either poor function or a nonfunctional liver graft) are not fully understood [6]. The extent and timing of ischemia, the type of liver undergoing I/R, and the existence of liver regeneration may alter the mechanisms of liver I/R injury and the effects of the treatment strategies assessed to date [12]. This point was exemplified by Ramalho et al. who demonstrated the loss of protection against liver damage of Ang-II receptor antagonists in conditions of partial hepatectomy (PH), while in conditions of I/R without hepatectomy, the Ang-II receptor antagonists decreased liver damage [13]. As is well known, the mechanisms of liver damage differ according to the percentage of the liver mass deprived of blood [14]. Therefore, experimental models that reproduce as closely as possible the clinical conditions in which these strategies are applied are likely to lead to the implementation of strategies in clinical practice in the relatively short term [1].

In this chapter, we aim to show that the mechanisms that govern liver I/R injury and regeneration depend on the experimental model applied. These models are valuable tools for elucidating the physiopathology of liver I/R injury and uncovering new therapeutic targets and drugs. A number of strategies for protecting the liver from I/R injury and for improving liver regeneration have been developed in animal models, some of which may find their way into clinical practice [6]. We stress that the type of ischemia (cold or warm) has an important influence in liver surgery, but most of the currently available reviews on the mechanisms of I/R do not distinguish between them [6]. In our view, this information may help to guide the design of future experimental models and treatment strategies in liver surgery for use in clinical practice.

2. Hepatic regeneration under warm ischemia

Following PH, hepatocytes that are normally quiescent enter the cell cycle in order to replace the part that has been removed. The original liver mass is restored after some 6–8 weeks (in humans) by tightly synchronized rounds of replication of the remaining hepatocytes [15]. Self-replication of the existing individual cell types is thought to be the key mechanism of regeneration after PH. However, it was recently suggested that hepatic progenitor cells may contribute to liver regeneration following PH [15]. A vast number of growth and metabolic factors and cytokines simultaneously regulate liver regeneration during PH. Under the influence of innate immunity components and gut-derived lipopolysaccharide, on Kupffer cells and stellate cells, tumor necrosis factor alpha, and interleukin 6, provided by those cells, prepares hepatocytes to respond to growth factors like epidermal growth factor and hepatocyte growth factor [15]. Among other auxiliary mitogens are norepinephrine, Notch and jagged proteins, vascular endothelial growth factor, platelet-derived growth factor, bile acids, insulin serotonin, estrogens leptin, triodothronine, and FGF1 and 2 [16]. Joint signals from these factors lead to the progression of the liver cell cycle, which in turn results in DNA synthesis and ultimately the proliferation of liver cells as mentioned above [15].

As is well known, remnant liver following PH can be used as an in vivo liver regeneration model in order to assess possible treatment strategies for improving postoperative outcomes after hepatectomy. Nonetheless, a two-third partial hepatectomy alone does not cause death in these models, and the remnant liver has the capacity to regenerate. In contrast, 30 min of liver ischemia just before PH exacerbates the remnant liver function, causing high mortality and negatively affecting liver weight restoration [1, 17]. It is also well known that vascular occlusion of the
hepatic hilum is often used to avoid hemorrhage in liver resection. However, vascular occlusion has been associated with warm ischemia damage, resulting in significant organ dysfunction and regenerative failure [12]. Hepatocytes are severely affected by I/R, especially in normothermic ischemia. Most of the early changes in anoxic hepatocytes take place in the mitochondria. Briefly, due to the unavailability of O$_2$ as a terminal electron carrier for the mitochondrial respiratory chain, the electron flow is immediately interrupted, thus reducing the respiratory chain. Since the mitochondria no longer accept electrons from substrates, pyridine nucleotides decrease, thus causing a rise in the intracellular NADH/NAD$^+$ ratio. The disruption of oxidative phosphorylation rapidly depletes cellular ATP, accelerates glycolysis, increases lactate formation, and alters H$^+$, Na$^+$, and Ca$^{2+}$ homeostasis and thus induces severe damage to the hepatocyte. Ischemia also causes a substantial rise in cAMP, a key factor in glucose metabolism. Via the action of cAMP-dependent protein kinase, cAMP causes the phosphorylation/deregulation of enzymes, which play a major role in the control of carbohydrate metabolism [18, 19]. Reperfusion injury derives mainly from toxic reactive oxygen species (ROS) generated on the reintroduction of O$_2$ to ischemic tissues. ROS are produced both from intracellular and from extracellular sources; in liver cells, the mitochondria are their major source [7, 20].

3. Preclinical studies in normothermic hepatic ischemia associated with hepatic resection

3.1 Animal species used

The results of animal studies can be extrapolated to human beings, even though there are limitations such as the differences in ischemia tolerance, the anatomy of the organ in different species, and differences in the surgical conditions applied in clinical practice and in experimental models [21]. Therefore, the correct choice of animal species and experimental model, and the standardization of the protocol according to the clinical issue under study, is particularly important [14]. Small animals like rats and mice are exceptionally useful because they are easy to handle, present minimal, financial, logistical or ethical problems, and allow genetic alterations such as the creation of transgenic and knock-out animals [14]. Larger animals (pigs, sheep, and dogs) have a more similar anatomy and physiology to humans, but their use is restricted by serious financial and logistical difficulties, ethical concerns, and the limited availability of immunological tools for use in these species [14, 21]. The age and sex of animals are also issues to consider. With regard to age, there are significant differences between younger (35–50 g) and older rats (250–400 g) in terms of their hepatic microcirculation at the different stages of ischemia, and with regard to sex, female rats are more sensitive to reperfusion injury than males after normothermic ischemia [14, 22, 23].

3.2 Experimental models of normothermic hepatic ischemia to evaluate the mechanisms involved

3.2.1 Global hepatic ischemia with portocaval decompression

The Pringle maneuver is often applied during liver resection, due to safety concerns. However, it has been associated with delayed liver failure and poor prognosis in patients undergoing major hepatectomy in conditions of prolonged liver ischemia [1, 13]. The global liver ischemia model with portal decompression provides an ideal simulation of the clinical condition of warm ischemia after the Pringle maneuver for liver resection and transplant [6, 14]. The first successful
shunt operation carried out in humans was by Vidal in 1903 [24]. Blakemore was among the first researchers to report successful portal-systemic anastomosis in rats, working mainly with endothelium-lined tubes [25]. Burnett et al. modified the technique to create a portocaval shunt [26]. In 1959, Bernstein and Cheiker developed the portosystemic shunt, which led portal blood into one of the iliac veins after functional hepatectomy [27]. In small animals, many other shunt techniques have been developed such as the portofemoral shunt or the mesentericocaval shunt via the jugular vein. In 1995, the splenocaval shunt was developed by Spiegel [28]. In large animals, on the other hand, a porto-femoro jugular bypass is frequently used [14, 29]. Results from experimental models of hepatic I/R injury alone are often extrapolated to clinical liver resection with PH and ischemia. However, in conventional experimental I/R models (for example, 70% partial hepatic ischemia), reperfusion ensues in the presence of nonischemic lobes [1]. Experimental models that combine PH and I/R injury rule out any contribution to recovery of the nonaffected liver tissue. Furthermore, in this model, posts ischemic recovery depends on the liver cell damage caused by the IR-injury and also on the stress caused by the liver resection and posthepatectomy liver regeneration [1, 30].

3.2.2 Global liver ischemia with spleen transposition

In 1970, Bengmark et al. developed this model for surgical treatment of portal hypertension [31]. In 1981, Meredith and Wade described a rat model that produced a portosystemic shunt in the anhepatic rat by transposition of the spleen, making a small incision in the left hypochondrium [32]. With the spleen inside a subcutaneous pouch, adequate portosystemic anastomoses emerge after some 2–3 weeks. The transposition induces the reversal of the blood flow in the splenic vein, which stimulates angiogenesis. Two weeks later, in the second step, a median laparotomy and temporary occlusion of the hepatoduodenal ligament are performed. This decompression by spleen transposition is easy to perform, because it does not require microsurgery. Within 2 or 3 weeks of surgery, the spleen is encapsulated without any signs of inflammation or bleeding. One drawback of this model is the long time span (3 weeks) until adequate portosystemic collaterals are large enough to take full control of portal vein flow. Furthermore, the effect of the changes in hepatic inflow on the collaterals remains unclear [6, 14, 33].

3.2.3 Partial hepatic resection under vascular occlusion

In 1982, Yamauchi et al. reported a hepatic ischemia model in which ischemia is induced by occlusion of the hepatic artery, the portal vein, and the bile duct of the left and median lobes. No extracorporeal shunt is required because the blood continues to flow through the right and caudal liver lobes. This model of partial ischemia (70%) has been extensively used in experimental studies of hepatic I/R [34–36]. An experimental model of 30% partial liver ischemia has also been used, in which occlusion at the hepatic artery and portal vein interrupts the supply of blood to the right lobe of the liver [19]. In the clinical setting, PH under I/R is normally performed to control bleeding during parenchymal dissection [6]. Therefore, an experimental model incorporating both hepatic regeneration and I/R injury can simulate the clinical situation of selective or hemihepatic vascular occlusion for liver resections. In this model, after left hepatic lobe resection, a microvascular clamp is placed across the portal triad supplying the median lobe (30%). Congestion of the bowel is prevented during the clamping because the portal flow through the right and caudate lobes is preserved. At the end of ischemia, the right lobe and caudate lobes are resected, and the clamp is released to achieve reperfusion of the median lobe. In this hepatic resection
model, portal decompression is not required, and certain important criteria are also met, such as reversibility, good reproducibility, and ease of execution [14, 19, 37].

### 3.3 Strategies applied in experimental models of normothermic ischemia

Many experimental studies have set out to develop in vivo pharmacological strategies for inhibiting the harmful effects of warm I/R [38–46]. Some of these studies are summarized in Table 1. However, none of them have been able to prevent hepatic I/R injury [6, 14]. However, it is important to develop strategies in experimental models that reproduce clinical practice conditions as closely as possible: for example, the use of intermittent clamping, and the combination of PH and I/R injury. Few of the studies carried out to date have complied with these requirements [12]. Some of these studies are summarized in Table 2. Recent breakthroughs in molecular biology are providing new opportunities for applying gene therapy to reduce liver I/R injury. The experimental data, however, have highlighted several problems inherent in gene therapy, including vector toxicity, difficulties in increasing transfection efficiency and protein expression at the appropriate site and time, and the difficulty of obtaining adequate mutants.

| Drug                                 | Ischemic time | Effect                      |
|--------------------------------------|---------------|-----------------------------|
| Cerulenin                            | 15 min        | ↓ UPC2, ↑ ATP               |
| Platinum nanoparticles               |               | ↓ Hepatic injury            |
| Exendin 4                            | 20 min        | ↓ Hepatic injury and autophagy |
| Catalase and derivatives             | 30 min        | ↓ Oxidative stress          |
| Apocynin                             |               | ↓ Oxidative stress          |
| Allopurinol                          |               | ↓ Oxidative stress          |
| N-Acetylcysteine                     | 40, 90 min    | ↓ Hepatic injury, oxidative stress and apoptosis |
| Dipyridamole                         | 45 min        | ↓ Hepatic injury            |
| 15-deoxy-Δ^{12,14}-prostaglandin J₂ | 60 min        | ↓ Hepatic injury and inflammation |
| Ago-miR-46a                          |               | ↓ Hepatic injury and apoptosis |
| Cold-inducible RNA-binding protein (CIRP) blockade | | ↓ Hepatic injury, apoptosis and inflammation |
| Anti-CD25 antibody                   |               | ↓ Hepatic injury and inflammation |
| Diannexin                            |               | ↓ Hepatic injury and inflammation |
| Ethyl pyruvate                       |               | ↓ Hepatic injury, apoptosis and autophagy |
| Fasting                              |               | ↓ Hepatic injury and inflammation; ↑ autophagy |
| Angiotensin II receptor antagonist    |               | ↓ Hepatic injury, apoptosis and inflammation |
| Riboflavin                           |               | ↓ Hepatic injury, oxidative stress and inflammation |
| α7 Nicotinic acetylcholine receptor agonist | | ↓ Hepatic injury, oxidative stress and inflammation |
| Omega–3 Fatty acid                   |               | ↓ Hepatic injury and inflammation; ↑ liver regeneration |
| Cobalt protoporphyrin                | 60, 90 min    | ↓ Hepatic injury and inflammation |
| Drug                                    | Ischemic time | Effect                                                                 |
|----------------------------------------|---------------|------------------------------------------------------------------------|
| Hydroxytyrosol                         | 75 min        | ↓ Hepatic injury, apoptosis, oxidative stress and inflammation         |
| miR-370 inhibitor                      |               | ↓ Hepatic injury and inflammation                                      |
| Augmenter of liver regeneration (ALR)  | 90 min        | ↓ Hepatic injury, apoptosis and inflammation                           |
| Carbon monoxide                        |               | ↓ Hepatic injury                                                       |
| Pan-selectin antagonist                |               | ↓ Inflammation                                                         |
| Erythropoietin                         |               | ↓ Hepatic injury and apoptosis                                         |
| Helium                                 |               | ↓ Hepatic injury; ↑ survival                                           |
| Low-dose LPS                           |               | ↓ Hepatic injury, apoptosis and inflammation                           |
| Protease-activated receptor 4 antagonist|               | ↓ Hepatic injury, apoptosis and inflammation                           |
| Vasoactive intestinal peptide neuropeptide |           | ↓ Hepatic injury, apoptosis and inflammation                           |
| ACE inhibitor                          | 30 min        | ↓ Oxidative stress                                                    |
| ROS scavenger                          |               | ↓ Apoptosis                                                            |
| Branched-chain amino acid (BCAA)       |               | ↓ Hepatic injury and inflammation                                      |
| Carvacrol                              |               | ↓ Hepatic injury, apoptosis and oxidative stress                       |
| CR2-CD59 (complement inhibitor)        |               | ↓ Hepatic injury; ↑ regeneration and survival                         |
| Hydrolysed whey peptide                |               | ↓ Hepatic injury, apoptosis and inflammation                           |
| Hyperbaric oxygen therapy              |               | ↓ Hepatic injury                                                       |
| Sivelestat sodium hydrate              |               | ↓ Hepatic injury and inflammation                                      |
| Liraglutide                            |               | ↓ Hepatic injury, apoptosis and inflammation                           |
| Allopurinol                            | 30, 60 min    | ↓ Oxidative stress                                                    |
| Diazoxide                              |               | ↓ Hepatic injury and inflammation                                      |
| PPARα agonist                          | 30, 60, 90 min| ↓ Oxidative stress and inflammation; ↑ autophagy                      |
| Propofol73                             |               | ↓ Hepatic injury and apoptosis                                         |
| Melatonin                              | 35, 40 min    | ↓ Hepatic injury, apoptosis and oxidative stress                       |
| Levosimendan                           | 40, 60 min    | ↓ Hepatic injury, apoptosis, oxidative stress and inflammation         |
| Carnosic acid                          | 45 min        | ↓ Hepatic injury                                                       |
| Limonin                                |               | ↓ Hepatic injury, oxidative stress and inflammation                   |
| Low-intensity laser therapy            |               | ↓ Hepatic injury and oxidative stress                                  |
| Rho-kinase inhibitor                   |               | ↓ Hepatic injury; ↑ survival                                           |
| Cardamonin                             |               | ↓ Hepatic injury, oxidative stress and inflammation                   |
| Quercetin                              |               | ↓ Hepatic Injury                                                      |
| Protoporphyrin                         |               | ↓ Hepatic Injury                                                      |
### Table 1. Pharmacological strategies used in experimental models of warm ischemia.

| Drug                                      | Ischemic time | Effect                                      |
|-------------------------------------------|---------------|---------------------------------------------|
| Sevoflurane                               | 40 min        | ↓ Hepatic injury                            |
| SOD                                       | 45, 60 min    | ↓ Inflammation                              |
| L-arginine                                |               | ↓ Inflammation                              |
| Tocopherol                                | 45, 90 min    | ↓ Oxidative stress and inflammation         |
| IL-10                                     | 60 min        | ↓ Oxidative stress and inflammation         |
| Anti-ICAM-1                                |               | ↓ Inflammation                              |
| Gabexate mesilate                         |               | ↓ Inflammation                              |
| Analogue of prostacyclin (OP-2507)        |               | ↓ Inflammation                              |
| n-3 PUFA                                  |               | ↓ Hepatic injury and oxidative stress       |
| Adiponectin                               |               | ↓ Hepatic injury, apoptosis and inflammation|
| Atorvastatin                              |               | ↓ Hepatic injury                            |
| Dexametadomidine                          |               | ↓ Hepatic injury and oxidative stress       |
| Dioscin                                   |               | ↓ Hepatic injury, apoptosis and inflammation|
| Fibrin-derived peptide B15-42             |               | ↓ Hepatic injury                            |
| L-α-glycerylphosphorylcholine (GPC)       |               | ↓ Hepatic injury and oxidative stress       |
| Rapamycin                                 |               | ↓ Hepatic injury; ↑ autophagy               |
| Rosmarinic acid                           |               | ↓ Hepatic injury, oxidative stress and inflammation |
| Sevoflurane                               | 60, 90 min    | ↓ Oxidative stress and inflammation         |
| Simvastatin                               |               | ↓ Hepatic injury                            |
| Crocin                                    |               | ↓ Hepatic injury and oxidative stress       |
| Tert-butylhydroquinone                    |               | ↓ Inflammation                              |
| Hydrogen-rich saline                      |               | ↓ Hepatic injury and oxidative stress       |
| Spermine NONOate                          | 60, 90 min    | ↓ Oxidative stress and inflammation         |
| FK506                                     |               | ↓ Inflammation                              |
| Chloroquine                               |               | ↓ Hepatic injury and oxidative stress       |
| Lithium                                   |               | ↓ Hepatic injury and inflammation           |
| AMPK activators                           | 90 min        | ↑ NO, ATP                                   |
| α-Lipoic acid                             |               | ↓ Apoptosis; ↑ liver regeneration           |
| Edaravone                                 |               | ↓ Hepatic injury and oxidative stress       |
| Reduced glutathione                       |               | ↓ Hepatic injury, apoptosis and oxidative stress |
| Sildenafil                                 |               | ↓ Hepatic injury, apoptosis and inflammation|
| Oleanolic                                 |               | ↓ Hepatic injury; ↑ survival                |
| Unacylated-ghrelin                        | 2, 6 and 24 h | ↓ Oxidative stress                          |
| Minocycline                               |               | ↓ Hepatic injury, oxidative stress and inflammation |

**Pigs**

| Drug                                      | Ischemic time | Effect                                      |
|-------------------------------------------|---------------|---------------------------------------------|
| Sevoflurane                               | 40 min        | ↓ Hepatic injury                            |
| Drug                                   | Ischemic time | Effect                                                                 |
|----------------------------------------|---------------|------------------------------------------------------------------------|
| Low dose of 2-complement receptor 1-related protein Y | 30 min        | ▼ Hepatic injury and oxidative stress; ▲ liver regeneration             |
| Melatonin                              | 60 min        | ▼ Hepatic injury; ▲ liver regeneration                                  |
| C1 esterase inhibitor                  |               | ▼ Hepatic injury; ▲ liver regeneration and survival                    |
| Hydrogen sulfide                       | 75, 90 min    | ▼ Hepatic injury and apoptosis; ▲ survival and liver regeneration       |
| Mice                                   |               |                                                                        |

| Drug                                   | Ischemic time | Effect                                                                 |
|----------------------------------------|---------------|------------------------------------------------------------------------|
| Low dose of 2-complement receptor 1-related protein Y | 30 min        | ▼ Hepatic injury and oxidative stress; ▲ liver regeneration             |
| Melatonin                              | 60 min        | ▼ Hepatic injury; ▲ liver regeneration                                  |
| C1 esterase inhibitor                  |               | ▼ Hepatic injury; ▲ liver regeneration and survival                    |
| Hydrogen sulfide                       | 75, 90 min    | ▼ Hepatic injury and apoptosis; ▲ survival and liver regeneration       |
| Rats                                   |               |                                                                        |

| Drug                                   | Ischemic time | Effect                                                                 |
|----------------------------------------|---------------|------------------------------------------------------------------------|
| CF102                                  | 10 min        | ▼ Hepatic injury, apoptosis and inflammation; ▲ liver regeneration      |
| Inchinkoto                             | 15, 30 min    | ▼ Hepatic injury, oxidative stress and inflammation                    |
| Anti-HMGB1                             | 20 min        | ▼ Hepatic injury; ▲ liver regeneration                                  |
| Thrombomodulin                         |               | ▼ Hepatic injury and apoptosis; ▲ liver regeneration                   |
| 2 mercaptoethane sulfonate             | 30 min        | ▼ Hepatic injury and oxidative stress; ▲ liver regeneration             |
| Butyrate                               | 30, 60 min    | ▼ Hepatic injury and inflammation                                      |
| Omega3 fatty acids                     | 40 min        | ▼ Hepatic injury and oxidative stress                                   |
| Polyamines                             |               | ▼ Necrosis, apoptosis and inflammation; ▲ liver regeneration            |
| Glucose or lipid emulsion              | 60 min        | ▼ Hepatic injury; ▲ liver regeneration                                  |
| Resistin or anti-visfatin antibodies   |               | ▼ Hepatic injury; ▲ liver regeneration                                  |
| Tauroursodeoxycholate                  |               | ▼ Oxidative stress                                                     |
| Sirolimus                              |               | ▼ Inflammation                                                         |
| Combined angiotensin II receptor type 1 and 2 antagonists |           | ▼ Hepatic injury and oxidative stress; ▲ liver regeneration             |
| Thymoquinone                           |               | ▼ Hepatic injury, apoptosis and oxidative stress                       |
| M3 AChR antagonist                     | 75, 90 min    | ▼ Hepatic injury and apoptosis and inflammation; ▲ survival and liver regeneration |
| IL-1 receptor antagonist               | 90 min        | ▼ Oxidative stress                                                     |
| Pigs                                   |               |                                                                        |

| Drug                                   | Ischemic time | Effect                                                                 |
|----------------------------------------|---------------|------------------------------------------------------------------------|
| Hydrogen inhalation                    | 20 min        | ▼ Hepatic injury, apoptosis and oxidative stress                       |
| Desferrioxamine                        | 150 min       | ▼ Oxidative stress; ▲ liver function                                   |
| Dogs                                   |               |                                                                        |

| Drug                                   | Ischemic time | Effect                                                                 |
|----------------------------------------|---------------|------------------------------------------------------------------------|
| FK 3311 (Cox-2 inhibitor)              | 60 min        | ▼ Inflammation                                                         |

Table 2. Pharmacological strategies used in experimental models of warm ischemia with partial hepatectomy.
4. Controversies on hepatic regeneration under warm or cold ischemia

In an attempt to expand the size of the donor pool, the use of living related liver transplantation (LDLT) has helped increase the number of donor livers, but, nonetheless, concerns persist about graft-size disparity and hepatic regeneration. In 1990, Broelsch et al. reported the first 20 series of LDLT in the USA [47]. In 1997, Lo et al. [48] performed the first successful LDLT using an extended right lobe from a living donor for an adult recipient [6]. In LDLT, liver graft must be successfully regenerated; however, cold I/R, which will take place during liver transplantation surgery, will reduce the regenerative capacity of the liver.

The clinical application of strategies designed at beachside will depend on the use of experimental models that resemble as much as possible the clinical conditions in which the strategy intends to be applied [12]. However, many investigators have used rodent models of PH under or without vascular occlusion to mimic some of the pathophysiological events that occur during LT [6]. To the best of our knowledge, pharmacological strategies, which were used in experimental models of hepatic regeneration under warm ischemia (Table 2), were not applied in experimental models of LDLT. However, only three drugs (sirolimus, Ang II receptor type 2 antagonist, and Omega-3) were applied in patients submitted to LDLT (see Table 3). In contrast with the benefits on liver regeneration observed in experimental models of PH under I/R [49], the administration of sirolimus in LDLT decreases liver regeneration in patients [50]. Indeed, sirolimus decreases liver injury in patients only in combination with cyclosporine [51]. Similarly, angiotensin II receptor type 2 antagonist does not reduce hepatic injury as opposed to the benefits obtained in preclinical studies of PH under vascular occlusion [13, 52]. By contrast, pharmacological treatment with omega-3 had benefits on hepatic injury in clinical LDLT and in preclinical studies after PH under vascular occlusion [53, 54]. In our view, these controversial results may be explained at least partially, by the differences in the mechanism responsible of I/R damage and liver regenerative failure dependently on the surgical procedure (LDLT versus PH + I/R). Of note, it would be extremely useful to make a clear distinction between the mechanisms for each surgical situation to design therapies that demonstrate its effectiveness under experimental conditions similar to what happens in clinical practice [12]. This will probably lead to translation of the best strategies to clinical practice in the short term [12].

| Drug                                      | Ischemic time | Effect                |
|-------------------------------------------|---------------|-----------------------|
| Sirolimus                                 | Not reported  | ↓ Liver regeneration  |
| Sirolimus + cyclosporine                  |               | ↓ Hepatic injury      |
| Angiotensin II receptor type 2 antagonist |               | ↑ Hepatic injury      |
| Omega 3                                   |               | ↓ Hepatic injury; ↑ liver regeneration |

Table 3. Pharmacological strategies used in living donor liver transplantation.
5. Conclusions

Although our knowledge about the mechanisms involved in the development of liver I/R injury and regenerative failure has significantly improved, and it has consequently been accompanied by a long list of potential therapeutic alternatives, I/R injury and regenerative failure after surgical procedure still represent a serious problem in the clinical practice. It should be considered that the mechanisms involved in hepatic I/R and regenerative failure very much depend on the experimental conditions used: which type of research is done, type of ischemia applied (warm or cold), period of ischemia (ranging from minutes to days), extension of hepatic ischemia (partial or total), graft subclinical situation (healthy, steatotic, aged,...), etc. Thus, new therapeutic strategies from experimental studies should be considered specific to the concrete experimental/surgical conditions used, and most probably, they cannot automatically be validated for all clinical situations requiring both vascular occlusion and liver regeneration [7]. We recognize the complication, but multidisciplinary research groups should devote additional efforts to better understand the cellular alterations and the crosstalk within the liver during the different clinical setting, requiring both vascular occlusion and liver regeneration to ultimately develop effectual therapeutic strategies aimed at reducing I/R damage and improving hepatic regeneration after liver surgery.

Acknowledgements

This research was supported by the Ministerio de Economía y Competitividad (project grant SAF-2015-64857-R) Madrid, Spain; the European Union (FondosFeder, “una manera de hacer Europa”); by CERCA Program/Generalitat de Catalunya; and by the Secretaria d’Universitats i Recerca (Grant 2017SGR-551) Barcelona, Spain. ME Cornide-Petronio has a Sara Borrell contract from the Instituto de Salud Carlos III (Grant CD15/00129), Madrid, Spain. MB Jiménez-Castro has a contract from the Programa de Promoción del talento y su empleabilidad from the Ministerio de Economía y Competitividad (Grant EMP-TU-2015-4167), Madrid, Spain. J Gracia-Sancho received continuous funding from the Instituto de Salud Carlos III (currently FIS PI17/00012) & the CIBEREHD, from Ministerio de Ciencia, Innovación y Universidades.

Conflict of interest

The authors declare that they have no conflict of interest.
Hepatic Regeneration Under Warm or Cold Ischemia Conditions: Controversies…
DOI: http://dx.doi.org/10.5772/intechopen.80340

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