Ischemic pre- and post-conditioning: current clinical applications

Pré et post conditionnement ischémique : applications cliniques actuelles

R. Thuret\textsuperscript{a,b,*}, T. Saint Yves\textsuperscript{a,c}, X. Tillou\textsuperscript{d}, N. Chataure\textsuperscript{a,e}, R. Thuillier\textsuperscript{a,e}, B. Barrou\textsuperscript{a,f}, C. Billault\textsuperscript{a,f}

\textsuperscript{a}Inserm U1082; université de Poitiers, faculté de Médecine et Pharmacie, 86000 Poitiers, France
\textsuperscript{b}Service d’Urologie et de Transplantation rénale, hôpital Lapeyronie, 34295 Montpellier, France
\textsuperscript{c}Service d’Urologie, CHU de Poitiers La Miletrie, 86021 Poitiers, France
\textsuperscript{d}Service d’Urologie, CHU Cote-de-Nacre, 14000 Caen, France
\textsuperscript{e}CHU de Poitiers, Département de Biochimie, 86000 Poitiers, France
\textsuperscript{f}Service d’Urologie et de Transplantation rénale, hôpital de la Pitié-Salpêtrière, 75013 Paris, France

Summary
Ischemic conditioning is a phenomenon through which short sequences of ischemia-reperfusion applied to an organ confer some degree of protection towards future ischemic insults. This phenomenon was first observed in the mid-1980s in cardiac surgery, and has been since widely studied in different settings. Different sort of ischemic conditioning exist: local vs remote, direct or pharmacological, and with different timeframes of protection. Ischemic conditioning seems especially suited to applications in transplantation since schedules of both cold and warm ischemia, as well as reperfusion, are carefully and easily controlled, and the benefits of protecting fragile organs against ischemia-reperfusion injuries might help widen the pool of possible grafts and ensure better graft function and survival. The pathways through which ischemic conditioning work are many, offering both preservation of cell energy, protection against oxidative stress, better blood flow to organs and protection against apoptosis. In the field of pharmacological conditioning, which tries to mimic the protective effects of traditional ischemic conditioning without the potential side-effects associated with vessel clamping, many common-use drugs

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*Corresponding author.
E-mail adress: rodolphethuret@gmail.com (R. Thuret).
Introduction

Organ transplantation face a major challenge in the discrepancy between the number of potential recipients and the number of potential donor, and this challenge has been addressed by widening the scope of potential organ donors to so-called “extended-criteria donors” (ECD). These include donors with co-morbidities potentially detrimental to the graft function, such as history of hypertension, vascular disease or diabetes in kidney transplantation, but also non-heart-beating donors (NHBD). Organs from these donors are more fragile than those from “standard-criteria donors” (SCD) and thus additional care has to be taken in preserving these organs, so as to minimize the risk of graft dysfunction or failure. Protective measures dealing with organ harvesting techniques, organ preservation and preservation solutions have been described previously in this work. However, another field, already used in human clinic in cardiovascular surgery settings and under evaluation in organ transplantation in both animal models and human clinic shows promising perspectives in organ protection.

Ischemic pre-conditioning (IPC) was first described in the context of heart surgery in the mid-1980s [1]. IPC is a phenomenon by which sequenced short ischemic periods followed by reperfusion confer protection against further ischemic insult to the organ. Studies have shown that this phenomenon is not limited to the heart but also takes place in the kidney, liver, brain and small intestine, and covers different mechanisms and pathways. Time between IPC and ischemic insult should first be taken into consideration: one can then described classic IPC (C-IPC), which typically confers a potent protection against further ischemia but is limited in time, usually 2 to 4 hours after initiation of the procedure [1], and the so called “second window of protection” (SWOP); this SWOP happens around 24 hours after the initial IPC procedure but offers more moderate protection. Site of the IPC procedure must also be taken into account, and has led to the description of both local IPC (LIPC) in which the organ vessels are directly clamped and remote IPC (RIPC) where the organ protection is secondary to vessel clamping in a different area [2].

While relatively easy to implement in a controlled, surgical setting such as transplantation or cardiac surgery, IPC is not well-suited to emergency settings, as the onset of myocardiac or brain infarction cannot be anticipated. Therefore of interest is the phenomenon of ischemic post-conditioning (IPoC), which was described subsequently to IPC. Sequential clamping and de-clamping of organ vessels after the ischemic insult can also confer some degree of protection, or higher repair potential to organs. Both local (LIPoC) and remote (RIPoC) IPoC have been described.

Direct clamping of vessels may not always be easily performed or may convey collateral risk to the recipient of surgery. Moreover, ischemic injury may also happen outside of
Local Ischemic Preconditioning: Mechanisms

IPC reduces ischemic preconditioning confers protection towards ischemia-reperfusion injury via multiple molecules and pathways. The commonly described theory involves cell-surface receptors to molecules such as adenosine, bradykinin, endothelin and opioids that after binding to their cell receptors trigger intra-cellular signalling pathways such as protein kinase C, MAP-Kinase, heat shock factor -1, NF-κ B [3]. IPC reduces the energy demand in cells during ischemia [4]. By maintaining high levels of ATP in cells, glycolysis levels are lowered, and Na⁺ / K⁺ ATPase pump function is preserved. When Na⁺ / K⁺ ATPase pump fails, ionic transfer between cells and extra-cellular environment is disrupted, with Na⁺ influx in the extracellular space, cell swelling and eventually cell death. Acidobasic equilibrium is also better preserved. During IPC, mitochondrial K<sub>ATP</sub> channels remain open, which helps reduce intra-mitochondrial calcium accumulation. IPC also induces up-regulation of transcription factors NF-κB, AP-1 and genes c-fos and jun. These confer a protective phenotype towards hypoxia, in part via increase of heat shock protein production.

IPC also confers protection against oxidative stress. IPC has been shown to reduce the conversion of xanthine dehydrogenase into xanthine oxidase, which in turn is responsible for production of reactive oxygen species deleterious to cells during the reperfusion phase. This phenomenon was observed in rat livers, mouse muscle and mouse intestine. In liver, IPC reduces the activation of Kupfer cells. IPC also decreases apoptotic cell death by lowering levels of TNF-α and modulating the caspase-dependant pathway. Protection against apoptotic cell death also happens via activation of the mitochondrial K<sub>ATP</sub> channels and inhibition of the mitochondrial permeability transition pore. On a larger level, IPC reduces arteriolar vasospasm and thus ensures better blood flow through capillaries during reperfusion. NO and eicosanoid levels are increased after IPC, which induce vasodilatation.

Remote Ischemic Preconditioning: Mechanisms

Remote ischemic preconditioning is a method through which brief cycles of ischemia-reperfusion in one organ is thought to confer protection against sustained ischemia in other organs. Mechanisms through which RIPC confers protection against subsequent ischemia are much less understood than those implicated in LIPC. Biochemical messengers released in the circulation are thought to be in part responsible for the protective effects [5]. The effects of RIPC were first demonstrated on the myocardium, with IPC sites as diverse as the hind limb, mesenteric artery, gut arteries or kidney artery. Effects were then investigated in other territories; for example, IPC of the hepatic or coronary artery has been shown to provide protection in kidneys or on stomach. RIPC has been shown to work even in organs that had become tolerant to IPC after multiple short-spaced ischemia-reperfusion cycles. The involvement of circulating molecules produced by the organ subjected to ischemia/reperfusion cycles has been promoted by studies in which effluents from hearts subjected to IPC were secondarily transferred to recipient hearts which then exhibited protection against a new ischemic insult. Opioids and K<sub>ATP</sub> channels were implicated in this transfer. Adenosin, NO, TNF-α, bradykinin, proteine kinase C, CGRP, capsaicin, heat shock proteins are also involved in RIPC. These molecules are differently implicated in early- and late-response RIPC. Both neurogenic and humoral pathways are implicated in RIPC, with some overlap between the two, but the importance of each one of these at given time points and in given organs in RIPC is unclear. The role of NO in RIPC is better understood, with induction of iNOS in remote organs after ischemic conditioning. When blocking NO activity or iNOS, RIPC protective effect was lost. Induction of iNOS is most probably through production of NF-κ B at the site of ischemic preconditioning, which is secondarily released in the circulation. Adenosin, which is both a trigger and a mediator of LIPC has also been shown to be implicated in RIPC, especially in RIPC of the heart and skeletal muscles, probably through its effects on K<sub>ATP</sub> channels. Different receptors to adenosin exist in different tissues, with probably different effector pathways in RIPC. Similarly, molecules whose roles have been defined in LIPC are probably also effective in RIPC, through different pathways.

Pharmacological conditioning

The concept of pharmacological preconditioning relies on applying medications that would mimic the protective mechanisms of IPC in humans. Many drugs have been tested in different settings and different organs, including the liver and the kidney. In kidney transplantation, no human studies have been so far performed. However, efficacy of pharmacological preconditioning has been shown with a number of molecules in animal models. Among those, erythropoietin has been shown to provide protection against ischemia-reperfusion injuries in a rat kidney transplantation model [6]. Glutamine [7], as well as sildenafil [8] or Xenon [9] have also been used in animal models. Several anesthetics such as sevoflurane [10] have also been shown to confer some degree of protection akin to IPC effects.

Preclinical studies

Small Animal Models

Kidney

Most of the studies of ischemic conditioning have been performed in small rodents, and have shown a protective effect of ischemic conditioning in its various forms on a large variety of organs. Torras et al first described the optimal IPC sequence in mouse in 2002 [11]: a 1-cycle schedule of 15-min ischemia followed by 10-min reperfusion. However, there is some great disparity in the protocols applied in most...
animal studies. A meta-analysis of the effect of IPC on animal kidneys was published recently by Wever et al [12]. Fifty-eight articles were finally included in this meta-analysis. There was a large variation between articles in terms of pre-conditioning strategy, including the study of C-IPC, SWOP or both, the timeframes between IPC and ischemic insult, and the type of IPC stimulus (unique or multiple sequence of ischemia-reperfusion). Both LIPC and RIPC were also evaluated, depending on the study. Ninety percent of studies were conducted on small rodents (mice or rats in eighty-three percent of cases). The overall results of these studies were that IPC significantly reduced serum creatinine, and that IPC maintained a beneficial effects when adjusting subgroups for IPC timing, with SWOP conferring a better protection than C-IPC or animal species, with better results observed in mice rather than rats. There were no differences in effects between LIPC and RIPC or any combination of the two, or in fractioned vs continuous IPC. IPC was also associated with lower BUN; in this sub-analysis, there was only a difference in results when comparing mice to rats, with mice having better results. IPC also proved to be effective when analysing histological lesions in kidneys; once again, this was true in all studied subgroups. While the studies reviewed in this meta-analysis seem to present a very positive effect of IPC, it is important to remember the high heterogeneity between the studies.

Liver

IPC in liver transplantation has also been extensively studied in mouse and rat models. Once more, IPC protocols vary between studies, with 5 to 10 min of ischemia followed by 10 to 15 min of reperfusion. This has been tested in cold and warm ischemia models, and IPC was associated with less liver damage and an improved survival [13-15]. In a transplantation setting, IPC has also been shown to improve graft survival in a rat model of orthotopic liver transplantation [16].

Large Animal Models

Following the good results of IPC protocols in small rodents, several groups studied the effects of IPC on kidneys in large animal models. However, results are much less stellar in these settings, and the beneficial effects of IPC on kidney function, both in transplantation and kidney surgery, are still unproven.

One of the first studies of IPC in large animals was conducted by Behrens et al in 2000 [17]. Pig kidneys were submitted to right laparoscopic nephrectomy followed by 60-minute clamping of the left kidney vessels, preceded by three 10 min clamping / 10 minute reperfusion of the left kidney vessels sequence. The 60-min ischemic period was then followed by 8-hour reperfusion, after which the pigs were sacrificed. IPC did not induce protection of the kidney evaluated by inulin clearance and histological dam- age. Orvieto et al showed similar results [18], irrespective of IPC protocol (sequential clamping-reperfusion sequences ranging from 25 to 60 minutes in total, followed by 90-minute ischemia). With a single 5-min clamping / 5-min reperfusion sequence followed by 60-minute ischemia in a similar porcine model, Hernandez et al also showed no improvement in renal function in the IPC group, as evaluated by serum creatinine levels and histopathologic findings [19].

IPC effects on renal function has also been evaluated in a canine model. Kosieradzki et al evaluated both C-IPC, SWOP and pharmacological IPC through the use of dipyridamole in an in situ ischemia/reperfusion (I/R) model, a transplantation model, and also studied the effect of IPC on isolated renal tubules [20]. In the in situ I/R model, C-IPC protocol was a 10 - min ischemia / 10-min reperfusion sequence followed by 45-min ischemia and 4-hour reperfusion, SWOP protocol was identical with a 24-hour de- lay between IPC and ischemic insult; in the transplantation model, kidneys were preconditioned with two 8-min ischemia / 5-min reperfusion cycle prior to organ retrieval; pharmacological protocol was direct infusion in the renal artery for 10 minutes followed by 45-min ischemia and 4-hour reperfusion. In the transplantation model, kidneys were then retrieved and stored for 24 hours in University of Wisconsin (UW) solution at 4 °C before transplantation in another animal. In the isolated tubules study, tubules were collected from freshly retrieved kidneys submitted to C-IPC and then stored in UW solution at 4 °C for 24 hours, followed by 1 hour rewarming. Animal studies failed to show any positive effect of either C-IPC or SWOP in whole-animal experiments (in situ I/R and trans- plantation models). However, IPC had a positive effect on cell viability in the isolated tubules model, indicating that other factors may cancel the IPC effect in whole-organ models.

Human Applications

While IPC seems to be a promising path to preserve organs submitted to ischemic injury, whether in emergency situations such as myocardial infarction or strokes or in a planned surgical settings, human applications of IPC have not always been as successful as preliminary animal studies would have led people to expect. First of all, IPC in animal experiments require precise timing of the conditioning itself and then of the ischemic insult. This is obviously not applicable in emergency situation. In scheduled surgical situations, it is far easier to integrate precise timing for conditioning and subsequent ischemia. Transplantation would be the favored field for such an application, as every step between organ procurement and final reperfusion of the organ is precisely controlled, and organs from ECD would greatly benefit from the added protection IPC would confer. Selzner et al recently published a review of IPC, IPOc and pharmacological conditioning in kidney and liver transplantation [3]. However, this study shows that while good results are readily achieved in animal models, and especially small animals, the results are not as consistent as one could have hoped in human practice.

Heart and Lung

IPC was first described in animal models applied to myocardial infarction, and heart surgery is probably the field in which most applications could be found: indeed, cardiac surgery aims to reduce ischemia-reperfusion injury, in cardio-aortic
bypass surgery predominantly, but is also in itself cause for ischemia-reperfusion injuries. Cardioplegia techniques, as well as several different anaesthetics protocols described in animal models, have been developed to minimize ischemia-reperfusion injury. Ischemic conditioning seems uncommonly suited to this goal. However, preconditioning, and per-conditioning are suited to scheduled surgery, but not to emergency situations such as represented by acute myocardial infarction. While many studies of IPC, RIPC and pharmacological IPC have been performed in humans, they have met with mixed results, and larger, multicentric trials are ongoing which may yield clearer results [21]. In the setting of acute myocardial infarction, postconditioning seems like a more viable option than IPC; three factors have to be taken into account: the delay after which the first ischemia-reperfusion cycle is performed, the duration and number of cycles, the duration of reperfusion between each cycle. These factors vary on different animal models, according in part to animal species, and have not been clearly defined in human surgery. Proof of the clinical relevance of IPC in acute myocardial infarction was first published in 2005, and further strengthened in retrospective studies. However, these effects have been described in coronary arteries dilatation and stenting procedures; whether the same effects could be applied through pharmacological IPC in myocardial infarction treated with fibrinolysis is still unclear [22].

Liver

In view of satisfactory results observed in IPC trials in liver transplantation in rats, several human studies of IPC in liver transplantation have been set up. However, results were nowhere near as favourable as in animal models. While IPC seem to confer some protection to the remaining parenchyma in patients undergoing liver resection [23-24], studies in transplantation did not show similar effects. Koneru et al reported no reduction of the severity of ischemia-reperfusion injury in liver submitted to a 5-min ischemia/5-min reperfusion cycle [25]. Azoulay et al used a 10-min ischemia/10-min reperfusion cycle, and while they showed a reduction of serum transaminase in the IPC group, there was no impact on graft or patient survival and IPC was found to be the only variable significantly associated with early graft dysfunction [26]. Andreani et al investigated the effect of IPC in living donor liver transplantation and could not find any advantage in the IPC group in terms of ischemic injury, primary graft non-function, acute cellular rejection, morbidity and mortality [27]. A meta-analysis of IPC in human liver transplantation published in 2008 [28] could not find any difference in terms on mortality, initial poor function, primary graft non-function, retransplantation rate, ICU stay length and duration of hospital stay between IPC and non-IPC groups.

Kidney

To our knowledge no study of IPC in kidney transplantation has been conducted in humans. Data relating to RIPC effects on kidneys in humans are mixed. Zimmerman et al showed that three cycle of 5-min ischemia / 5-min reperfusion of the lower extremity in patients undergoing cardiopulmonary bypass-assisted cardiac surgery prevented the onset of acute kidney injury [29]. Walsh et al failed to show similar clinical effects in patients undergoing endovascular aneurysm repair, while showing a reduction of urinary markers of renal injury in preconditioned patients; the authors speculate that the small size of the study may explain this lack of clinical translation of IPC effect [30]. However, both Choi et al and Pedersen et al failed to show a protective effect of RIPC on kidneys in cardiac surgery [31-32]. In a different context, RIPC has been successfully employed to mitigate contrast medium-induced kidney injury [33-34].

Conclusion

Ischemic conditioning under its different forms provides a stimulating field of research for improving organ quality, by enhancing protection against ischemia-reperfusion injury in IPC and by promoting organ repair in IPoC. Transplantation seems like the ideal setting as both timed IPC intervention in the donor and controlled IPoC in the recipient are easily feasible. However, initial enthusiasm with results in small animal models must be tempered as there is at the moment few human clinical application outside of heart surgery. Studies in human liver transplantation failed to show a positive effect of IPC, and renal effect of LIPC is uncertain in large animal models commonly used as the closest surrogates to human transplantation. Studies of the effect of RIPC on renal function outside of the transplantation field have also been inconclusive. Further evaluation is thus warranted to determine if the extra-cardiac effects of ischemic conditioning observed in small rodents can be successfully translated to human practice.

Disclosure of interest

The authors have no conflicts of interest to declare in relation to this review.

References

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