

# The effect of ischemic preconditioning and postconditioning on testicular torsion-detorsion injury

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## Abstract

The primary pathophysiological event in testicular torsion is ischemia-reperfusion (I-R) injury, which is caused by the twisted spermatic cord and its release. Moreover, testicular torsion is most likely mediated by reactive oxygen species. Ischemic preconditioning (IPreC) is a phenomenon in which a prior ischemic stress renders the organ resistant to a subsequent ischemic insult. Ischemic postconditioning (IPostC) is defined as rapid intermittent interruptions of blood flow at the early phase of reperfusion, which mechanically alters reperfusion hydrodynamics. IPreC as well as IPostC provide powerful endogenous protection in many tissues against I-R injury. In this review, we explain the possible mechanisms involved in testicular I-R due to torsion-detorsion and aspects of IPreC and IPostC in the testis. IPreC is clinically feasible only when ischemia is predictable. However, unlike ischemia, reperfusion tends to have a more predictable onset. IPostC is a simple, harmless method that provides a new tool to protect organs from testicular I-R injury.

**Keywords:** Testicular torsion; Ischemia-reperfusion; Ischemic preconditioning; Ischemic postconditioning

## 1. Introduction

A child or adolescent with symptoms including acute scrotal pain, tenderness, or swelling should be looked upon as an emergency situation requiring prompt evaluation, differential diagnosis, and potentially immediate surgical exploration. One of the main causes of an acute scrotum in children and adolescent is testicular torsion. The occurrence of testicular torsion has been estimated to be as high as 1 in 4000 males by the age of 25, and has been implicated in testicular injury and infertility [1]. Testicular torsion requires an emergency operation to avoid loss of germ cells and ischemic testis [2]. The surgical procedure can lead to orchectomy of the ipsilateral torqued testis, and infertility of the contralateral (not torqued) testis [3]. Usually, the first signs of testicular necrosis appear within 6-12 h of the testicular torsion crisis, and thus a delay in diagnosis and treatment can result in lower ability of spermatogenesis, or infertility. Despite improvement in early diagnosis of testicular torsion and changes in clinical management (i.e. earlier surgical intervention) infertility remains one of the main consequences [4,5]. The degree of fertility loss in an individual with testicular torsion depends on the extent of ischemia and the subsequent damage to the contralateral testis

[6-8]. The primary pathophysiological event in testicular torsion is ischemia-reperfusion (I-R) injury caused by the twisted spermatic cord and its release. As well, testicular torsion is most likely mediated by reactive oxygen species (ROS) [9]. The DNA of germ cells is particularly sensitive to oxidative damage [10]. In addition, torsion-detorsion has been associated with decreased spermatogenesis, alteration of the production of various hormones, and induction of infertility [11-13].

## 2. Unilateral testicular torsion and reactive oxygen species (ROS)

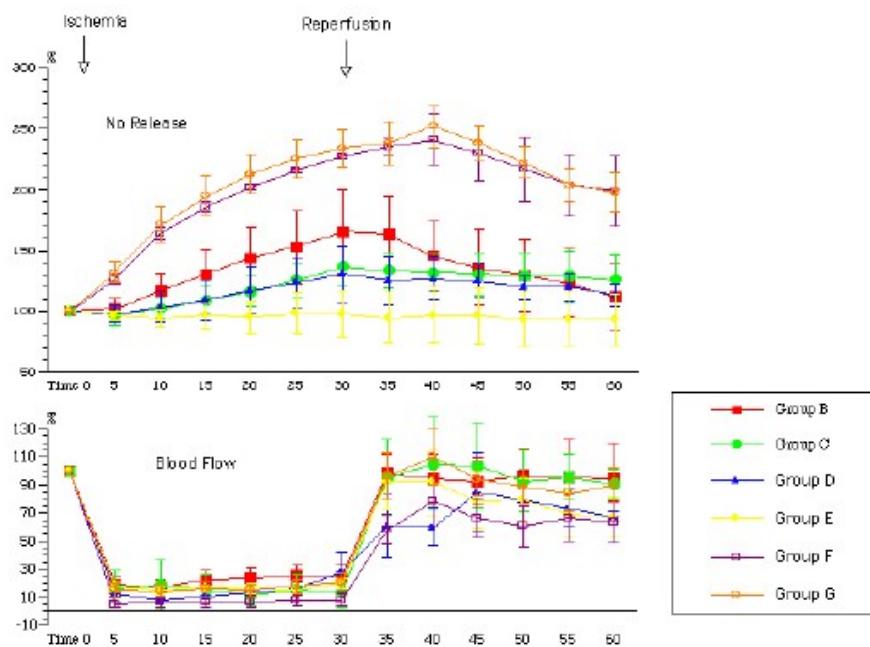
I-R injury in the testis is related to abnormal signal transduction due to testicular torsion-detorsion and is associated with ROS over generation [14]. I-R also contributes to cellular dysfunction and initiates the apoptosis/necrosis cascade with subsequent inflammatory infiltration. Reperfusion injury is an integrated response to blood flow restoration after ischemia. Also, reperfusion injury is initiated at the early moments of reperfusion, which can potentially last for several days [15]. Neutrophils which recruit to the testes after torsion-detorsion are potent generators of ROS [16]. I-R injury induces the production of numerous toxic substances in the microcirculation of various types of tissue. In

addition, vascular endothelial cell injury and microcirculation disorders can occur during reperfusion, and can induce organ derangement. The production of free radicals such as ROS or nitric oxide (NO) are associated with factors that can cause I-R injury. The ROS derive from mature granulocytes can adhere to the vascular endothelium [17,18]. The crucial role of ROS in the induction of germ cell death has been demonstrated by the use of antioxidants to scavenge ROS prior to testis reperfusion [19]. Enzymatic antioxidant defense systems, such as superoxide dismutase, catalase and glutathione peroxidase, protect tissues from ROS [3]. Furthermore, antioxidant pretreatment of experimental animals with superoxide dismutase, catalase, imvastatin [20], and melatonin [21] are effective in preventing testicular reperfusion injury [22].

### **3. Reports in the effect of other drug against testicular torsion-detorsion injury**

There are reports describing the effects of protective agents against testicular torsion-detorsion injury. Sarica and coworkers reported that a calcium-channel blocker, verapamil, prevented unilateral testicular torsion injury [23]. Moreover, Sarioglu-Buke and colleagues

reported that capsaicin effectively prevented apoptosis in the both ipsilateral and contralateral testis after unilateral testicular torsion [24]. Subsequently, effects of dexamethasone (glucocorticoid class of steroid) molsidomine (vasodilator and NO donor) selenium and sildenafil (phosphodiesterase type 5 inhibitor) against testicular torsion-detorsion injury have been reported [7,25,26]. Previously, our laboratory has shown that L-arginine significantly meliorated I-R induced injury in the testis, suggesting that NO has a cytoprotective effect against I-R injury in the testis [27]. Figure 1 shows a real time monitoring of NO and blood flow in the ipsilateral testis during I-R. Clamping of the left testicular artery in the rat resulted in a decreased blood flow of 5-20% compared to the basal level measured before clamping. After removal of the clip, the blood flow recovered from 50 to 100% of the basal level within one minute in all groups. Immediately following clamping of the testicular artery, NO release rapidly increased and plateaued within approximately 30 min. When a NO electrode was inserted into the rat testis treated with 10, 30 and 100 mg/kg i.p. of L-NAME, a non-selective inhibitor of nitric oxide synthase, NO release was decreased in a dose-dependent manner.



**Figure 1.** Blood flow and NO release during ischemia-reperfusion (I-R) in the testis. B: 30 min ischemia-30 min reperfusion (I-R) rats, C: I-R rats treated with L-NAME (10 mg/kg i.p.), D: I-R rats treated with L-NAME (30 mg/kg i.p.), E: I-R rats treated with L-NAME (100 mg/kg i.p.), F: I-R rats treated with L-Arg (10 mg/kg i.p.), G: I-R rats treated with L-Arg (30 mg/kg i.p.). Blood flow and NO release were expressed as % of the basal level. Data are shown as mean  $\pm$  SEM of six to eight separated determinations in each group. L-Arg: L-arginine [27].

Groups C, D and E correspond to 10, 30 and 100 mg/kg, respectively. In contrast to treatment with L-NAME, L-arginine treatment (10 or 30 mg/kg i.p.) significantly increased NO release in a dose-dependent manner (groups F or G). After removing the clip, NO release treated with L-NAME returned almost to basal levels whereas NO release treated with L-arginine (10 or 30 mg/kg i.p.) remained significantly higher than basal levels during these 30 minutes. Tamamura and coworkers reported that edaravone, a free radical scavenger, significantly ameliorated I-R induced testicular damage by reducing the oxidative stress (see Table 1) [13]. Figure 2 shows histological data. Extensive cell swelling, tubular vacuolation, necrosis, and loss of germ cell maturation were observed in the I-R(0) and I-R(1) groups. In contrast, high-dose treatment with edaravone (I-R(10) group) significantly reduced these I-R-induced changes. Tsounapi and coworkers reported that sivelestat, a neutrophil elastase inhibitor, directly inhibits the accumulation and activation of neutrophils. Additionally, sivelestat offers efficient protection against the production of oxygen radicals and cytokines not only in the ipsilateral but also in the contralateral testis (Table 2) [28].

#### **4. The influence of unilateral torsion-detorsion injury against contralateral testis**

Prolonged unilateral testicular torsion with more than 4 h of 720 degree unilateral

testicular torsion is enough to create tissue injury in both ipsilateral and contralateral testes in experimental animal models [29,30]. Also, prolonged unilateral testicular torsion is not followed by a full recovery of blood flow in the ipsilateral testis 24 h after detorsion [31-33]. Although many studies suggest that the contralateral non-torted testis is damaged after unilateral testicular torsion [33-36], some studies have shown that this phenomenon is not always observed [31]. The physiological and biochemical mechanisms of contralateral testicular injury (including the role of ROS on such damage) are unclear [37]. Some authors have reported that decreased testicular blood flow and subsequent tissue hypoxia contribute to contralateral testicular injury after testicular torsion-detorsion [38]. Kizillican and colleagues showed that a short period of unilateral torsion (10 min) produced a decrease in contralateral blood flow [39]. Furthermore, Tanyel and coworkers reported that the contralateral testis could be damaged by a reflexive decrease in blood flow from the activated sympathetic system [39]. However, our previous report strongly disagreed with this hypothesis because in the contralateral testis the testicular blood flow did not show any variation during the ischemia period or the initial two hours of reperfusion of ipsilateral testis utilizing laser Doppler flowmeter [28].

**Table 1.** MDA, 8-OHdG and MPO levels in the testis [13]

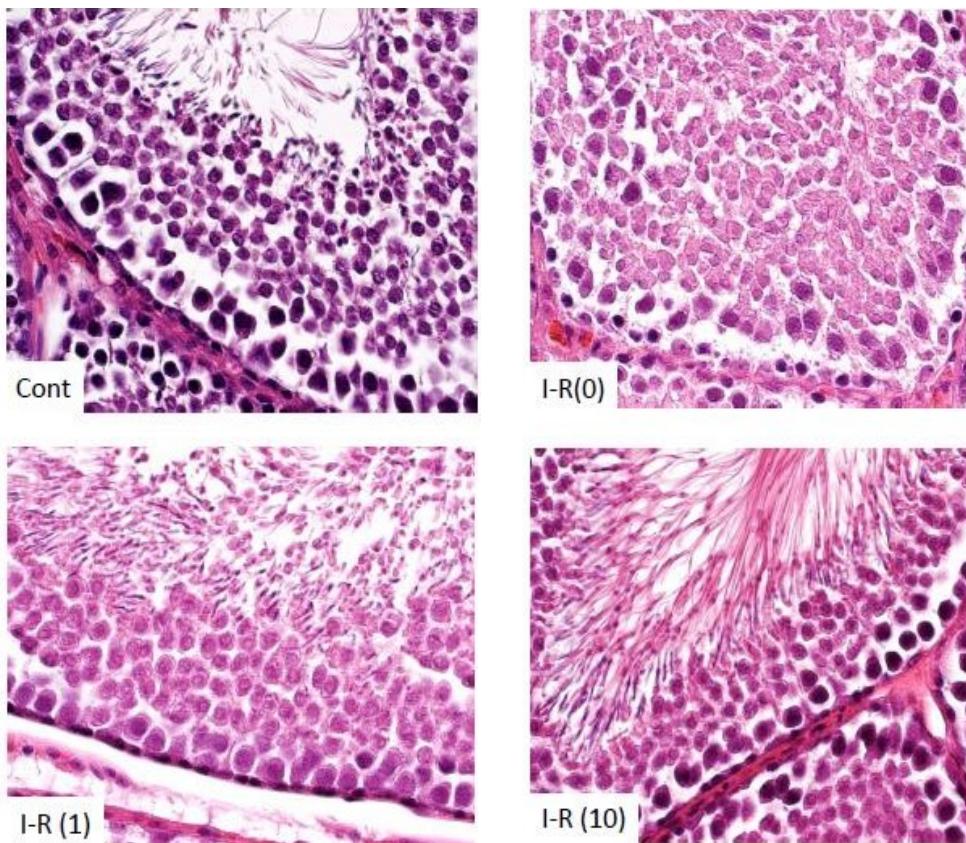
	<b>MDA (nmol/mg protein)</b>	<b>8-OHdG (pg/µg DNA)</b>	<b>MPO (ng/mg protein)</b>
Cont	1.02 ± 0.09	4.84 ± 0.46	0.57 ± 0.04
I-R(0)	2.30 ± 0.35 *	6.74 ± 0.23 *	8.53 ± 2.69 *
I-R(1)	1.71 ± 0.39	4.96 ± 0.72	5.43 ± 2.76 †
I-R(10)	1.46 ± 0.20	5.15 ± 0.34	4.09 ± 1.06 †

A sham-operated control group administered edaravone at 10 mg/kg i.p. (Cont group); a group administered vehicle and then subjected to induction of 30-min ischemia and 60-min reperfusion (I-R(0) group); and groups administered edaravone at 1 or 10 mg/kg i.p. and then subjected to 30-min ischemia and 60-min reperfusion (I-R(1) and I-R(10) groups, respectively). Sixty min prior to induction of ischemia, edaravone was administrated intraperitoneally.

Data are shown as mean ± SEM of five separate determinations in each group. MDA and MPO concentrations are normalized with protein content and 8-OHdG is normalized with DNA contents, respectively.

\* significantly different from Cont and I-R(10) group. (p<0.05)

† significantly different from Cont group. (p<0.05)



**Figure 2.** Histological changes evaluated by hematoxylin-eosin (HE) staining. A sham-operated control group administered edaravone at 10 mg/kg i.p. (Cont group); a group administered vehicle and then subjected to induction of 30-min ischemia and 60-min reperfusion (I-R(0) group); and groups administered edaravone at 1 or 10 mg/kg i.p. and then subjected to 30-min ischemia and 60-min reperfusion (I-R(1) and I-R(10) groups, respectively). Sixty min prior to induction of ischemia, edaravone was administrated intraperitoneally. Original magnification: x400 [13].

Due to the aforementioned reports, the influence of the contralateral testis following unilateral testicular I-R remains controversial. Other groups have reported the important role of immunological responses in pathogenesis [34,35]. Wallace and coworkers have suggested alternative explanations including the release of acrosomal enzymes or a neurohumoral mechanism and have also proposed the term “sympathetic orchiopathy” [36]. Moreover, Jeong and colleagues reported that puberty (ages 5 to 6 weeks) rats had more severe contralateral testicular damage than adult rats after testicular torsion because immunological, humoral and neural differentiation and maturation vary by age. After testicular torsion, immunological protection of puberty rats from contralateral testicular damage such as testicular torsion will decrease due to immature Sertoli cells [40]. It might be possible that the severity of contralateral damage caused by immunological activity is different from that of adult rats [21].

##### **5. Definition and mechanism of ischemic preconditioning**

Over two decades ago, Murry’s group reported that short cycles of ischemia and reperfusion before an occlusion of a coronary artery reduced infarct size, and this was called ischemic preconditioning (IPreC) [41]. Similarly, the concurrent protection was actually found in human due to the fact that IPreC causes adaptation to ischemia in all mammals [42]. IPreC limits the severity of I-R injury. Thus, after IPreC, the extent of the area of a subsequent infarction, I-R arrhythmias, and contractile dysfunction is reduced [43]. IPreC mechanisms of action have been extensively reviewed. Several features and pathways of this process are now clear but some elements still remain unknown. Many studies on distinct organ and species indicate the empirical knowledge about common or different mechanisms. In the literature there are inconsistencies related to differences among the animal species, tissue, and animal model.

**Table 2.** Measurement of testosterone and MDA levels, expression of HSP-70 and MPO activity in both ipsilateral and contralateral rat testis [28].

		<b>Control</b>	<b>I-R-A</b>	<b>I-R15-A</b>	<b>I-R60-A</b>	<b>I-R-B</b>	<b>I-R15-B</b>	<b>I-R60-B</b>
<b>T</b>	<b>RT</b>	17.80±10.22	-	-	-	1.30±0.75 <sup>a</sup>	2.16±1.73 <sup>a</sup>	4.70±4.05 <sup>a</sup>
	<b>LT</b>	17.55±10.8	-	-	-	1.01±0.47 <sup>a</sup>	2.81±1.42 <sup>a</sup>	0.49±0.28 <sup>a</sup>
<b>MDA</b>	<b>RT</b>	2.37±0.22	4.60±0.41 <sup>a</sup>	4.61±0.37 <sup>a</sup>	3.66±0.38 <sup>a</sup>	10.43±0.82 <sup>a,b</sup>	10.31±0.43 <sup>a,b</sup>	6.72±1.22 <sup>a</sup>
	<b>LT</b>	2.37±0.22	4.42±0.35 <sup>a</sup>	4.25±0.29 <sup>a</sup>	3.26±0.35 <sup>a</sup>	7.60±0.35 <sup>a,c</sup>	5.19±0.85 <sup>a,c</sup>	2.85±0.22 <sup>a,c</sup>
<b>HSP-70</b>	<b>RT</b>	2.52±0.27	9.92±1.26 <sup>a</sup>	9.38±1.17 <sup>a</sup>	11.81±1.10 <sup>a</sup>	11.34±0.79 <sup>a</sup>	15.52±1.07 <sup>a</sup>	14.19±0.83 <sup>a</sup>
	<b>LT</b>	2.52±0.27	10.93±1.11 <sup>a</sup>	8.00±1.18 <sup>a</sup>	10.85±1.16 <sup>a</sup>	13.06±1.23 <sup>a</sup>	12.16±0.62 <sup>a</sup>	12.16±0.77 <sup>a</sup>
<b>MPO</b>	<b>RT</b>	43.80±6.71	350.74±133.39 <sup>a</sup>	37.53±13.78 <sup>d</sup>	97.10±42.73	94.00±24.72 <sup>a</sup>	52.24±11.50	69.38±12.89
	<b>LT</b>	43.80±6.71	119.49±60.46	29.26±5.32	29.30±13.52	141.97±62.14	35.59±6.37	61.78±8.60

Through a right inguinal incision, the right spermatic cord was identified. The right testicular artery and vein were clamped for 90 minutes with a small Sugita standard aneurysm clip (Mizuho Ikakogyo, Tokyo, Japan) with a holding force of 145g. The animals were divided into sham operated control rats (control group; n=6), I-R animals (I-R group; n=12), I-R animals that received intra-abdominally sivelestat (15 mg/kg) (I-R15 group; n=12), and I-R animals that received intra-abdominally sivelestat (60 mg/kg) (I-R60 group; n=12). In subpopulations of I-R, I-R15, and I-R60 groups, after ischemia, reperfusion was performed for 2 h (I-R-A groups, n=6; I-R15-A, n=6; and I-R60-A, n=6, respectively) or 48 h (I-R-B, n=6; I-R15-B, n=6; and I-R60-B groups, n=6,respectively).

The data are shown as mean ± SEM. T: testosterone, RT: right testis, LT: left testis.

“a” significantly different from the control group ( $p<0.05$ ).

“b” significantly different from I-R60-B group ( $p<0.05$ ).

“c” significantly different ( $p<0.05$ ) from any of the other cells sharing the same superscript.

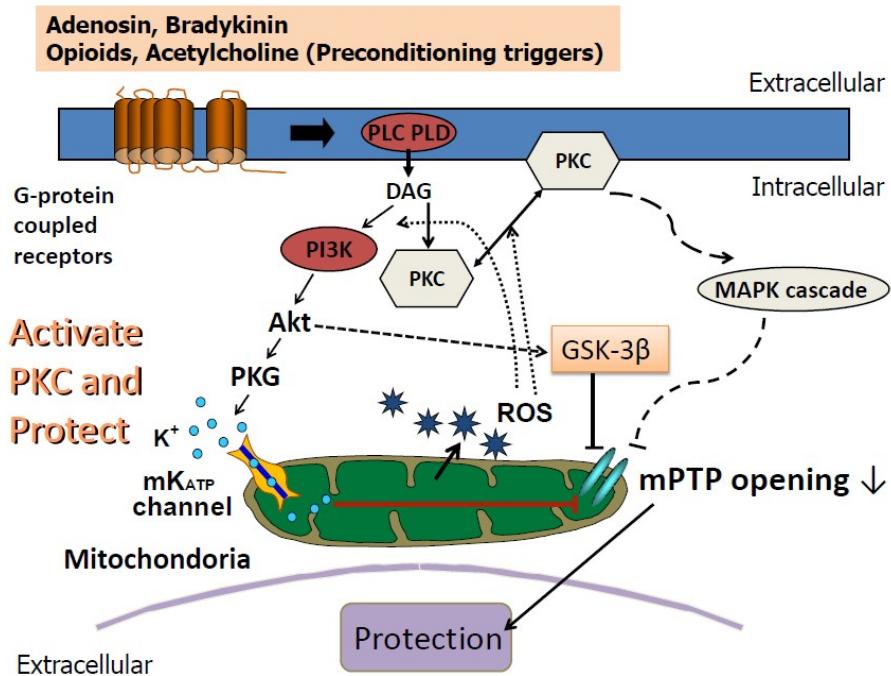
“d” significantly different from I-R-A group ( $p<0.05$ ).

Initial IPreC protocols in the heart were unwieldy as authors usually designed four, five or six short and alternative cycles of I-R. Although this procedure is very appealing, none of these studies have support for it or cannot be used to extrapolate from it to other organs. In recent years, one ischemia and one reperfusion window in IPreC protocol has been shown to have a protective effect in other organs, including the liver and the kidney [44-47]. The ischemia adaptation is biphasic, and consists of protection less than 2 h prior to the sustained ischemia (classic preconditioning or first window of protection) and a delayed time frame 24-72 h later (delayed preconditioning or second window of protection (SWOP)) [42]. IPreC can also be obtained by inducing ischemia on distant organs (remote preconditioning) or by pharmacological treatment prior to initial ischemia (pharmacological preconditioning) [48-53]. The benefit of IPreC relies on both infarct size reduction and functional improvement. In addition, IPreC improves endothelial function in both classic and delayed time, as a general effect as well as locally in the preconditioned organ [54-56]. Moreover, a lot of systemic stimuli by IPreC are evoked with similar protection such as substances acting on G-protein coupled receptors (adenosine and bradykinin), proinflammatory substances (lipopolysaccharide, tumor necrosis factor alpha and monophosphoryl lipid A), and changes in oxygen tension (anoxia, hypoxia and hyperoxia) [42]. In fact, autacoids, such as adenosine, bradykinin, opioids and platelet activating factor produce a response to the cycles of brief I-R. Autacoids also play a pivotal role in IPreC protection. Their receptors induce signal transduction pathways that ultimately block the opening of mitochondrial permeability transition pores (mPTP) during the reperfusion phase after the infracting ischemia [57,58]. Opening of mPTP can be coupled with a large amount of cell death since it completely disrupts mitochondrial function and releases ROS [59-63]. Cell death in preconditioned heart is less than that in non-preconditioned heart, which went identical ischemic insult. Thus, IPreC protection requires a complex signaling cascade, which includes the opening of sarcolemmal or mitochondrial ATP-sensitive potassium channels ( $\text{sarcK}_{\text{ATP}}$  or  $\text{mitoK}_{\text{ATP}}$ ) [64-69]. Both of these  $\text{K}_{\text{ATP}}$  channels are regulated by PKC. Moreover PKC is activated by nitric oxide, and also by oxygen free radicals generated after mitoK ATP channel opening [70]. Diazoxide, a mitoK<sub>ATP</sub> opener, pharmacologically preconditions the heart [64,66]. Also, nicorandil is a K<sub>ATP</sub> channel opener that activates the sarcK<sub>ATP</sub> and mitoK<sub>ATP</sub>

channels. These drugs can be given before or during ischemia to prevent I-R injury. These results confirmed that redox signaling is involved in other organs by PreC [71,72]. IPreC utilizes endogenous as well as distant mechanisms in skeletal muscle, liver, lung, kidney, intestine and brain in animal models to protect from I-R injury [73]. In addition, some groups have confirmed the efficacy of IPreC in cardiac surgery and percutaneous coronary interventions in humans [74]. Figure 3 shows possible mechanisms of IPreC in the cell.

## **6. Definition and mechanism of postconditioning**

Although it has been shown that IPreC can make ischemic myocardium resistant to I-R injury, the need for a pretreatment could be limited due to its clinical application. IPreC is of little practical use as the onset of an infarction is usually unpredictable. During late 1980s and 1990s researchers intensively investigated whether or not pharmacologically modified reperfusion could reduce reperfusion injury. Meanwhile, Zhao and coworkers demonstrated that several brief cycles of ischemia and reperfusion at the start of reperfusion after sustained ischemia reduced infarct size in dogs with an efficacy comparable to that of IPreC [75,76]. This procedure has been called postconditioning. Based on the knowledge of gradual reperfusion and IPreC, Vinten-Johansen's group introduced the ischemic postconditioning (IPostC) treatment against I-R injury [77]. In that study, the IPostC protocol was 30 s of reperfusion followed by 30 s of coronary occlusion, which were repeated for three cycles at the very onset of reperfusion. The IPostC concept consists of at least three factors including the delay after which the first re-occlusion is established, the duration and number of re-occlusions, and the duration of the interspersed reperfusion [78]. IPostC still reduced infarct size when the delay to the first re-occlusion ranged from 1 to 3 min. Many groups have reported that the IPostC were initiated within 1 min of reperfusion, and within this 1 min time frame no impact of a delay on infarct size reduction was noted [79-83]. Also, IPostC with increased cycles and/or extended duration of I-R within each cycle may impair the outcome of IPostC. Additional ischemia relative to the duration of the index ischemia may limit the reduction of the infarct size. IPostC using several cycles of 5 min reperfusion and 5 min ischemia did not improve the damaging effect of I-R injury [77,78]. The efficacy of these ischemic or pharmacological interventions occurring with a 5-10 min delay in reperfusion, resulted in markedly limited



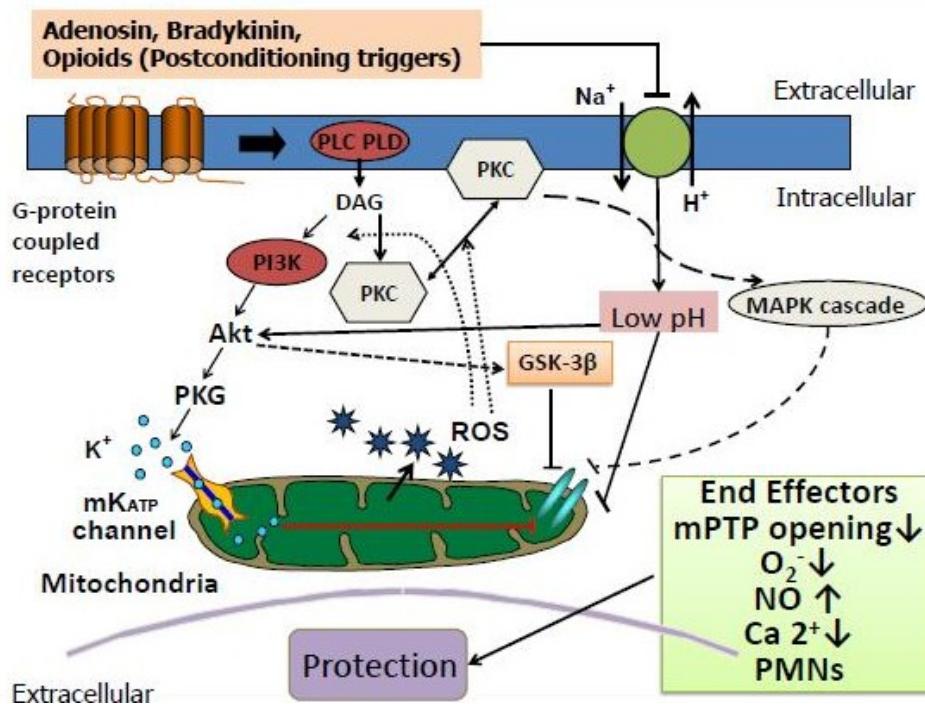
**Figure 3.** Possible signal transduction pathways of IPreC in the cell.

addition of cardioprotection to reperfusion per second. These mechanical interventions such as gradual reperfusion and IPostC, and several pharmacological interventions have been studied against reperfusion injury. Based on the knowledge acquired with the studies in IPreC, IPostC represents a promising tool to inhibit I-R injury. Recent studies demonstrate for the first time that IPostC can protect against endothelial I-R injury in humans [84,85]. Experimental studies suggest that IPostC is able to inhibit the detrimental effects of lethal I-R injury such as calcium overload, oxidative stress, rapid pH correction, endothelial dysfunction and inflammation. The main effects of IPostC-induced protection with respect to the preservation of mitochondrial integrity include reduced sensitivity to the increased intracellular calcium ( $Ca^{2+}$ ) levels triggered by I-R injury, restoration of redox balance and nitric oxide-mediated vasorelaxation [86]. Data from many research groups have provided evidence that IPostC is able to activate cell-surface receptors such as adenosine, bradykinin and opioids that lead to protective effects via signal transduction pathways and through signaling molecules, which thereafter recruit signal transduction pathways such as the PI3K-Akt, MEK1/2-Erk (extracellular-signal regulated kinase) 1/2, and

cGMP-PKG kinase cascades. The above signal transduction pathways are known to terminate on the mitochondria. Early acidosis and kinases such as GSK-3beta prevents mPTP opening [87-91]. In other words, the first few minutes of reperfusion represents a window of opportunity for triggering the many mediators, which in concert will lead to protection against reperfusion injury. Figure 4 shows the possible mechanisms of IPostC in the cell.

#### 7. Similarities and differences in cellular signal transduction between IPreC and IPostC

Although understanding the precise cellular signaling events is still in evolution, similarities exist between IPreC and IPostC despite the fundamental differences in the timing of protection. In particular, the first few minutes of reperfusion following the ischemia appear crucial to both IPreC and IPostC induced protection. Much evidence indicates that components of the so-called RISK (reperfusion injury salvage kinase) pathway play a role both in IPreC and in IPostC. IPreC and IPostC also reduce the oxidant-induced injury. Moreover, they attenuate the local inflammatory response to reperfusion [75,79,83]. IPreC and IPostC activate a signal transduction pathway involving the PI3K-Akt and Erk1/2, which has been termed the RISK pathway. The RISK pathway terminates with the inhibition of the met opening



**Figure 4.** Possible signal transduction pathways IPostC in the cell.

at reperfusion to afford protection in both IPreC and IPostC [87-91]. However, some differences between the pathways activated by IPreC and IPostC also exist [75]. It is likely that the ROS signaling involved in pre- and post-ischemic phases are not the same. Since exogenous ROS are able to induce IPreC but not IPostC, it is also likely that compartmentalization plays a pivotal role in the latter case. In particular, the concomitance of acidosis, NO formation, mPTP inhibition, and ROS generation seems mandatory in IPostC [43]. In addition, Yang and colleagues showed that Erk is involved in IPostC, but not in IPreC [81]. Some groups showed that a redox signaling may be involved in IPostC, but the ROS involved may not be the same of those involved in IPreC [92,93]. (see Figures 3 and 4)

#### 8. Pharmacological postconditioning

Although understanding of the mechanisms of IPostC has been unraveled completely, the possibility to induce IPostC pharmacologically would be arising. In fact, several studies are focused on the effects of protective drugs administered either before, or during the index ischemia or at the onset of reperfusion. Kin and coworkers provided strong evidence to support the involvement of some adenosine receptor subtypes in IPostC [94]. Hausenloy and Yellon reviewed that an

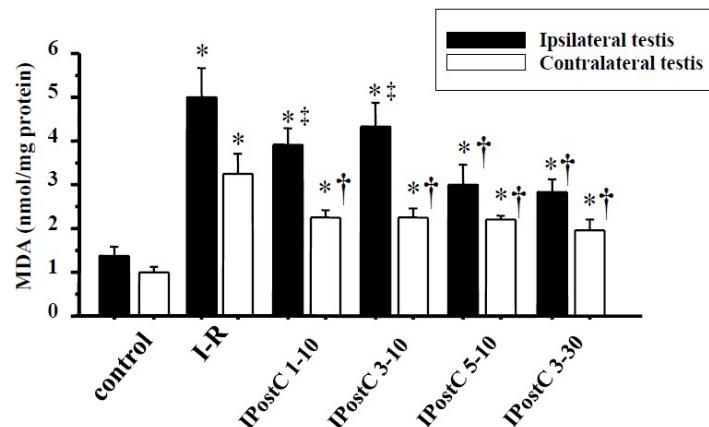
increasing number of agents including insulin, erythropoietin, adipocytokines, adenosine, volatile anesthetics natriuretic peptides and 'statins', when administered at the time of myocardial reperfusion, reduce myocardial infarct size through the activation of the RISK pathway [91]. Successful pharmacological IPostC in humans in the surgical setting has been reported by Jin and coworkers using adenosine given within 1 min of aortic cross-clamp removal [95]. Understanding the IPostC mechanisms may improve the protective effects of drug administration that is otherwise limited by systemic hemodynamic side effects or other undesirable side effects of these agents.

#### 9. IPreC and IPostC with respect to against the testicular I-R injury

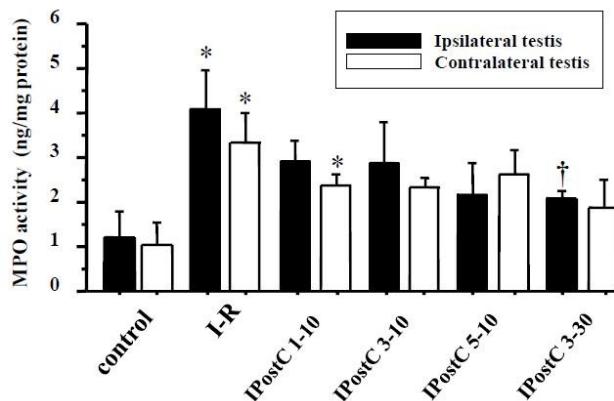
Similarly, some researchers reported the effects of IPreC on testicular I-R induced damage. Ceylan and colleagues noted no protective effects of IPreC in rat testes during 90 min of 720-degree torsion [96], while Sahinkanat and coworkers reported that IPreC protects testicular tissue [97]. Although some studies show that IPreC is effective, especially in the reperfusion phase in other tissue, these reports did not include observations in the

reperfusion phase. Akcera et al. reported that gradual detorsion has a tendency to decrease testicular I-R injury in the rat torsion-detorsion model [98]. Their results showed that the gradual detorsion (controlled reperfusion) of ischemic testis may be a method for preventing reperfusion injury. The data from that study supported our hypothesis that IPostC, a variation of controlled reperfusion requiring a similar surgical manipulation, presents protective effect against I-R injury in the testis. Recently, our study showed that IPreC and IPostC have protective effects against I-R induced biochemical and histological changes in rat testes including observations in the reperfusion [99]. However, IPreC is clinically feasible only when testicular torsion is predictable [73]. The concept of IPostC is attractive from a testicular surgical point of view. Interventions at the time of reperfusion are easy to perform when surgical repair of patients with testicular torsion. Many groups suggest that the early moments of reperfusion are important in the pathogenesis of post-ischemic injury and that early manipulation of reperfusion phase can reduce I-R related injury [76,100]. Recently, we have demonstrated that IPostC after unilateral testicular I-R injury has protective effect on ipsilateral and contralateral testes [101]. IPreC as well as IPostC inhibit production of ROS and neutrophil infiltration in the testis [102]. In a previous study from our laboratory, an IPostC protocol was applied (Figures 5 and 6), which consisted of five cycles of 10 s of reperfusion followed by 10 s of ischemia [99]. However, the optimal number and duration of the intermittent interruptions of blood flow during testicular IPostC remains unknown. It has been shown that among the different experimental protocols, IPostC 3-30

protocol (three cycles of 30 s reperfusion-30 s ischemia) presented the maximal protective effect against I-R-induced biochemical and histological alterations in both ipsilateral and contralateral testes. IPostC 3-30 protocol significantly inhibited these parameters. We reported that the IPostC 3-30 protocol significantly decreased the tissue levels of lipid peroxidation, neutrophil infiltration and apoptosis in both ipsilateral and contralateral testes caused by unilateral I-R injury. Figures 5 and 6 show data of MDA and MPO concentrations, respectively, in the experimental testis. However, it has not been reported whether the deleterious effects of I-R injury were attenuated or whether some beneficial mechanisms were triggered following testicular IPostC compensating the I-R injury-related damage. Further studies are needed to clarify the potential mechanisms responsible for the beneficial effect of IPostC in both ipsilateral and contralateral testes after unilateral testicular I-R injury. Although among the different IPostC protocols, the IPostC 3-30 protocol showed the maximal protective effects for bilateral testes. It is still unclear if this protocol offers optimal protective effects against testicular I-R injury (Figures 7-9). Extensive tubular vacuolation, necrosis and loss of maturation of germ cells were observed in the I-R group (Figure 8). IPostC 3-30 treatment dramatically reduced bilaterally the alterations observed in the I-R group. Apoptosis in bilateral testicular samples was characterized by TUNEL technique. A large number of TUNEL-positive germinal cells mostly at the spermatogonium and spermatocyte stage were observed in testicular samples bilaterally in I-R group (Figure 9). TUNEL-positive germ cells in bilateral testicular samples



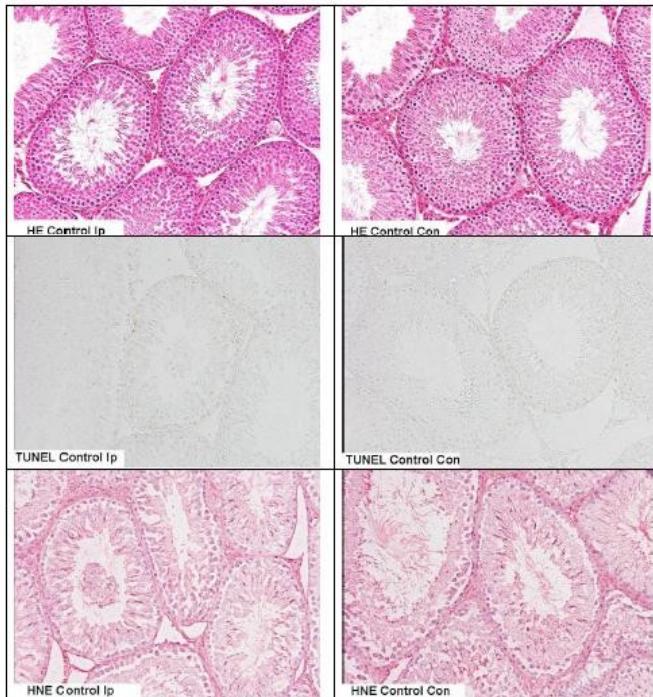
**Figure 5.** Mean values of tissue MDA concentration in both ipsilateral and contralateral testes of all groups. MDA concentration was normalized with protein content. Data are shown as mean  $\pm$  SEM of six separate determinations in each group. \* significantly different from control group. ( $p<0.05$ ) † significantly different from I-R group. ( $p<0.05$ ) ‡ significantly different from contralateral testis in the same group. ( $p<0.05$ ) [101].



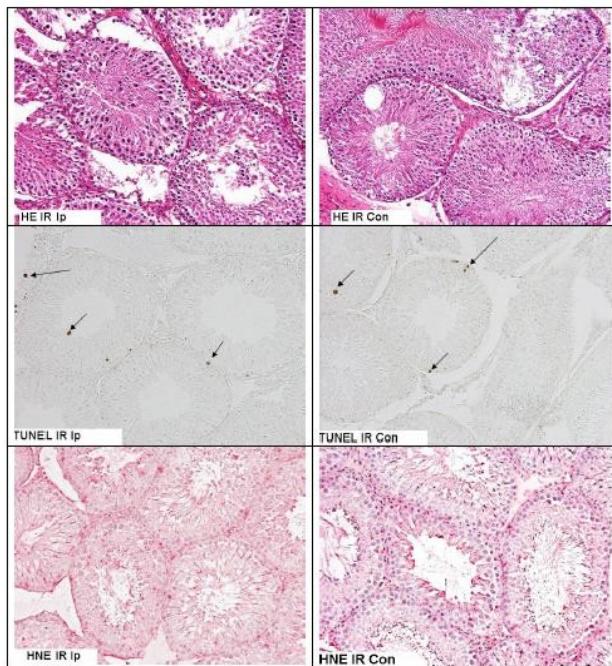
**Figure 6.** Mean values of tissue MPO activity in both ipsilateral and contralateral testis of all groups. MPO activity was normalized with protein content. Data are shown as mean  $\pm$  SEM of six separate determinations in each group. \* significantly different from control group. ( $p<0.05$ ) † significantly different from I-R group. ( $p<0.05$ ) ‡ significantly different from contralateral testis in the same group. ( $p<0.05$ ) [101].

in the IPostC 3-30 were significantly lower compared with I-R group (Figures 7 and 8). In bilateral testicular samples of I-R group, 4-HNE (a marker of lipid peroxidation) immunoreactive cells in the basement membrane of the seminiferous tubules, germ cells, and Leydig cells were greatly increased compared with the control group (Figure 9). 4-HNE immunoreactivity in IPostC 3-30 group was decreased compared with I-R group. Additional investigations are required to identify the most

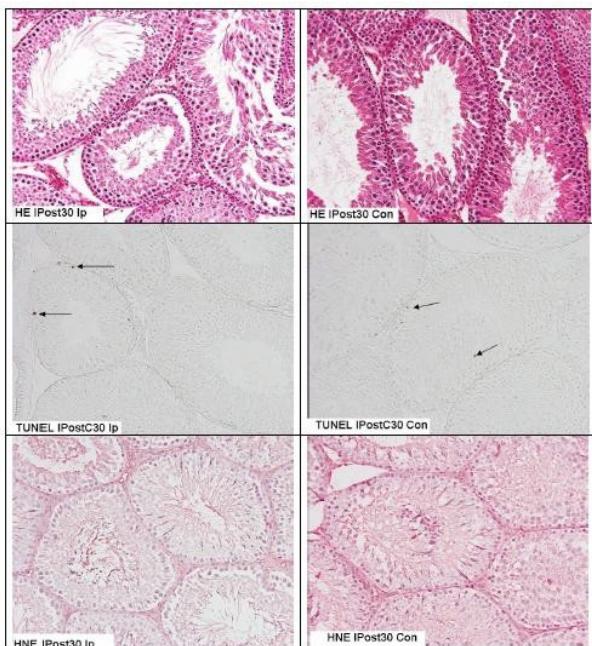
appropriate time intervals and duration as well as the number of cycles during IPostC management [101]. To date, extensive investigatory efforts have aimed to find effective strategies and drugs to ameliorate or even prevent testicular I-R injury. However, apart from cooling the scrotum, no other method has thus far been successfully applied in clinical practice [103]. The application of IPostC appears to be a simple and promising strategy to reduce testicular I-R injury.



**Figure 7.** Hematoxylin-eosin (HE), TUNEL and immunohistochemical staining of 4-Hydroxy-2-Nonenal (4-HNE) in both ipsilateral and contralateral testis of the control group. Original magnification: x200. Ip: ipsilateral testis, Con: contralateral testis, IPostC: ischemic postconditioning, HNE: 4-HNE [101].



**Figure 8.** Hematoxylin-eosin (HE), TUNEL and immunohistochemical staining of 4-Hydroxy-2-Nonenal (4-HNE) in both ipsilateral and contralateral testes of the I-R group. TUNEL-positive germinal cells in the seminiferous tubules (black arrows). Original magnification: x200. Ip: ipsilateral testis, Con: contralateral testis, IPostC: ischemic postconditioning, HNE: 4-HNE [101].



**Figure 9.** Hematoxylin-eosin (HE), TUNEL and immunohistochemical staining of 4-Hydroxy-2-Nonenal (4-HNE) in both ipsilateral and contralateral testes of the IPostC 3-30 group. TUNEL-positive germinal cells in the seminiferous tubules (black arrows). Original magnification: x200. Ip: ipsilateral testis, Con: contralateral testis, IPostC: ischemic postconditioning, HNE: 4-HNE [101].

**10. Future perspectives of intervention of torsion-detorsion in the testis**

There is still a need to find new treatments to improve testicular protection during open testis surgery. However, it is obscure whether the principle of IPostC is a suitable method to achieve this goal. Although we reported that experimental IPostC effectively protects testicular I-R injury, we did not check if this procedure would have the same protective effect during routine human testicular surgery. Further work is clearly required to assess the potential benefit of IPostC in humans. If IPostC on testicular torsion detorsion should become an important and widespread technique of testicular protection, large-scale studies are necessary to provide strong evidence of the beneficial effects of this procedure in human testes as well as in other organs. However, there is always the potential danger to damage the testicular artery by the repeated clamping and declamping. For this reason, IPostC might never gain widespread acceptance as a routine technique in the majority of patients. The key is to find pharmacological ways to induce IPostC. Pharmacological PostC has the potential advantage of reducing the requirement for invasive therapy associated with unavoidable endothelial trauma and possible embolic events. Thus, it is important to reveal the underlying IPostC signal transduction of protection and induce this pharmacologically. More experimental studies providing mechanistic insights are required.

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