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Remote Ischemic Preconditioning for Kidney Protection: GSK3 β -Centric Insights Into the Mechanism of Action

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Abstract

Preventing acute kidney injury (AKI) in high-risk patients following medical interventions is a paramount challenge for clinical practice. Recent data from animal experiments and clinical trials indicate that remote ischemic preconditioning (IPC), represented by limb IPC, confers a protective action on the kidney. IPC is effective in reducing the risk of AKI following cardiovascular interventions and the use of iodinated radiocontrast media. Nevertheless, the underlying mechanisms for this protective effect remain elusive. A protective signal is conveyed from the remote site undergoing IPC, like the limb, to target organs, like the kidney, *via* multiple potential communication pathways, which may involve a humoral, neuronal, and systemic mechanisms. Diverse transmitting pathways trigger a variety of signaling cascades, including the reperfusion injury salvage kinase and survivor activating factor enhancement pathways, all of which converge on glycogen synthase kinase (GSK)3 β . Inhibition of GSK3 β subsequent to IPC reinforces the Nrf2-mediated antioxidant defense, diminishes the NF κ B-dependent pro-inflammatory response, and exerts prosurvival effects ensuing from the desensitized mitochondria permeability transition. Thus, therapeutic targeting of GSK3 β by IPC or by pharmacologic preconditioning with existing FDA-approved drugs having GSK3 β inhibitory activities might represent a pragmatic and cost-effective adjuvant strategy for kidney protection and prophylaxis against AKI.

Keywords

acute kidney injury (AKI); remote ischemic preconditioning (IPC); limb IPC; ischemia/reperfusion; glycogen synthase kinase 3 β (GSK3 β); NF κ B; Nrf2; inflammation; mitochondria permeability transition (MPT); lithium; renal protection; review

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BACKGROUND

Acute kidney injury (AKI) is a common and potentially life-threatening complication annually affecting about 2,000 to 3,000 per million population, with two-thirds of cases occurring in intensive care units^{1,2}. Common causes of AKI include sepsis, ischemia/reperfusion injury, trauma, and exposure to nephrotoxic agents^{1,2}. In examining the epidemiologic risk factors, it is apparent that patients with older age, hypovolemia, heart dysfunction, preexisting chronic kidney disease (CKD), and exposure to nephrotoxic medications are more susceptible to developing AKI following diagnostic or therapeutic medical interventions (i.e. iatrogenic AKI)¹. Specific medical interventions linked to AKI include intravascular use of iodinated radiocontrast media, administration of nephrotoxic medications, and major surgeries involving cardiopulmonary bypass (CPB) or aortic cross clamping^{1,2}.

In the past 30 years, clinical management of AKI has been principally confined to treatment of symptoms and general supportive care including fluid resuscitation and kidney replacement therapy. These treatments are, however, of limited utility with unsatisfying therapeutic efficacy, given the poor prognosis. Although only a minority of patients with AKI requires hemodialysis after their initial hospital discharge, long-term follow-up studies show that the number of patients whose kidney recovery is incomplete has been underestimated and that AKI *per se* is an independent risk factor for subsequent transition to CKD^{2–4}. Therefore, it is imperative to develop a novel, pragmatic, and effective therapy for prophylaxis against AKI in these susceptible patients. Recently, a burgeoning body of evidence from both experimental and clinical studies points to ischemic preconditioning (IPC) as a promising and feasible approach to kidney protection and prophylaxis against AKI⁵.

CASE VIGNETTE

A 65-year-old man with a history of diabetes and hypertension for over 30 years presented to the emergency room with unstable angina pectoris. Laboratory testing revealed an elevated level of cardiac enzymes and serum creatinine level of 2.1 mg/dL (186 μ mol/L; corresponding to an estimated glomerular filtration rate [eGFR] of 32 mL/min/1.73 m² as calculated using the CKD-EPI creatinine equation⁶), consistent with stage 3 CKD. Urinalysis demonstrated an albumin-creatinine ratio of 2.6 mg/mg. The patient underwent urgent coronary angiogram, which revealed 90% stenosis of right coronary artery (RCA) and 75% stenosis of left anterior descending branch. An attempt at percutaneous coronary angioplasty of the RCA failed. The patient was referred for surgical coronary artery bypass grafting (CABG) with CPB but was considered to be a poor candidate for surgery because of high risk of AKI (risk score of 8 using the Thakar model of dialysis risk after cardiac surgery⁷). The patient was subsequently maintained on nonsurgical treatments, including insulin, furosemide, valsartan, metoprolol, amlodipine, acetyl salicylic acid, and lovastatin.

Although not currently standard of care, remote IPC may prove very helpful for patients like the one presented above. In future clinical practice, the approach to this patient might change. After induction of anesthesia for CABG surgery, this patient might undergo 4 cycles

of a 5-minute period of upper arm ischemia, brought about placing a 9-cm blood pressure cuff around the upper arm and inflating it to a pressure 50mmHg greater than his systolic blood pressure. Each period of ischemia would be followed by a 5-minute period of reperfusion induced by deflation of the blood pressure cuff. Remote IPC would occur in the anesthetic room during patient monitoring and placement of intravascular and bladder catheters. Immediately after the remote IPC protocol is completed, the patient would undergo CABG surgery with a significant reduction in the risk of AKI.

PATHOGENESIS

Ischemic preconditioning (IPC) is an innate tissue adaptation, whereby brief episodes of ischemic insult to a tissue or solid organ make both local and remote organs more resistant to a later prolonged exposure to the same or other injuries⁸. The concept of IPC was first advanced in 1986 by Murry *et al*⁹, who described a protective effect of repeated brief episodes of coronary artery ischemia/reperfusion on a subsequent myocardial infarction induced by sustained occlusion of the coronary artery in dogs. Before the introduction of IPC, Zager *et al*¹⁰ had already made a similar observation in the kidney, namely that in rats, prior exposure of the kidney to ischemia confers protection against additional ischemic kidney insults. In fact, it has been known for almost a century that a previous sublethal heavy metal exposure renders the kidney resistant to the additional injury¹¹.

More recently, studies have demonstrated that brief ischemia imposed even on non-target organs, most commonly in the limbs, exerts a protective effect on remote solid organs (eg, heart, lung, kidney or intestine) against injuries associated with ischemia and with other insults, including toxicants, hemorrhagic shock/resuscitation, and iodinated radiocontrast media¹². Limb IPC achieved by cycling between inflating and deflating blood pressure cuffs on either the arm or leg is a cost-free, straightforward, and attractive protocol. This technique has been reproducibly shown to attenuate acute injuries in the heart and in other organs, including the kidney¹³. A meta-analysis by Wever *et al*¹⁴ examined a total of 58 experimental studies on the effect of IPC (including limb IPC) in animal models of AKI and concluded that IPC has a protective effect on AKI induced by ischemia reperfusion injury. IPC successfully prevents reductions in kidney function as assessed by serum creatinine and serum urea nitrogen and it also minimizes kidney histological damage. Moreover, the kidney-protective properties of IPC seem to be most effective in animals when the IPC stimulus is applied 24 hours before the ischemic injury (late window of protection)¹⁴.

In an effort to evaluate the safety and effectiveness of IPC in humans, 13 randomized controlled trials (1,334 participants) have been completed so far in patients at risk of AKI following cardiac or vascular interventions. In 11 of the 13 trials (1,216 participants), limb IPC was found to reduce the risk of AKI compared with the control group (risk ratio, 0.70; 95% CI, 0.48–1.02)¹⁵. A substantial trend toward statistical significance suggests that limb IPC might be beneficial for prevention of AKI, although more adequately powered trials are still needed to validate its effectiveness¹⁵. The kidney-protective effect of limb IPC seem more pronounced in patients who are at high risk for AKI. This is supported by the recent Renal Protection (RenPro) Trial¹⁶, in which 100 adult patients with stable angina pectoris were recruited for elective coronary angiography using the contrast agent iohexol, a

nephrotoxic iodinated radiocontrast media. This group of patients also had preexisting CKD and thus was at an increased risk of developing superimposed AKI.¹⁶ A total of 26 patients developed contrast-induced AKI, 6 (12%) in the limb IPC group and 20 (40%) in the control group. Multivariable analysis suggested limb IPC as a strong independent contributor to prevention of contrast-induced AKI¹⁶.

Notwithstanding the positive findings made by most of the clinical and animal studies, a few clinical trials^{17–21} have demonstrated no benefit of IPC in preventing AKI. The conflicting results may be attributable to confounding variables like IPC protocols and patient characteristics. To date, the optimal protocol for remote IPC to trigger organ protection in humans remains unknown, but at least in rats, 3 or 4 (not 1 or 2) cycles of 5-minute remote IPC are necessary to confer heart protection²². Most of the clinical trials showing beneficial effects of IPC employed more than 3 cycles of 5 to 10-minute IPC, whereas in a study of 2 cycles of 10-minute iliac clamping, there was no evidence of any favorable changes in kidney outcome indices in patients undergoing open abdominal aortic aneurysm repair¹⁹. Also to be determined is whether use of upper versus lower limbs makes a difference in remote IPC preconditioning. Loukogeorgakis *et al*²³ have shown that when using the leg rather than the arm for conditioning stimulus, more cycles are required to reach a preconditioning threshold and protect against ischemia/reperfusion-induced endothelial dysfunction in healthy volunteers. Anesthesia might be another confounding variable in examining the kidney effect of remote IPC. In some studies, such as those involving cardiac surgery, limb IPC was performed after anesthetic induction and, thus, patients were pain free²¹. However, as suggested by some experimental studies, pain may strongly stimulate pre-conditioning²⁴, and remote IPC is dependent, in part, on intact local neural pathways²⁵. In addition, some inhalational anesthetics can themselves cause preconditioning and protect from kidney injury²⁶. This may mask the effect of remote IPC if IPC is performed during anesthesia. Thus, future studies are required to verify if variations in the mode of remote IPC, like the number of IPC cycles, upper versus lower limb IPC, or anesthesia, make a difference in the kidney-protective effects of IPC.

Different patient characteristics might be another important contributor to the contradictory findings from different trials. For instance, remote IPC is seemingly beneficial in patients at intermediate or high risk for AKI, whereas little kidney protective effect can be seen in patients with low risk or those who do not have reduced kidney function²⁷. This variable should be validated in future studies by stratifying according to patients' propensity for AKI. Furthermore, recent evidence suggests that, at least for cardioprotection, remote IPC is likely more effective in male than female patients²⁸. We don't as yet know if gender disparity also exists with IPC-associated kidney protection; however, it seems that clinical trials favoring a kidney protective effect for IPC often recruited more male patients^{29,30} and most of the negative studies^{17–20} enrolled more female patients.

In view of the limitations of previous clinical trials as well as some of the previously described confounding factors, more studies aiming to provide definitive evidence in defining the role of remote IPC are in progress. Among these trials, the Remote Ischaemic Preconditioning for Heart Surgery (RIPHeart) study³¹ is a prospective, randomized, double-blind, multicentre, controlled trial including 2,070 adult cardiac surgical patients. It has been

meticulously designed and adequately powered to determine whether remote IPC may improve clinical outcomes, including AKI, following all types of cardiac surgeries in which CPB is used.

Despite a number of reproducible observations suggesting the benefit of IPC in AKI, the underlying molecular mechanisms accounting for the kidney protective activity remain largely unknown. The most intriguing aspect of remote IPC is how the protective signal generated by remote IPC is transmitted to the target organs like the kidney. To date, there have been many studies investigating the mechanisms of remote IPC on heart protection³². From available evidence, the substance generating the remote IPC-induced protective activity is dialyzable, transferable, and receptor-mediated, suggesting the involvement of one or more blood-borne humoral mediators³³. These mediators purportedly include an as-yet unidentified small (~3.5–15 kDa) hydrophobic molecule released during brief IPC, which is cardioprotective when purified and enriched by reverse phase chromatography and administered to naïve hearts¹². In addition, autonomic reflex and other neurogenic pathways that are stimulated by the release of opioid, adenosine, and bradykinin may be involved in IPC-induced organ protection. In support of this view, blocking the autonomic ganglion abolishes the cardioprotective effects of remote IPC³³. Moreover, systemic immune modulation and an anti-inflammatory effect on immune-competent cells following IPC might also contribute to the protection of target organs against subsequent acute injury³³. The mediating pathways by which the protective signal is carried from the site subjected to IPC (e.g. limbs) to target organs may differ among experimental models and may involve humoral, neuronal, and systemic mechanisms (Figure 1).

RECENT ADVANCES

Regardless of the exact communication pathways transmitting the protective signal of IPC to the kidney, it is nevertheless important to decipher the molecular signaling mechanisms behind IPC so that pharmaceuticals can be developed as an alternative means of preconditioning that is able to precisely and effectively target the responsible signal transducers. This is particularly relevant for patients with preexisting CKD and comorbidities, like peripheral vascular disease, hypercoagulability, and diabetes mellitus. If subjected to limb IPC, in particular lower-limb IPC, these patients would be at significant risk for thrombosis, rhabdomyolysis, limb ischemia, nerve injury and other complications^{34–36,37}.

IPC triggers a number of cell signaling transducers that contribute to the organ protective effects. Within the complex signaling network, the RISK (reperfusion injury salvage kinase) and SAFE (survivor activating factor enhancement) pathways have emerged as two major prosurvival signaling cascades mediating the beneficial action of IPC³⁸. Activation of receptor tyrosine kinase or G-protein–coupled receptor by IPC in target organs triggers phosphoinositide 3-kinase (PI3K)/Akt and mitogen-activated protein kinases (MAPK)/extracellular regulated kinase (ERK) signal transduction as part of the RISK pathway (although the role of ERK is debatable). In parallel, IPC increases the levels of growth factors and cytokines like tumor necrosis factor (TNF)- α , which bind cell surface receptors in target organs and activate JAK (Janus kinase) and STAT3 (signal transducer and activator

of transcription 3), as part of the SAFE pathway. Inhibition of either the RISK or the SAFE pathway totally abolishes the protective activity of IPC^{39,40}, consistent with a cross-talk between the two pathways. Whether these two pathways converge on the same signaling transducer needs to be clarified, but glycogen synthase kinase (GSK) 3 β has been shown to be a convergence point for the RISK and SAFE pathways⁴¹, justifying the choice of this signaling transducer as a target to both potentiate prosurvival cascades and maximize organ protection.

To understand the mechanism of action responsible for kidney protection elicited by remote IPC, Liu *et al*⁴² dissected the RISK signaling cascades^{43,44}. Rats with preexisting CKD caused by partial nephrectomy were subjected to limb IPC prior to iohexol-induced AKI. The limb IPC-induced kidney protection could be abrogated by pretreatment with wortmannin (a specific inhibitor of PI3K) but not by U0126 (a ERK1/2 inhibitor)⁴². This observation was confirmed using another selective inhibitor of PI3K, LY294002, and another ERK1/2 inhibitor, PD98059. Moreover, the limb IPC-induced kidney protection was found to be associated with inhibitory phosphorylation of the signaling transducer GSK3 β , which is downstream of the PI3K/Akt signaling pathway. Inhibition of GSK3 β by SB216763, a selective small molecule inhibitor, mimicked the kidney protective effect of limb IPC and likewise attenuated loss of kidney function and prevented the acute kidney histological injury elicited by iohexol⁴².

GSK3 β is a highly conserved serine/threonine protein kinase that is ubiquitously expressed and was found in the early 1980s to phosphorylate glycogen synthase and regulate glucose metabolism^{45,46}. GSK3 β is constitutively active in quiescent cells, while both the Wnt signaling pathway and PI3K/Akt downregulate its activity *via* inhibitory phosphorylation of the serine at amino acid 9 (ie, near the amino terminus). Its activity can be amplified by reactive oxygen species following oxidative injury^{47,48}. Interest in GSK3 β has heightened considerably following the finding that it is an important regulator of not just glycogen metabolism but also several other key cellular events such as signal transduction, insulin action, gene transcription, protein translation, cytoskeletal organization, cell cycle progression, and cell death and survival⁴⁵. In addition, GSK3 β has been implicated in a multitude of pathophysiologic processes, including embryo development, tissue injury, repair, and regeneration.

As a redox-sensitive serine/threonine protein kinase, GSK3 β is interconnected with multiple cellular signaling cascades, including the Wnt, Nrf2 antioxidant response, and NF (nuclear factor) κ B pathways, and more⁴⁶. A number of transcription factors, such as Nrf2 (NRF2 in humans, ie, the product of the *NFE2L2* gene) and the NF κ B subunit RelA/p65, have been found to be cognate substrates for GSK3 β and are subjected to GSK3 β -directed phosphorylation and regulation of transcriptional activity⁴⁹(Figure 1). Studies from our and other groups have indicated that GSK3 β determines RelA/p65 phosphorylation at serine 468, thereby specifying the transcription of an array of NF κ B target molecules involved in immune reaction and inflammatory response^{50–52}.

In studies we have performed in animal models, inhibition of GSK3 β mitigates pro-inflammatory NF κ B activation in kidney tubules⁵⁰ and the glomerulus⁵³, exerts an anti-

inflammatory and immunoregulatory activity (Figure 2), but largely preserves other NF κ B-dependent biological activities, including prosurvival/anti-apoptosis and anti-inflammation, resulting in attenuated kidney histologic evidence of kidney injury⁵⁰. In addition, GSK3 β is responsible for phosphorylation, nuclear exclusion, and degradation of Nrf2⁵⁴, which drives the primary cellular defense signaling against the cytotoxic effects of oxidative stress in mammalian cells^{55,56}. Inhibition of GSK3 β has been shown to promote the Nrf2-dependent antioxidant response and confer protection in several organ systems (Figure 3)^{56,57}. Our studies have found that GSK3 β inhibition by lithium reinforces the NRF2 antioxidant response and ameliorates hepatic injury in patients with hepatitis C virus infection⁵⁷.

Kidney cells have a discrete mitochondrial pool of GSK3 β ^{48,58,59}. Components of the mitochondria permeability transition (MPT) pore, including cyclophilin F and the voltage-dependent anion channel (VDAC), possess GSK3 β phosphorylation consensus motifs and serve as putative substrates for GSK3 β ^{48,58–61}. By controlling the phosphorylation and activity of cyclophilin F and VDAC, GSK3 β may thereby determine how readily the MPT pore opens. Since induction of the MPT pore opening can lead to cell death, GSK3 β can dictate the sensitivity of cells to pro-death stimuli. In our work, inhibition of GSK3 β by selective inhibitors desensitizes the MPT pore to stimuli that would ordinarily cause it open, ameliorates mitochondria dysfunction in kidney tubular and glomerular cells, attenuates kidney dysfunction and proteinuria, and improves the histology of kidney tubules and the glomerulus following injury by the pro-oxidant paraquat⁴⁸ and also high-dose diclofenac⁵⁸ and adriamycin⁵⁹ (Figure 4)^{48,58,59}. Consistent with this observation, inhibition of GSK3 β by limb IPC or by SB216763 (the small molecule inhibitor) ameliorates mitochondrial injury in kidney tubules after iohexol exposure in rats, enhances the tubular expression of Nrf2, reduces oxidative stress, blunts NF κ B activation, and lessens kidney inflammation.⁴²

Besides IPC, a multitude of treatments and drugs are known to attenuate acute organ injury via inhibition of GSK3 β . These include insulin, estrogen, valproic acid, lithium, and more^{62,63}. Moreover, most of the communication pathways utilized by IPC for remote organ protection (Figure 1), such as adenosine, opioid, nitric oxide, reactive oxygen species, and autonomic reflex, are capable of inducing inhibitory phosphorylation of GSK3 β ⁶⁴. Thus, GSK3 β is likely the point of convergence for multiple protective pathways. Of note, GSK3 exists in two isoforms, GSK3 α and GSK3 β , both of which are widely expressed in almost all organ systems^{45,65}, including the kidney. Although GSK3 α and GSK3 β display 84% structural homology and are similarly regulated and functionally redundant in many aspects of cellular signaling⁶⁵, it seems that only GSK3 β is involved in mediating the protective effect of IPC and other organ protective treatments⁶⁶. Indeed, in cardiac myocytes, knockdown of GSK3 α fails to provide any protective effects, even though the protective effects of insulin and hypoxic pre-conditioning remain intact. Knockdown of GSK3 β by itself achieves levels of protection comparable to the effects of insulin⁶⁶. Moreover, insulin does not increase the level of protection above that already achieved in the GSK3 β -silenced cells. Conversely, in murine cardiac myocytes expressing a mutant form of GSK3 β that cannot be inhibited (the serine 9 residue is replaced by an alanine), the cellular protective effect of hypoxic preconditioning is markedly blunted⁶⁶. Therefore, inhibition of the β , but not the α , isoform of GSK3 is responsible for the protective signaling in IPC. Accordingly, therapeutic targeting of GSK3 β by using pharmacological inhibitors is an attractive

substitute for IPC as a prophylactic measure against AKI, in particular for patients unable to tolerate IPC.

Recent structure-activity relationship studies have advanced the development of a number of highly selective small-molecule chemical inhibitors of GSK3 β . However, most of these novel inhibitors are currently in preclinical evaluation and may take years to enter clinical trials. Fortunately, several US Food and Drug Administration (FDA)-approved agents, like lithium, are able to effectively block the activity of GSK3 β ^{67,68}. The lightest alkali metal on earth, lithium is the best-known GSK3 β inhibitor⁶⁷. It is an essential trace element required for physical and mental health in humans⁶⁹ and has been in clinical use for a half century as an FDA-approved first-line treatment for bipolar affective disorders⁷⁰. The beneficial effect of lithium in diverse organ systems has been noted for decades. For example, lithium exhibits a neuroprotective and neurotrophic activity⁷¹ and reinforces neural repair in acute brain injury (e.g. stroke or ischemia)^{72,73} as well as in chronic neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's diseases^{74,75}. In addition, lithium can ameliorate neutropenia associated with cancer chemotherapy⁷⁶ and has been one of the treatment choices for neutropenia⁷⁷. Recent research has deciphered the mechanism of this general protective activity: lithium targets GSK3 β , which as discussed previously is a key cell signaling molecule central to the pathogenesis of multiple human diseases, including AKI.

Studies from our and other laboratories have demonstrated that lithium-induced blockade of GSK3 β can prevent AKI arising from endotoxin⁷⁸, nephrotoxic drugs⁷⁹ and ischemia/reperfusion injury⁷⁹. As an alkali ion, lithium requires high blood levels to pass through the blood brain barrier⁸⁰, thus the FDA-approved lithium dose for psychiatric disorders is quite high, with a relatively narrow therapeutic index. Long-term (usually >10 years) high-dose lithium therapy primarily for psychiatric disorders is occasionally associated with some kidney adverse effects, such as nephrogenic diabetes insipidus, interstitial nephritis, and even glomerular disease⁸¹. However, according to a large-scale epidemiology study of two regions in Sweden with a population of 2.7 million, end-stage kidney disease was uncommon in lithium-treated patients, although the incidence (0.53%) was noted to be higher than in the general population⁸². In our experience with animal models, the kidney-protective dose of lithium is much lower than its neurobiologic dose⁸³. Short-term treatment with low-dose lithium (approximately 1/3 to 1/2 of the neurobiologic dose) results in no detectable changes in kidney histology or function in healthy mice, but clearly prevents kidney histologic injury in mouse models of AKI⁷⁹. Therefore, it seems that short-term use of low-dose lithium might be a promising approach for preventing AKI. Taken together, if the pivotal role of GSK3 β in mediating the kidney protection by IPC is validated, preconditioning with lithium might be a feasible and cost-effective alternative to IPC, especially for patients at risk of complications if subjected to IPC.

SUMMARY

There is increasing scientific evidence supporting a role for IPC in protecting against AKI. The possible underlying mechanisms are less clear because of the limitations in many of these studies. First, all of the clinical trials published so far are small-scale low-powered

studies, which unavoidably undermine the reliability of the observations¹⁵. We believe that large-scale multicenter randomized controlled trials with high power would be worthwhile to conclusively validate the safety, effectiveness, and efficacy of IPC in preventing AKI. Second, published mechanistic studies have relied exclusively on the use of pharmacological inhibitors⁴², thus should be interpreted with the caveat that these inhibitors may act in a concentration-dependent way and may have other, as-yet unknown, functions. Researchers should consider using transgenic mice with kidney tubule-specific conditional knockout of the kinases being studied in order to validate the results in in-depth confirmatory studies. Mice with kidney tubule-specific conditional knockout of GSK3 β have been well established⁸⁴ and demonstrated resistance to mercury-induced AKI⁸⁵. Whether ischemia/reperfusion injury–, cardiac surgery–, or contrast media–induced AKI is preventable in this line of knockout mice merits further investigation.

To date, available evidence demonstrates only that GSK3 β inhibition is sufficient for IPC-associated kidney protection⁴². It remains unknown whether inhibitory phosphorylation of GSK3 β is required and essential for the kidney protective activity of IPC. To address this question, gene-targeted knock-in mice with mutant (impervious to inhibition) GSK3 β ⁸⁶ may be considered.

Furthermore, recent studies have indicated that there is cross-talk between the RISK and SAFE pathways and that the JAK-STAT3 pathway is regulated by the activity of PI3K-Akt-GSK3 β cascade in the IPC-induced cardioprotection^{87,88}. Future studies are needed to explore if the SAFE pathway is involved and is under the regulation by GSK3 β in IPC-conferred kidney protection.

Despite these pitfalls, the key findings of the latest studies exploring kidney protection by IPC are very consistent with the mechanism of action of IPC revealed so far for heart protection, where GSK3 β inactivation has been found to mediate a variety of divergent protection signaling pathways triggered by IPC to prevent myocardial injury^{66,89,90}.

In conjunction with recent evidence indicating a general benefit of targeting GSK3 β in various organ systems^{46,91–93}, it seems that GSK3 β is situated at the convergence point of multiple protective pathways of IPC. Therapeutic targeting of GSK3 β by IPC (Figure 1) or by using existing FDA-approved drugs with GSK3 β inhibitory activities, such as lithium⁷⁹, may represent a promising and pragmatic strategy for kidney protection and prevention of AKI. In future clinical practice, patients with high risk of AKI like the one presented in the case vignette may routinely undergo remote IPC as a standard preprocedural prophylactic measure to prevent AKI prior to medical interventions such as major surgery involving CPB or the use of contrast media.

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Figure 1. GSK3β is a convergence point of multiple protective signaling pathways triggered by limb ischemia preconditioning (IPC)

The protective signals of limb IPC may be transmitted to remote target organs like the kidney via different communication mechanisms, including humoral mediators, neurogenic pathways, and systemic regulation of immune-competent cells. These mediating pathways function on the kidney and activate the reperfusion injury salvage kinase (RISK) pathway (involving PI3K/Akt signaling) and the survivor activating factor enhancement (SAFE) pathway (involving JAK/STAT3 signaling), both of which have been shown to converge on GSK3β. GSK3β is situated at the nexus of a number of pivotal signaling cascades and controls NFκB phosphorylation, Nrf2 nuclear exclusion and degradation, as well as the opening of the mitochondria permeability transition (MPT) pore. Inhibition of GSK3β in the kidney following limb IPC desensitizes MPT, reduces proinflammatory NFκB activation, and stabilizes Nrf2 in the nucleus in kidney cells, resulting in pro-survival/anti-apoptotic, anti-inflammatory/immunomodulatory and antioxidant effects, which contribute to limb IPC-conferred kidney protection against various injuries elicited by contrast media or other insults. GSK3β, glycogen synthase kinase 3β; JAK, Janus kinase; PI3K, phosphoinositide 3-

kinase; RISK, reperfusion injury salvage kinase; SAFE, survivor activating factor enhancement; STAT3, signal transducer and activator of transcription 3.

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Figure 2. Ischemic preconditioning prevents inflammation in an acutely injured kidney by regulating the NF κ B-dependent inflammatory response

NF κ B RelA/p65 is a cognate substrate of GSK3 β and can be phosphorylated by GSK3 β at serine 468, which has been shown to specify the expression of an array of NF κ B target genes involved in kidney inflammation. Upon injury, the activity of the redox-sensitive GSK3 β is elevated. This promotes the phosphorylation of NF κ B RelA/p65, and amplifies the transcription of pro-inflammatory genes, including chemokines, cytokines and inflammatory mediators. Ischemic preconditioning inhibits GSK3 β by inducing phosphorylation at serine 9 via a number of communication pathways and possibly through reperfusion injury salvage kinase (RISK) and survivor activating factor enhancement (SAFE) signaling. GSK3 β inhibition subsequent to ischemic preconditioning will attenuate NF κ B RelA/p65 phosphorylation, repress the expression of proinflammatory molecules, and prevent inflammatory injury in the kidney, resulting in a kidney-protective effect. AKI, acute kidney injury; GSK3 β , glycogen synthase kinase 3 β .

Figure 3. Ischemic preconditioning exerts kidney protection and prevents acute kidney injury by regulating the GSK3 β -dictated Nrf2 detoxification/antioxidant cellular defense pathway Nrf2, as a cognate substrate of GSK3 β , can be phosphorylated directly by GSK3 β or indirectly by Fyn kinase, which requires phosphorylation and activation by GSK3 β . Phosphorylation of Nrf2 facilitates its nuclear export, which is followed by its ubiquitination and degradation. Upon injury, the activity of the redox-sensitive GSK3 β is upregulated. This will promote Nrf2 phosphorylation, nuclear exit, and degradation, resulting in a reduced antioxidant gene expression and response. Ischemic preconditioning inhibits GSK3 β by inducing phosphorylation at serine 9. This effect may be attained via a number of communication pathways and possibly through reperfusion injury salvage kinase (RISK) and survivor activating factor enhancement (SAFE) signaling. GSK3 β inhibition diminishes Nrf2 phosphorylation, mitigate the nuclear export of Nrf2, enhance Nrf2 nuclear accumulation, and the antioxidant response, culminating in a kidney protective effect. AKI, acute kidney injury; GSK3 β , glycogen synthase kinase 3 β .

Figure 4. Ischemic preconditioning protects against acute kidney injury by preventing mitochondria dysfunction and damage

There is a discrete mitochondrial pool of GSK3β. Structural components of the mitochondrial permeability transition pore, including cyclophilin F and voltage-dependent anion channel (VDAC), possess multiple GSK3β phosphorylation consensus motifs and serve as cognate substrates for GSK3β. Phosphorylation of cyclophilin F and VDAC by GSK3β has been shown to reduce the threshold MPT pore (sensitizing it to open more readily). Upon oxidative injury, the activity of the redox sensitive GSK3β is elevated. This promotes the phosphorylation of cyclophilin F and VDAC, subsequently augments MPT and potentiates cell death. Ischemic preconditioning inhibits GSK3β by inducing phosphorylation at serine 9 via a number of communication pathways and possibly through reperfusion injury salvage kinase (RISK) and survivor activating factor enhancement (SAFE) signaling. GSK3β inhibition reduces the phosphorylation of cyclophilin F and VDAC, desensitizes MPT, and gives kidney cells resistance to prodeath insults, contributing to a kidney-protective effect. AKI, acute kidney injury; Cyp-F, cyclophilin F; GSK3β, glycogen synthase kinase 3β; MPT, mitochondria permeability transition; VDAC, voltage-dependent anion channel.