Limb Ischemia Protects Against Contrast-Induced Nephropathy

Running title: Bonventre; Limb ischemia protects against CIN

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Contrast-induced acute kidney injury (AKI), or nephropathy, (CIN) is frequently diagnosed in the setting of coronary angiography. The incidence varies in the literature partly related to the differences in diagnostic criteria used for CIN. In a recently published manuscript in Circulation Maioli et al.¹ used a definition of $\geq 0.5$ mg/dl over baseline serum creatinine within 3 days of the administration of contrast medium and found an incidence of 12.1% among 1490 patients who had an baseline estimated creatinine clearance of $<60$ ml/min. It has become increasingly recognized that even mild forms of AKI are associated with adverse short and long term outcomes including onset or progression of chronic kidney disease and more rapid progression to end stage kidney disease²,³. Patients with CIN have an increase in short-term and long-term mortality, whether the renal dysfunction is acute or chronic and after adjustments for other comorbidities⁴. While it has been concluded by many that the development of CIN may identify other conditions that are more responsible for the adverse outcomes, there are increasing data from randomized trials that CIN may directly contribute to the increased risk of cardiovascular and renal adverse outcomes⁵. In previously mentioned study by Maioli et al., the authors reported that persistent renal dysfunction, defined as a relative decrease of creatinine clearance of $\geq 25\%$ from baseline at 3 months after coronary angiography, occurred in 18.6% of CIN patients¹. These patients with persistent renal dysfunction had a higher incidence of death at 5 years when compared to those whose renal functional impairment was transient or those who did not develop CIN. In another study the adjusted odds ratio of sustained decline in kidney function 3 months after coronary angiography was more than 4-fold in patients who had mild AKI ($\geq 0.3$ mg/l or 50-99% increase in serum creatinine) and more than 17-fold for those with moderate or severe AKI ($\geq 100\%$ increase in creatinine)⁶. Experimental models in animals provide pathophysiological explanations for how the effects of acute injury can lead to chronic
inflammation, vascular rarefaction, tubular cell atrophy, interstitial fibrosis, and 
glomerulosclerosis. It is therefore very important to avoid the kidney injury associated with 
contrast administration.

Er et al., in this issue of Circulation, report that remote ischemic preconditioning protects 
the kidneys against CIN. Preconditioning represents an activation by the organism of intrinsic 
defense mechanisms to cope with pathological conditions. Ischemic preconditioning is the 
phenomenon whereby a prior ischemic insult renders the organ resistant to a subsequent ischemic 
insult. Renal protection afforded by prior renal injury was described exactly one century ago, in 
1912, by Suzuki who noted that the kidney became resistant to uranium nephrotoxicity if the 
animal had previously been exposed to a sublethal dose of uranium. In referring to the work of 
Suzuki, Aschoff attributed the resistance to uranium toxicity to a resistance of the renal 
epithelium and proposed this to be a defense mechanism of the kidney. There have been a 
number of studies over the years demonstrating that preconditioning with a number of renal 
toxicants led to protection against injury associated with a second exposure to the same toxicant 
or to another nephrotoxicant. It is not, however, a universal finding that toxins confer 
resistance to subsequent insults.

Zager and colleagues performed the first experiments evaluating the effects of prior 
exposure of the kidney to ischemia on subsequent susceptibility to ischemic injury in rats a short 
time later. Our laboratory created a mouse model in which prior exposure to ischemia 
protects against a second ischemic insult imposed 8 or 15 days later. Unilateral ischemia was 
also protective against a subsequent ischemic insult to that kidney, revealing that systemic 
uremia was not necessary for protection. Unilateral ischemia, however, did not protect the 
contralateral kidney against ischemic injury 6 or 8 days later. In a subsequent study we found
that protection afforded by 30 min of ischemic preconditioning (inducing severe functional and histological damage) lasted for at least up to 12 weeks\textsuperscript{17}. Some of the cellular processes and signaling mechanisms proposed to explain preconditioning in the kidney and other organs are listed in Table 1.

While it is possible in humans that preconditioning can be carried out directly on the kidney, e.g. by inducing ischemia prior to allograft placement or transiently obstructing the native kidney prior to a procedure which will result in ischemia\textsuperscript{18}, it is nevertheless more clinically tractable to develop methods in patients in which preconditioning can be induced pharmacologically or by minimally invasive remote ischemic preconditioning protocols. Remote ischemic preconditioning is a therapeutic strategy by which protection can be afforded in one vascular bed by ischemia to another vascular bed in the same organ or a different organ. In 1993 it was reported that temporary occlusion of the circumflex artery 4 times for 5 min each followed by 5 min of reperfusion, leads to protection of myocardium in the distribution of the left anterior descending coronary artery after ischemia to that vessel, demonstrating that ischemia in one vascular bed protects tissue in another bed\textsuperscript{19}. Since then there have been a large number of studies demonstrating that ischemia to one organ protects against ischemia to another\textsuperscript{20}. Patients undergoing elective abdominal aortic aneurysm surgery were randomly assigned to either two cycles of intermittent cross clamping of the common iliac artery with 10 min of ischemia and 10 min reperfusion serving as the remote preconditioning influence. Remote preconditioning resulted in less postoperative myocardial injury, myocardial infarction, and renal impairment\textsuperscript{21}.

If the limb is used for preconditioning, ischemia can be carried out reasonably safely and a highly clinically relevant inexpensive therapeutic approach is established. A number of studies involving limb ischemia have been performed in animals. Wever and colleagues
evaluated the effects of unilateral or bilateral limb ischemia for 12 min of ischemia followed by 12 min of reperfusion, or 3 periods of 4 min of ischemia followed by 4 min of reperfusion, on kidney injury induced by 25 min of ischemia commencing at the end of the preconditioning in the rat. After 24 hrs of reperfusion renal function was improved by 30-60% in both bilateral preconditioning groups and one unilateral fractionated preconditioning group. Renal tubular damage was less in the protected preconditioned groups.

Er et al., in this issue of Circulation, carried out a double blind study of the role of remote preconditioning on the development of acute kidney injury in patients with impaired renal function who receive contrast medium for elective coronary angiography. Half of the patients were given 4 cycles of 5-min inflation of an upper arm blood pressure cuff followed by 5 min of deflation to produce transient and repetitive limb ischemia. In the preconditioning group, standard upper-arm blood-pressure cuffs were inflated to 50 mm Hg above systolic blood pressure whereas in the sham group the cuff was inflated to diastolic pressure levels then deflated to 10 mmHg. The primary end point was the development of CIN as defined by an increase in serum creatinine ≥ 25% or ≥ 0.5 mg/dL above baseline at 48 hr after contrast-medium exposure.

The authors evaluated 100 patients with either baseline serum creatinine >1.4 mg/dL and/or estimated GFR <60 mL/min/1.73 m². Remote preconditioning resulted in protection against CIN. Of 50 patients in the control group, 20 (40%) developed AKI and of 50 patients in the remote ischemic preconditioning group only 6 (12%) developed AKI. The composite cardiovascular endpoint (death, rehospitalization or hemodialysis during a 6 week follow-up period) occurred more often in the control group. All patients in the study received oral N-acetylcysteine, 600 mg twice orally the day before, and the day of, coronary angiography. The patients also received continuous intravenous saline infusion 12 hours before to 12 hours after
coronary angiography. The levels of NGAL in the urine increased at 24 and 48 hours in both the control and the preconditioned groups with greater increases in neutrophil gelatinase-associated lipocalin (NGAL) found in the control groups. There was also a greater increase in serum cystatin C in the control group at 24 and 48 hr after the contrast administration. The lower levels of serum cystatin and urinary NGAL are consistent with a protection of GFR and less kidney injury.

The study results are very interesting since the protective effects of remote preconditioning suggests an intervention that could be relatively easily applied in routine medical practice. There have been a number of studies examining the potential protective effect of remote preconditioning by cycling of inflation and deflation of blood pressure cuffs on either the arm or leg with varying results. Many of these studies have focused on the heart but some have been targeted to evaluating kidney protection in patients undergoing cardiac surgery. In the latter study by Choi et al. in 76 patients undergoing complex valvular heart surgery, remote leg ischemic preconditioning (38 patients) in did not reduce the degree of renal injury, as reflected by urinary biomarkers, or incidence of acute kidney injury, whereas it did reduce myocardial injury and intensive care unit stay.

There are, however, a number of issues related to the Er et al. study which dictate that confirmatory findings will be necessary before considering this approach. The number of patients in this pilot study was quite small and it will become apparent over time whether the results will be confirmed as a second extended RenProII-Trial will test the effects of remote preconditioning on cardiovascular mortality and morbidity. One might suspect that repeated episodes of 5 min of ischemia at 50 mmHg above systolic pressure would be not well tolerated by all patients. The incidence of AKI in the control group is higher than a number of reports of patients with chronic
kidney disease who are given contrast agents especially since the patients were all hydrated. This might be explained somewhat by the mean age of 73 years in both control and remote preconditioning group, heart failure in 84% and diabetes in 64% in both control and treated groups in the RenPro-Trial. On the other hand the incidence is consistent with that found by Mehran et al.\textsuperscript{25} for the average risk score of 13 in the RenPro pilot study. In the study by Maioli and colleagues\textsuperscript{1}, where the incidence of CIN in 1490 patients with baseline GFR less than 60 ml/min was 11.5%, the patients had a lower contrast nephropathy risk than the patients in the Er et al. study\textsuperscript{10}. Er et al. used low-osmolar contrast agent (Iohexol) whereas Maioli et al. used Iodixanol, a non-ionic, dimeric iso-osmolar contrast medium. In a meta-analysis it has been reported that the risk for CIN with intra-arterial administration to patients with renal insufficiency, was greater with Iohexol than for Iodixanol\textsuperscript{26}.

What might be the mechanism of remote preconditioning induced protection against CIN? As indicated in Table 1, there have been a number of mechanisms implicated as mediators of the protection afforded by remote ischemic preconditioning. One should consider what might happen after transient muscle ischemia which could represent a circulating influence that would affect kidney susceptibility to contrast. There is evidence that brief forearm ischemia results in downregulation of pro-inflammatory genes and up-regulation of anti-inflammatory genes in circulating human leukocytes\textsuperscript{27}. It is likely that vasodilatory factors are released locally which may have effects at a distance. It has been reported that transient limb ischaemia releases a low molecular mass (<15 kDa), hydrophobic, circulating factor that induces protection against ischemia/reperfusion injury across species, is independent of local neurogenic activity, and requires opioid-receptor activation for manifestation of protection of cardiac cells\textsuperscript{28}. The factors responsible for advantageous effects of remote ischemic preconditioning on the kidney
represent a subject that demands more study particularly since identification of the responsible protective factor(s) would provide a therapeutic approach for prevention of CIN as well as potentially prevention of AKI in other clinical settings.

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**References:**


Table 1. Factors implicated as protective mediators of remote ischemic preconditioning

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<tr>
<th>Adenosine</th>
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<tr>
<td>Nitric oxide</td>
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<tr>
<td>Protein kinase C (PKC)</td>
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<td>Extracellular signal related kinase (ERK)</td>
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<td>AKT (protein kinase B)</td>
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<td>Mitochondrial ATP-sensitive potassium channel (K$_{ATP}$ channel)</td>
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<td>Mitochondrial connexin 43</td>
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<td>Antioxidants</td>
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<td>Hypoxia inducible factors (HIFs)</td>
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<td>Heat shock proteins</td>
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<td>Sirtuin activity (SIRT1)</td>
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<tr>
<td>Autophagy</td>
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<tr>
<td>Decrease in genes regulating inflammation (cytokine synthesis, leukocyte chemotaxis, adhesion, exocytosis, innate immune signaling pathways)</td>
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