Ischemia With Intermittent Reperfusion Reduces Functional and Morphologic Damage Following Renal Ischemia in the Rat

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Attempts to minimize ischemic injury by interrupting a given ischemic period might be compromised if repeated bouts of reperfusion injury occurred. To determine whether intermittent ischemia improved or worsened functional and morphologic outcome of renal ischemia, halothane-anesthetized rats underwent a right nephrectomy and placement of a snare about the left renal vascular pedicle at 37° C. Eleven animals underwent 45 minutes of continuous renal ischemia (C-ISC), whereas 10 animals received 45 minutes of vessel occlusion interrupted (I-ISC) at 15 and 30 minutes by snare release and 5 minutes of reperfusion. A group of three sham rats underwent the above procedure but did not have the snare tightened. Blood samples were drawn preoperatively and 24, 48, and 72 hours postoperatively for creatinine analysis. At 72 hours the animals were sacrificed and their kidneys morphologically evaluated. The C-ISC group had a significantly higher mean postoperative plasma creatinine ($p < 0.01$) as well as significantly higher plasma creatinine levels at 24 ($p < 0.005$) and 48 hours ($p < 0.05$) than did the I-ISC group. The C-ISC group also demonstrated significantly greater histologic damage than the I-ISC group ($p < 0.002$) when assessed by a pathologist blinded to the intervention. Sham rats did not demonstrate functional or morphologic damage. These data demonstrate a significantly improved outcome when 45 minutes of renal ischemia is interrupted by periods of reperfusion. We are led to conclude that in this setting reperfusion injury did not overwhelm the salutary effects of interrupting the 45-minute ischemic event. (Ann Vasc Surg 1993;7:150-155.)

Although the incidence of postoperative acute renal failure (ARF) complicating vascular surgery has decreased in recent years, it is not rare. When it does occur, it remains a significant problem with often dire consequences.[1] In a series of 116 ruptured abdominal aortic aneurysms, abnormal serum creatinine levels (>1.8 mg/dl) were documented in 72% of surviving patients. In this experience a creatinine level >4 mg/dl was associated with a mortality rate of 72%.[2] In the series of Crawford et al.[3] comprising 605 patients with thoracoabdominal aortic aneurysms requiring a period of suprarenal cross-clamping, it was noted that 5% of individuals with normal preoperative blood creatinine levels required dialysis after operation. In those patients with an elevated preoperative blood creatinine level (≥2 mg/dl), 17% required dialysis postoperatively and, of these, 62% were dead within 90 days. Preoperative renal function,[3] blood loss, and clamp time[2] correlated best with postoperative renal failure in these series. Preoperative renal function and blood loss are not typically subject to direct surgical manipulation, whereas clamp time may be lessened and/or

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kidneys may be intermittently reperfused during the ischemic period.

A number of modalities are currently in clinical or experimental use to either reduce or prevent postoperative ARF. They include vigorous hydration, diuretics, angiotensin converting enzyme inhibitors, profound renal hypothermia,[4] and most recently in an effort to reduce reperfusion injury, free radical scavengers.[5-9] Yet a number of straightforward interventions that might reduce the incidence of postoperative ARF have not been systematically evaluated. In this regard, interrupting the ischemic period with intermittent reperfusion might be seen as beneficial. Conversely, if intermittent reperfusion occasioned increased reperfusion injury from the repeated ischemia-reperfusion cycles, it might prove deleterious.

Although the problem has obvious operating room importance, there are mechanistic considerations as well. Ischemia-reperfusion damage vs. beneficial preconditioning effects are critical issues in the systematic study of ischemia. Protective preconditioning effects have been described in some settings for cardiac muscle and suggested for brain. The issues are not clear or universal and most certainly not well defined for renal ischemic episodes. In this study we examined the effect of intermittent reperfusion on the function and morphology of rodent kidneys exposed to 45 minutes of ischemia.

METHODS

Operative protocol. Twenty-four adult male Sprague-Dawley rats weighing 300 to 400 g were anesthetized with halothane via chamber induction (1.5%) and mask maintenance (0.75%). Core temperature was continuously monitored with a rectal probe and meticulously maintained at 37 ± 1° C with a thermal pad and an external heat source. For both ischemic protocols and the sham protocol, each animal underwent a right nephrectomy and isolation of the left renal pedicle through bilateral flank incision. The left renal artery and vein were isolated and encircled with a snare of polyethylene tubing (PE 10 in a sheath of PE 160). In the ischemic groups the snare was tightened and secured. Eleven animals underwent 45 minutes of renal ischemia by continuous occlusion (C-ISC), whereas 10 animals received 45 minutes of renal pedicle occlusion interrupted (I-ISC) at 15 and 30 minutes of occlusion by release of the snare and reperfusion of the kidney for a period of 5 minutes each. The snare was then reapplied, tightened, and secured. A group of three sham rats underwent the above procedure but did not have the snare tightened. Confirmation of ischemia and reperfusion was based on direct observation of kidney color changes, after which the kidney was replaced within the abdominal cavity to minimize any cooling effects. Prior to the occlusion period, anesthesia was reduced until extremity withdrawal was demonstrated. This reduced and standardized the anesthetic level at which occlusion would proceed. Flank incisions were closed and the rats were recovered with free access to food and water for 72 hours. Two rats were excluded from analysis: one for surgical error in the C-ISC group and one with a 24-hour postoperative creatinine of 4.8 mg/dl, which rejected it from the I-ISC data set by the Q-value criteria[10] for statistical outliers.

Blood was drawn from the tail vein preoperatively for measurement of plasma glucose and creatinine and postoperatively at 24 and 48 hours for measurement of plasma creatinine levels. Animals surviving 72 hours were anesthetized with halothane as before. Blood was drawn for plasma creatinine and hematocrit. The abdomen was opened through a midline incision, and the left and right flanks were examined for hemorrhage, torsion of the remaining kidney, and patency of the inferior vena cava. The left kidney was removed, bisected through its long axis, and immediately fixed in 10% buffered formalin. Any animal that died prior to 72 hours was examined in a similar fashion during a postmortem. Animal care complied with the "Principles of Laboratory Animal Care" and the "Guide for the Care and Use of Laboratory Animals."

Morphologic assessment. Fixed kidneys were embedded in paraffin, cut into 4 μm sections, and stained with hematoxylin and eosin. Tissues prepared in this fashion did not exhibit any confounding histologic autolysis. Two long sections of each kidney were examined by a pathologist blinded to the intervention. Morphologic evaluation of ischemic damage was assessed by grading the extent of necrosis of the proximal convoluted tubules as outlined by Jablonski et al.[11] and adapted here in Table I. Grading was performed according to the predominant pattern present.

Biochemical assays. Plasma creatinine levels were determined by a quantitative colorimetric assay first described by Jeffe[12] and later modified by Slot[13] and Heinegard and Tiderstrom[14] (Sigma Kit No. 555; Bausch and Lomb Spectronic 20). Calibration curves were constructed and demonstrated a calculated R^2 of 0.993 (Sigma creatinine standards No. 925-3 and No. 925-15). Plasma

Table I. Description and results of histopathologic grades for the C-ISC, I-ISC, and sham rats*

Grade	Description	C-ISC	I-ISC	Sham
0	Normal			XXX
1	Mitoses and necrosis of individual cells		XXXXX	
2	Necrosis of all cells in adjacent proximal convoluted tubules with survival of surrounding tubules	XX	XXXX	
3	Necrosis confined to the distal third of the proximal convoluted tubule with a band of necrosis extending across the inner cortex and outer medulla	XXX		
4	Necrosis affecting all three segments of the proximal convoluted tubule	XXXX		

NOTE: The X's represent the number of animals in each group demonstrating a grade of histologic damage.
*Modified from Jablonski P, Howden BO, Rae DA, et al. An experimental model for assessment of renal recovery from warm ischemia. Transplantation 1983;35:198-204.

glucose levels were determined through quantification of hydrogen peroxide following the enzymatic conversion of glucose and oxygen to gluconic acid and hydrogen peroxide (glucose analyzer, YSI model No. 23A).

Statistical analysis. All values were expressed as mean ± standard error of the mean. Comparisons of biochemical end points between C-ISC and I-ISC groups were assessed by Student's *t* test. Histopathologic grades between groups were assessed by Mann-Whitney *U* analysis. Any decision to exclude outlying data was based on the Q test.[10] Statistical analysis was performed with the Statview 512 program on a Macintosh computer.

RESULTS

Fig. 1 demonstrates the effect of ischemia on plasma creatinine in three groups of rats: C-ISC (n = 10), I-ISC (n = 9) , and sham (n = 3). The I-ISC and C-ISC groups did not differ with respect to either preocclusion plasma glucose levels (204 ± 14 and 224 ± 20 mg/dl, respectively) or hematocrit at the time of sacrifice (36 ± 1.5 and 37 ± 0.5%, respectively). Both groups demonstrated an increase in creatinine following the ischemic insult. However, the C-ISC group had a significantly higher 24-hour plasma creatinine ($p < 0.005$) than the I-ISC group. At 48 hours the C-ISC group (n = 9) continued to show higher creatinine levels than the I-ISC group (n = 9) ($p < 0.05$). This trend continued to 72 hours, but at that point the plasma creatinine levels of the two groups were no longer significantly different. None of the sham group's individual postoperative creatinine levels was statistically different from the group's preocclusion value. One animal in the C-ISC group was dead by the 48-hour time point. There were no deaths in the I-ISC group.

Fig. 2 is a comparison of the mean postoperative plasma creatinine levels for the C-ISC, I-ISC, and

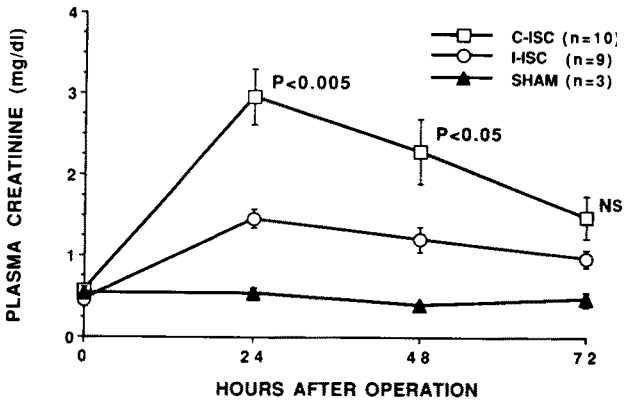

Fig. 1. The effect of interrupted ischemia on rats operated on at 37° C. Both the C-ISC and the I-ISC groups showed an increase in creatinine following the ischemic insult. However, the plasma creatinine levels from the C-ISC group tracked significantly higher than the I-ISC group for the first 48 hours following surgery. The creatinine levels from the sham group were not altered by the operative procedure.

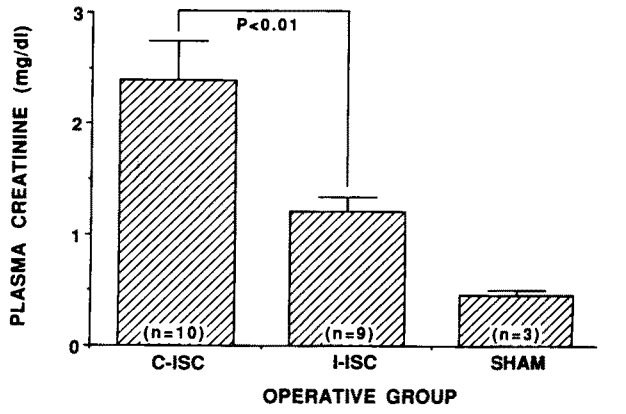

Fig. 2. The effect of interrupted ischemia on the mean of the postoperative creatinine levels for the C-ISC, I-ISC, and sham groups. The C-ISC group had a significantly higher mean postoperative plasma creatinine than the I-ISC group did. The mean postoperative plasma creatinine for the sham group was not different from its control value.

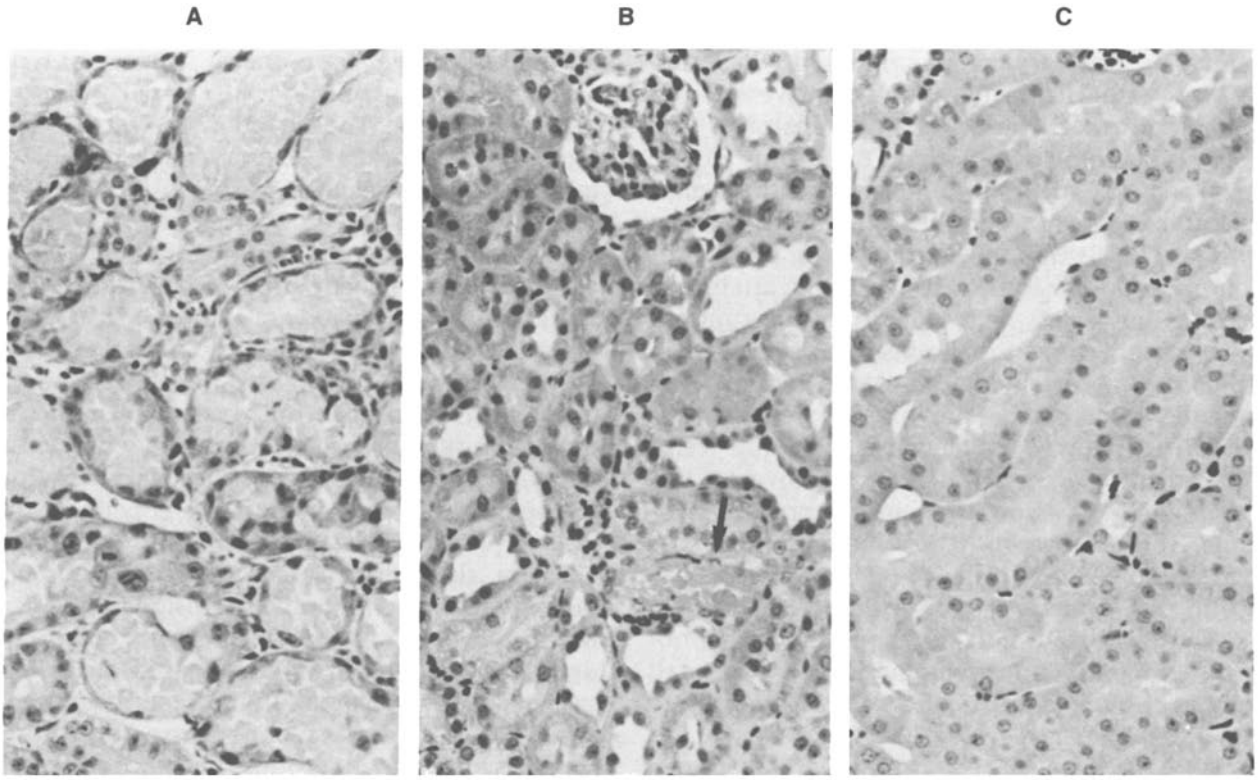

Fig. 3. Photomicrographs demonstrating the histology of renal damage. **A,** Renal cortex from a kidney subject to continuous ischemia demonstrating grade 4 damage to the proximal tubules. All of the tubules are lined by necrotic or regenerating epithelium. **B,** Renal cortex from a kidney subject to intermittent ischemia demonstrating grade 2 damage. Occasional tubules lined by necrotic or regenerating epithelium (arrow) are surrounded by adjacent, intact tubules. **C,** Renal cortex from the sham animal is free of tubular damage.

the sham groups and is based on the average value of the postocclusion creatinine levels for each rat. The C-ISC group had significantly higher mean postoperative creatinine levels than the I-ISC group ($p < 0.01$). The sham group's mean postoperative creatinine was not different from its preocclusion level.

On histologic evaluation the I-ISC group demonstrated a lesser degree of acute proximal tubular damage than did the C-ISC group (Table I) ($p < 0.002$, Mann-Whitney U). Two of the nine C-ISC animals had grade 2 damage to the proximal tubules, three had grade 3, and four demonstrated grade 4 damage (Fig. 3, *A*). In contrast, of the nine I-ISC animals, five demonstrated grade 1 proximal tubular damage and four demonstrated grade 2 damage (Fig. 3, *B*). None demonstrated grade 3 or 4 changes. The sham animals did not show histologic evidence of acute tubular injury (Fig. 3, *C*).

In addition, focal renal infarcts were observed in five animals of the I-ISC group, three animals of the C-ISC group, and in one animal of the sham group. These were distinguished from acute tubular damage by their focality, their subcapsular location (occasionally extending into the medulla), and the involvement of all elements of the renal parenchyma rather than proximal tubules alone. The presence of infarcts did not correlate with the grade of tubular damage in the remainder of the renal parenchyma or the functional parameters discussed above and are presumed to be incidental sequelae of the surgical process. Thus we did not include these occurrences in our morphologic analysis for ischemia.

DISCUSSION

Clinical relevance. This study demonstrated improved functional and morphologic outcome in kidneys subjected to 45 minutes of warm ischemia by interrupting the ischemic period with brief episodes of reperfusion. While at first glance this may intuitively seem self-apparent, the potential for injury due to repetitive ischemia-reperfusion

cycles is very real. The effects of intermittent renal pedicle occlusion were examined in the early 1970s,[15-17] but the results at that time were somewhat conflicting. Yoho et al.[17] demonstrated that dogs undergoing two 1-hour occlusions of the renal pedicle with 3 minutes of intervening reperfusion had a lower mortality rate and blood urea nitrogen levels than dogs undergoing 2 hours of continuous occlusion. Schirmer,[15] in an attempt to account for Yoho's data, noted that intermittent reperfusion lessens the diminution of respiration in the renal cortex and glycolysis in the renal medulla immediately following the occlusive period. Truss,[2] however, at 3 weeks following occlusion failed to demonstrate any improvement in renal glycolysis associated with interrupted ischemia. The present study of interrupted ischemia was unique in that it had, in addition, a control group that underwent continuous ischemia (C-ISC), it evaluated both functional and morphologic endpoints, and it maintained the animals long enough (3 days) to allow for recovery from the initial insult.

More recently, Thornton and Zager[18] examined the effects of 35 minutes of renal pedicle occlusion with or without various periods of intermittent reperfusion on tissue levels of adenine nucleotide breakdown products and on functional and morphologic end points. The animals undergoing intermittent ischemia demonstrated a significant reduction in breakdown products at the end of the ischemic period. However, this difference did not persist, and 30 minutes after ischemia the continuous and intermittent groups demonstrated similar levels of total adenine nucleotides (the sum of ATP + ADP + AMP). Also, with this model, Thornton and Zager failed to demonstrate any functional or morphologic difference associated with intermittent reperfusion. Our study differs in that both our ischemic periods and reperfusion periods were longer than that of Thornton and Zager's. Thus the worse functional and morphologic outcome found in our study may simply be due to a more severe ischemic insult. The protection observed may then be attributed to the longer reperfusion periods. That intermittent reperfusion may actually be protective is suggested by the significantly higher level of end-ischemic total adenine nucleotide content found in the intermittent reperfusion group by Thornton and Zager. Their data thus suggest a mechanism for our results in that intermittent reperfusion may better sustain total adenine nucleotides during a prolonged ischemic event.

Model considerations. We chose 45 minutes of renal pedicle occlusion because it is a reasonable approximation of clinically relevant ischemic periods and is known to produce a substantial renal insult yet allow survival for functional and histologic assessments. The elevation in plasma creatinine and mortality rate seen in this study with 45 minutes of renal pedicle occlusion at 37° C and contralateral nephrectomy agrees with that previously reported by our laboratory and that seen by other authors.[11] This period of occlusion represents a moderate to severe ischemic insult. The important and potentially confounding variables of hyperglycemia,[19,20] volume loss, and temperature effects were controlled and ruled out by monitoring the preocclusion plasma glucose, the hematocrit at the time of sacrifice, and body core temperature throughout the ischemic period. None of these variables was different in either group. In addition, the kidney was returned to the cavity during each ischemic and reperfusion period so as to minimize any protective effect of cooling. Indeed the reperfused kidneys would have been warmed by the two additional reperfusion periods, hence favoring a more adverse effect in the reperfused (warmed) group. This was not the case and the reperfused kidneys were less damaged.

Other authors have attempted to alter the functional outcome of renal ischemia by varying the manner in which the kidney experiences the ischemic event. Zager et al.[21] examined the response of rats having experienced one episode of brief ischemia (15 minutes) to an additional ischemic episode (25 minutes). If the initial ischemic event occurred 3.5 to 24 hours prior to the subsequent ischemic event, no sensitization to the ischemia occurred. However, if the initial ischemic event occurred just 30 minutes prior to the subsequent ischemic event, significant increases in functional damage was seen. Our data coupled with Zager's would suggest that although renal function may be preserved by interrupted ischemia, it is not preserved by preconditioning the kidney with a short ischemic episode.

A number of authors have demonstrated significant success in protecting the ischemic kidney with free radical scavengers.[5-9] The proposed mechanism for this protection involves reperfusion injury and has been well reviewed.[9] In this study, the I-ISC group received a total of three reperfusion periods, whereas the C-ISC group received one reperfusion period. The I-ISC group demonstrated significantly less functional and morphologic damage than did the C-ISC group. These findings would suggest that at least in this model reperfusion injury does not play a major role. It is possible that with three ischemic periods of 15 minutes each the

cell's natural ability to compensate for free radicals was not abolished. Further, multiple episodes of reperfusion may allow for washout of toxic metabolites and/or the maintenance of ATP and the adenine nucleotide pool. If so, it is not surprising that the I-ISC group fared better than the C-ISC group. More work is necessary to elucidate the mechanisms of damage in this model.

Interrupted ischemia as a means to reduce overall ischemic damage has been attempted in other tissues, most notably the myocardium. Reimer et al.[22] noted that four 10-minute episodes of left circumflex coronary artery occlusion separated by 20 minutes of reperfusion produced no necrosis and less adenine nucleotide depletion than 40 minutes of continuous occlusion. In contrast, Asimakis et al.,[23] in an isolated perfused rat heart, showed that interrupted ischemia does lead to a cumulative loss of adenine nucleotides in isolated mitochondria. As they suggest, this difference in results may be due to the model and/or the site of the nucleotide assay: Reimer et al. used whole subendocardium, whereas Asimakis et al. used isolated mitochondria.

CONCLUSION

We demonstrated a significantly improved outcome following 45 minutes of warm renal ischemia with intermittent reperfusion. The clinical advantage of this strategy is significant. Its ease of application and low attendant risk demands its clinical evaluation. It may now be possible to reduce the frequency of postoperative ARF in settings where intermittent perfusion is possible. Further investigations targeting critical mechanistic issues for these potentially protective effects are in order.

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