

# Lack of Evidence for a Remote Effect of Renal Ischemia/Reperfusion Acute Kidney Injury on Outcome from Temporary Focal Cerebral Ischemia in the Rat

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**Objective:** Acute kidney injury (AKI) and ischemic stroke may occur in the same cardiac surgical patient. It is not known if an interaction exists between these organ injuries. Isolated renal ischemia/reperfusion is associated with dysfunction in remote, otherwise normal organs, including the brain. In a rat model of simultaneous bilateral renal artery occlusion (BRAO) and middle cerebral artery occlusion (MCAO), the authors tested the hypothesis that AKI would worsen experimental stroke outcome.

**Design:** Sixty thermoregulated anesthetized rats were randomized to (1) 40-minute BRAO, (2) 80-minute MCAO, or (3) simultaneous BRAO + MCAO. Serum creatinine was measured at baseline and 2 and 7 days after organ reperfusion. Neurologic function and brain and kidney histologies were measured on day 7. In a parallel study, serum cytokines were measured over 16 hours.

**Setting:** Laboratory.

**Participants:** Male Wistar rats.

**Interventions:** Combined or isolated BRAO and MCAO.

COMBINED KIDNEY AND BRAIN INJURY can occur in different clinical circumstances, including anoxia, endocarditis, cardiac arrest, exsanguination, and surgery. Simultaneous insults of the kidney and brain are of particular interest in cardiac surgery, in which stroke occurs in 1%-4% of all cardiac operations<sup>1-3</sup> and in as many as 9.7% of high-risk cases.<sup>4</sup> Acute kidney injury (AKI) is even more common after cardiac surgery, with 8%-14% of patients sustaining at least a moderate impairment of renal filtration (eg,  $\geq 1$ -mg/dL postoperative accumulation of serum creatinine) and 1%-3% requiring dialysis.<sup>5-8</sup> Although AKI and stroke are independent predictors of mortality<sup>9,10</sup> and can result from atheroembolic events,<sup>11,12</sup> the possibility that the pathophysiology of these perioperative complications may interact has been suggested.<sup>13</sup>

Experimental evidence has established a relation among AKI, inflammation, and remote organ dysfunction.<sup>14-19</sup> In the brain, Liu et al<sup>20</sup> observed increased proinflammatory cytokine expression, microgliosis, and blood-brain barrier disruption in otherwise normal mice subjected to AKI. These physiologic changes also are integral to the evolution of ischemic brain injury. Supporting this, there are numerous other reports of encephalopathic events associated with uremia that are of relevance to the known pathomechanisms of cerebral ischemia.<sup>21-24</sup> To date, a potential interaction between AKI and ischemic brain injury has not been explored experimentally. A better understanding of the relation between AKI and ischemic stroke would facilitate the design of more targeted organ protection interventions. To this end, the authors developed a rat model of tandem bilateral renal artery occlusion (BRAO) and middle cerebral artery occlusion (MCAO) to test the hypothesis that neurologic dysfunction and cerebral infarct volume would worsen when focal cerebral ischemia occurs in the setting of AKI.

**Measurements and Main Results:** AKI was similar between the BRAO and BRAO + MCAO groups, with greater 48-hour creatinine increases ( $p < 0.02$ ) and renal histopathologic scores ( $p < 0.001$ ) in these groups than with MCAO alone. Neurologic scores correlated with cerebral infarct size ( $p = 0.0001$ ). There were no differences in neurologic score ( $p = 0.53$ ) and cerebral infarct volume ( $p = 0.21$ ) between the MCAO and BRAO + MCAO groups. There was no association between cerebral infarct size or neurologic score and 48-hour creatinine increase. Interleukin-6 was increased during reperfusion ( $p < 0.0001$ ), but a difference among groups was absent ( $p = 0.41$ ).

**Conclusions:** In contrast to the effects reported for AKI on normal remote organs, AKI had no influence on infarct size or neurologic function after experimental ischemic cerebral stroke.

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**KEY WORDS:** brain, ischemia, acute kidney injury, remote organ injury

## METHODS

The following studies were approved by the Duke University animal care and use committee. Male Wistar rats (9-10 weeks of age; Harlan-Sprague-Dawley, Indianapolis, IN) were housed in a temperature-controlled environment with an artificial light/dark cycle (12 h). They were fasted from food to standardize the intraschismic blood glucose concentration but were allowed free access to water for 12 hours before ischemia. Rats then were anesthetized with 5% isoflurane in 30% O<sub>2</sub>/N<sub>2</sub>. The trachea was intubated, and the lungs were ventilated mechanically. During surgery, anesthesia was maintained with 1.3%-1.8% isoflurane.

The pericranial temperature was controlled at  $37.5 \pm 0.1^\circ\text{C}$  by surface heating and cooling using a 22-gauge needle thermistor placed percutaneously adjacent to the skull. The tail artery was cannulated to monitor mean arterial blood pressure continuously. Arterial blood was sampled intermittently for measurements of blood gases, hematocrit, and glucose.

Experiments were performed to determine the duration (30, 40, or 50 min) of BRAO sufficient to cause AKI. Rats ( $n = 4$  per BRAO duration

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group and  $n = 2$  in sham group) underwent escalating BRAO durations. Rats were anesthetized with isoflurane in 30% O<sub>2</sub>/balance N<sub>2</sub>, the trachea was intubated, and the lungs were ventilated mechanically to maintain normocapnia. Arterial blood was withdrawn to define serum creatinine concentration (see below). To achieve BRAO, rats were positioned prone. Bilateral flank incisions were made approximately 1 cm lateral to the spinous processes, and retroperitoneal fat was dissected from the paraspinous muscles. The renal pedicles were exposed. Heparin (50 IU) was given to prevent thrombus formation. Smooth vascular clamps were placed over the proximal portion of the 2 renal arteries. Correct positioning was confirmed visually by the observed blanching of each kidney through the operating microscope. After 30, 40, or 50 minutes, the clamps were removed to allow renal reperfusion, confirmed by the observation of blanching reversal. The wounds were infiltrated with 0.25% bupivacaine and closed with deep and superficial sutures. The arterial catheter was removed. The animals were allowed to awaken, the trachea was extubated, and a recovery interval of 48 hours was allowed.

The rats then were anesthetized with isoflurane. Arterial blood was sampled for serum creatinine concentration. Blood samples (500  $\mu$ L) were allowed to coagulate at room temperature and then centrifuged at 13,000g for 10 minutes; serum was collected and stored at  $-70^{\circ}\text{C}$ . Spectrophotometric measurements (510 nm) of serum creatinine were made in duplicate for each time point using a Direct Creatinine Reagent Set (Eagle Diagnostics, Desoto, TX).

The animals then underwent *in situ* formalin fixation. The kidneys were excised and embedded in paraffin. Coronal sections (5- $\mu$ m thickness) were collected in quadruplicate from the rostral and caudal locations. Sections were stained with hematoxylin and eosin. An observer blinded to group assignment, using a system developed by Hamar et al,<sup>25</sup> scored histologic damage. Fifty proximal renal tubules from each kidney were assigned a grade of 0 (no damage), 1 (mild damage with rounding epithelial cells and a dilated tubular lumen), 2 (severe damage with flattened epithelial cells and loss of nuclear staining), or 3 (destroyed tubules with flat epithelial cells lacking nuclear staining). The values then were averaged across all 50 tubules sampled in a single kidney to yield a Hamar injury score. The most severe injury score of the 2 kidneys from each animal was used for subsequent analyses.

Baseline serum creatinine values (mean  $\pm$  standard deviation) were  $0.47 \pm 0.19$  mg/dL (sham),  $0.50 \pm 0.12$  mg/dL (30-min BRAO),  $0.44 \pm 0.20$  mg/dL (40-min BRAO), and  $0.62 \pm 0.18$  mg/dL (50-min BRAO). Creatinine values at 48 hours were  $0.37 \pm 0.12$  mg/dL (sham),  $1.88 \pm 0.34$  mg/dL (30-min BRAO),  $2.08 \pm 2.09$  mg/dL (40-min BRAO), and  $3.70 \pm 1.88$  mg/dL (50-min BRAO). Hamar scores at 48 hours were  $0.11 \pm 0.16$  (sham),  $1.75 \pm 0.46$  (30-min BRAO),  $1.62 \pm 0.76$  (40-min BRAO), and  $2.55 \pm 0.57$  (50-min BRAO).

Using these data as a guide, permutations of MCAO (80 or 90 min; for MCAO procedures, see below) and BRAO (40 or 50 min) durations were performed in combination across a series of animals. Animals receiving 80-minute MCAO and 40-minute BRAO consistently showed a mortality rate near 0 over a 7-day observation interval. Increasing ischemia duration by 10 minutes (renal or brain) caused frequent mortality. Thus, 40-minute BRAO and 80-minute MCAO were selected for the formal study protocol.

After anesthesia induction with isoflurane, endotracheal intubation, onset of mechanical ventilation, and placement of a tail artery catheter as described earlier, all rats underwent surgical preparation for MCAO, as previously described.<sup>26</sup> Using an operating microscope, a midline ventral cervical skin incision was made, and the right common carotid artery was identified. The internal carotid artery was dissected distally until the origin of the pterygopalatine artery was visualized. The external carotid artery was ligated and bisected to create a small stump. A 30-minute interval was allowed for physiologic stabilization. Heparin

(50 IU) was given through the arterial catheter to prevent unregulated thrombosis.

Rats then were assigned randomly to undergo MCAO only, BRAO only, or MCAO plus BRAO ( $n = 20$  per group). In the MCAO and BRAO groups, a 0.25-mm diameter nylon filament, coated with silicon (0.38-mm diameter), was inserted into the external carotid artery stump and advanced 19-20 mm from the carotid artery bifurcation into the internal carotid artery until resistance was felt. In the BRAO group, a filament was not introduced. A timer was started.

All rats were turned prone and underwent surgical exposure of the renal pedicles as described earlier. Ten minutes after the MCAO timer was started, vascular clamps were placed over the proximal portion of the 2 renal arteries in the BRAO and MCAO + BRAO groups. Correct positioning was confirmed by the observed blanching of each kidney. No renal artery clamp was placed in the MCAO group. After 40 minutes, the clamps were removed to allow renal reperfusion. Flank incisions were closed using deep and superficial sutures.

All rats were positioned supine. At 80 minutes after the MCAO timer was started, the filament was removed, and the wound was closed with suture. All wounds were infiltrated with 0.25% bupivacaine. Isoflurane was discontinued, the animals were allowed to awaken, and the trachea was extubated. Rats were returned to their cages after a 1-hour recovery interval. Buprenorphine, 0.1 mg/kg per day intraperitoneally, was provided for rats showing signs of pain or distress.

Physiologic values were recorded 30 minutes before MCAO onset (or the sham MCAO equivalent), at the midpoint of MCAO, and 5 minutes after onset of MCA reperfusion. Serum creatinine was measured before MCAO onset and at 48 hours and 7 days after MCAO (or the sham equivalent).

At 7 days after ischemia, the authors used a previously described scoring system that evaluates general status (spontaneous activity, body symmetry, gait), simple motor deficit (forelimb asymmetry, circling, hind-limb placement), complex motor deficit (vertical screen climbing, beam walking), and sensory deficit (hind limb, trunk, vibrissae, and face touch).<sup>27</sup> The score given to each animal at the completion of testing was the sum of the 4 individual scores, with 0 being the minimum (best) score and 48 the maximum possible (worst) score. This scoring system has been shown to correlate with cerebral infarct size.<sup>27</sup> The same experienced observer, who was blinded to group assignment, assigned all scores.

After neurologic evaluation, the rats were anesthetized using 5% isoflurane. A median sternotomy was performed to expose the heart. A metal cannula was inserted into the left ventricle and advanced into the ascending aorta. An incision was made in the right atrium, and normal saline, 30 mL, was infused to clear blood from the vasculature. Then, the animal was decapitated. The brain was removed, frozen at  $-20^{\circ}\text{C}$  in 2-methylbutane, and stored at  $-80^{\circ}\text{C}$  for later analysis. The body of the rat then was infused with additional normal saline, 100 mL, and then 10% buffered formalin, 100 mL. The kidneys were removed, placed in 10% buffered formalin, and stored at  $4^{\circ}\text{C}$ .

Serial quadruplicate 20- $\mu$ m-thick coronal brain sections were taken using a refrigerated cryotome at 720- $\mu$ m intervals over the rostral-caudal extent of the infarct. The sections were dried and stained with hematoxylin and eosin. A representative section from each 720- $\mu$ m interval was digitized with a video camera controlled by an image analyzer (M2 Turnkey System; Imaging Research, St Catharines, ONT, Canada). The image of each section was stored as a 1,280- $\times$  960-pixel matrix and displayed on a video monitor. With the observer blinded to the experimental conditions, the following regions of interest were outlined with a cursor: the noninfarcted ipsilateral cerebral cortex, the noninfarcted ipsilateral subcortex, the contralateral cerebral cortex, and the contralateral subcortex. The area within each region of interest (square millimeters) was determined by an automated counting of the calibrated pixels contained within the region of interest. The ipsilateral

noninfarcted cortex and subcortex areas were subtracted from the corresponding contralateral region of interest values. Infarct volumes (cubic millimeters) were computed as running sums of the subtracted infarct area multiplied by the known interval (eg, 720  $\mu\text{m}$ ) between sections over the rostral-caudal extent of the infarct calculated as an orthogonal projection.<sup>28</sup>

A separate cohort of animals ( $n = 6$  each for the MCAO, BRAO, and MCAO + BRAO groups) was treated as described earlier, with the following exceptions. Blood samples (500  $\mu\text{L}$ ) were taken at baseline and 1, 2, 6, and 16 hours after ischemia from a chronically positioned jugular venous catheter. Whole-blood samples were centrifuged at 3,000 rpm for 10 minutes to isolate serum, which was removed and stored at  $-80^\circ\text{C}$ . These samples were processed by an enzyme-linked immunosorbent assay to determine serum concentrations of interleukin-1 $\beta$  (IL-1 $\beta$ ; sensitivity  $>12.5$  pg/mL), interleukin-6 (IL-6; sensitivity  $>12.5$  pg/mL), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ; sensitivity  $>6.3$  pg/mL; SearchLight Sample Testing Service; Pierce Biotechnology, Woburn, MA).

Physiologic values (Table 1) were compared qualitatively to preserve statistical power. Statistical analysis was performed using StatView 5.0.1 (SAS Institute Inc, Cary, NC). The Hamar score value from the most severely injured kidney was selected from each animal for intergroup comparisons of renal histologic damage. Neurologic and Hamar scores were compared among groups using the Kruskal-Wallis H statistic. One-way analysis of variance was used to compare cerebral infarct volumes. In the presence of a significant F ratio, post hoc testing was performed using the Scheffe test. The Pearson rank-sum correlation coefficient was used to compare neurologic scores and cerebral infarct sizes, left versus right kidney Hamar scores, and Hamar score with 48-hour serum creatinine values within animals across groups. To explore for a potential association between the magnitude of renal dysfunction and the cerebral infarct size or neurologic function in the MCAO + BRAO group, linear regression analysis was performed using 48-hour serum creatinine as the independent factor. Repeated

measures analysis of variance was used to compare serum creatinine, IL-6, and TNF- $\alpha$  values among groups, with time as the repeat variable. Parametric values are reported as mean  $\pm$  standard deviation. Non-parametric values (neurologic and Hamar scores) are reported as median  $\pm$  interquartile range.

## RESULTS

Body weight, arterial blood gases/pH, blood glucose, pericranial temperature, blood pressure, and hematocrit values were similar among the groups (Table 1).

There was a main effect of group on serum creatinine ( $p = 0.005$ ) and an interaction between group and time ( $p = 0.0002$ ). BRAO, with or without MCAO, increased serum creatinine concentrations at 48 hours compared with MCAO alone ( $p < 0.03$ ), without a difference between the BRAO and MCAO + BRAO groups ( $p = 0.07$ ). Creatinine values in the 2 groups recovered to baseline by 7 days after reperfusion (Fig 1A). Hamar renal histologic scores demonstrated more severe kidney injury in the treatment groups that received BRAO ( $2.12 \pm 0.12$  and MCAO + BRAO  $2.12 \pm 0.20$  v MCAO alone  $1.51 \pm 0.57$ ;  $p < 0.0001$ ). MCAO + BRAO did not worsen Hamar scores versus BRAO alone ( $p = 0.92$ ). Histologic injuries correlated highly between the left and right kidneys for all animals (right kidney Hamar score,  $0.233 + 0.889 \times$  left kidney Hamar score;  $R^2 = 0.795$ ; Fig 1B). Day 7 Hamar scores were associated closely with 48-hour serum creatinine values ( $p = 0.0001$ ;  $\rho = 0.617$ ).

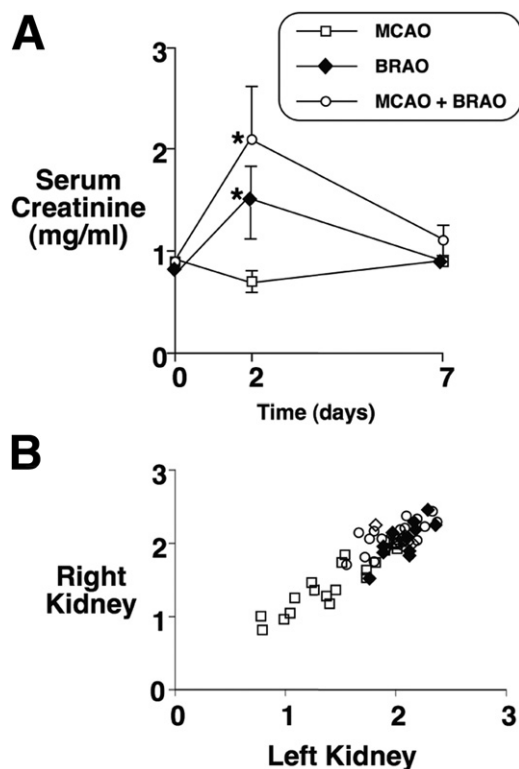
Neurologic scores were worse in the MCAO ( $13 \pm 10$ ;  $p = 0.0004$ ) and MCAO + BRAO ( $15 \pm 11$ ;  $p < 0.0001$ ) groups versus the BRAO group ( $2 \pm 2$ ). No difference between the

**Table 1. Physiologic Values**

	MCAO	BRAO	MCAO + BRAO
30 min before ischemia			
Animal weight (g)	271 $\pm$ 7	274 $\pm$ 8	271 $\pm$ 6
PaCO <sub>2</sub> (mmHg)	37 $\pm$ 2	39 $\pm$ 4	37 $\pm$ 3
PaO <sub>2</sub> (mmHg)	169 $\pm$ 9	157 $\pm$ 19	164 $\pm$ 10
Arterial pH	7.38 $\pm$ 0.03	7.37 $\pm$ 0.04	7.38 $\pm$ 0.04
Systolic pressure (mmHg)	92 $\pm$ 10	97 $\pm$ 10	90 $\pm$ 14
Diastolic pressure (mmHg)	78 $\pm$ 9	83 $\pm$ 8	78 $\pm$ 12
Blood glucose (mg/dL)	84 $\pm$ 17	91 $\pm$ 18	81 $\pm$ 11
Hematocrit (%)	41 $\pm$ 1	41 $\pm$ 2	41 $\pm$ 1
During ischemia			
PaCO <sub>2</sub> (mmHg)	39 $\pm$ 4	37 $\pm$ 3	38 $\pm$ 4
PaO <sub>2</sub> (mmHg)	164 $\pm$ 13	167 $\pm$ 15	173 $\pm$ 10
Arterial pH	7.34 $\pm$ 0.03	7.32 $\pm$ 0.03	7.32 $\pm$ 0.04
Blood glucose (mg/dL)	87 $\pm$ 21	95 $\pm$ 19	84 $\pm$ 17
Systolic pressure (mmHg)	88 $\pm$ 9	91 $\pm$ 12	93 $\pm$ 12
Diastolic pressure (mmHg)	67 $\pm$ 6	72 $\pm$ 10	69 $\pm$ 8
Pericranial temperature ( $^\circ\text{C}$ )	37.5 $\pm$ 0.2	37.5 $\pm$ 0.2	37.5 $\pm$ 0.2
Rectal temperature ( $^\circ\text{C}$ )	37.5 $\pm$ 0.2	37.5 $\pm$ 0.2	37.5 $\pm$ 0.2
5 min after ischemia			
Hematocrit (%)	40 $\pm$ 2	39 $\pm$ 2	40 $\pm$ 2
Systolic pressure (mmHg)	87 $\pm$ 7	87 $\pm$ 7	86 $\pm$ 10
Diastolic pressure (mmHg)	70 $\pm$ 6	69 $\pm$ 5	63 $\pm$ 8

NOTE. Values are presented as mean  $\pm$  standard deviation ( $n = 20$  per group).

Abbreviations: BRAO, 40-minute bilateral renal artery occlusion; MCAO, 80-minute middle cerebral artery occlusion; PaCO<sub>2</sub>, partial pressure of arterial carbon dioxide; PaO<sub>2</sub>, partial pressure of arterial oxygen.



**Fig 1.** (A) Serum creatinine concentrations at baseline and 48 hours and 7 days after renal ischemia (or sham surgery); \* $p < 0.03$  versus middle cerebral artery occlusion (MCAO). (B) Scatter plot of left and right kidney Hamar score values for individual rats in the groups with 80-minute middle cerebral artery occlusion without bilateral renal artery occlusion (BRAO; squares), 40-minute bilateral renal artery occlusion without middle cerebral artery occlusion (diamonds), and 80-minute middle cerebral artery occlusion combined with 40-minute bilateral renal artery occlusion (circles). 0, no histologic injury; 3, destroyed tubules with flat epithelial cells lacking nuclear staining.

MCAO + BRAO and MCAO groups was detected ( $p = 0.43$ ; Fig 2).

Total cerebral infarct sizes were larger in the MCAO ( $150 \pm 72 \text{ mm}^3$ ;  $p < 0.0001$ ) and MCAO + BRAO ( $127 \pm 71 \text{ mm}^3$ ;  $p < 0.0001$ ) groups versus the BRAO group ( $3 \pm 11 \text{ mm}^3$ ). No difference between the MCAO and MCAO + BRAO groups was detected ( $p = 0.453$ ). Similar patterns were present in the cortex and subcortex (Fig 3). Neurologic scores correlated with cerebral infarct size ( $p < 0.0001$ ;  $\rho = 0.835$ ). In the MCAO + BRAO group, there was no association between 48-hour creatinine and total infarct size or 7-day neurologic score (Fig 4).

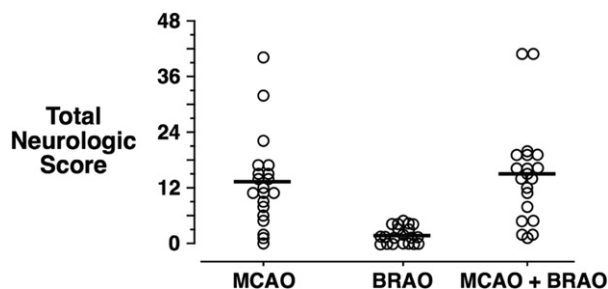
Serum IL-1 $\beta$  concentrations were below the limit of detection and thus are not reported. Figure 5 depicts serum IL-6 and TNF- $\alpha$  values. IL-6 increased as a function of the reperfusion interval, with restoration toward baseline values by 16 hours after ischemia. A main effect was present for time ( $p < 0.0001$ ) and but not for group ( $p = 0.41$ ). An interaction between factors was absent ( $p = 0.43$ ). TNF- $\alpha$  values did not reach statistical significance for a difference among groups ( $p = 0.36$ ) or as a function of time ( $p = 0.08$ ).

## DISCUSSION

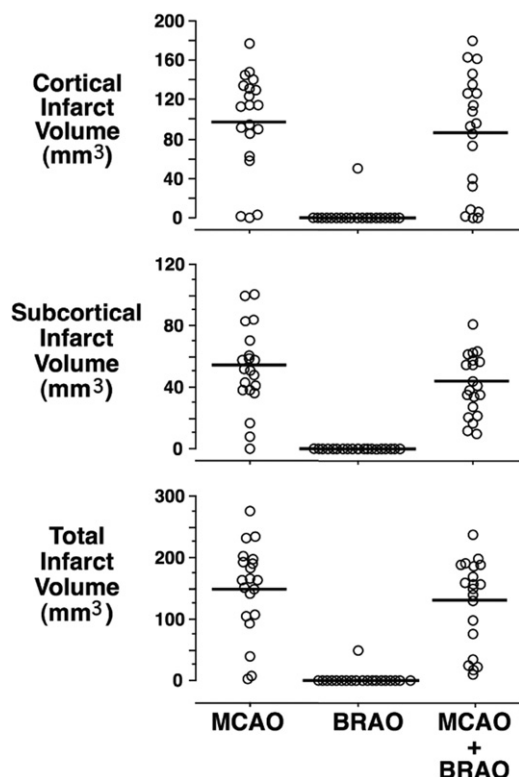
In the present study, experimental AKI did not affect the infarct size or neurologic dysfunction resulting from 80-minute MCAO in the rat. Similarly, the presence of a brain ischemic insult did not worsen AKI resulting from 40-minute BRAO. The results contrast with experimentally measured adverse effects of AKI in remote organs and are the first to evaluate specifically an interaction between AKI and focal ischemic stroke. Increased serum concentrations of proinflammatory cytokines are putative mediators of remote organ AKI effects and are believed to increase in part from impaired filtration and clearance as uremic toxins.<sup>29</sup> As observed in previous work with experimental renal artery occlusion,<sup>30</sup> the present study demonstrated increased serum IL-6 concentrations after AKI. Nevertheless, the present study failed to show altered cerebral infarct sizes or neurologic deficits in rats recovering from tandem MCAO and AKI.

In clinical settings, renal and neurologic impairments may co-exist. Improvements in cognitive performance occur with dialysis and renal transplantation in patients with chronic renal failure.<sup>31</sup> AKI has been associated with a worsened long-term outcome after cardiopulmonary bypass, myocardial infarction, aneurysmal subarachnoid hemorrhage, and stroke.<sup>5,32-34</sup> Although the list of toxic substances known to accumulate with impaired renal filtration continues to expand,<sup>29</sup> comorbidities inherent to patients with kidney disease complicate attempts to determine direct, clinically relevant, adverse effects of uremic toxins.

Experimental models present an opportunity to study AKI in isolation and have identified remote toxic effects of renal dysfunction on the lung,<sup>35,36</sup> heart,<sup>37</sup> liver,<sup>38,39</sup> intestine,<sup>40</sup> the contralateral kidney,<sup>41</sup> and the brain.<sup>23</sup> However, the picture remains complex. In rats subjected to 30-minute BRAO, Kelly<sup>14</sup> reported increased circulating TNF- $\alpha$ , an upregulation of myocardial markers of tissue inflammation, and the development of left ventricular systolic and diastolic dysfunctions. The exposure of a Langendorff rat heart preparation to human



**Fig 2.** Total neurologic score (0, no deficit) measured 7 days after 80-minute middle cerebral artery occlusion (MCAO), 40-minute bilateral renal artery occlusion (BRAO), or 80-minute middle cerebral artery occlusion plus 40-minute bilateral renal artery occlusion. Open circles represent values for individual rats. Horizontal lines indicate group mean values. Neurologic scores were worse in the middle cerebral artery occlusion ( $p = 0.0004$ ) and middle cerebral artery occlusion plus bilateral renal artery occlusion groups ( $p < 0.0001$ ) versus the bilateral renal artery occlusion group. Values for the middle cerebral artery occlusion plus bilateral renal artery occlusion and middle cerebral artery occlusion groups were not different ( $p = 0.43$ ).



**Fig 3.** Cortical, subcortical, and total cerebral infarct volumes measured 7 days after 80-minute middle cerebral artery occlusion (MCAO), 40-minute bilateral renal artery occlusion (BRAO), or 80-min middle cerebral artery occlusion plus 40-minute bilateral renal artery occlusion. Open circles represent values for individual rats. Horizontal lines indicate group mean values. No difference between the middle cerebral artery occlusion and the middle cerebral artery occlusion plus bilateral renal artery occlusion groups was detected.

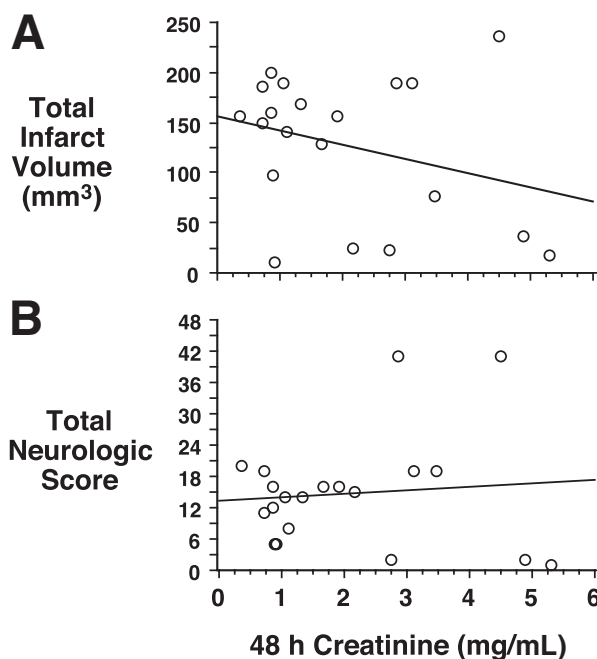
dialysis hemofiltrate identified cardiac depressant factors that were most evident in patients with moderate or severe acute renal dysfunction (associated with heart failure) compared with chronic renal failure.<sup>37</sup> In contrast, human dialysis hemofiltrate from patients with acute renal failure inhibited *in vitro* polymorphonuclear leukocyte chemotaxis, oxidative metabolism, and apoptosis, which could influence stroke evolution favorably.<sup>42</sup> In otherwise healthy animals, renal ischemia/reperfusion causes systolic and diastolic myocardial dysfunction,<sup>14</sup> increased pulmonary vascular permeability, alveolar hemorrhage, interstitial edema, and red blood cell sludging<sup>35</sup> and impairs alveolar fluid clearance pathways.<sup>15</sup> Numerous markers of hepatic dysfunction and increased hepatic TNF- $\alpha$  levels are evident after as little as 30 minutes of renal ischemia/reperfusion.<sup>38</sup> Increased circulating cytokines TNF- $\alpha$  and IL-6 levels are known predictors of adverse outcomes from cardiac surgery.<sup>43</sup> The authors could find no investigation of these effects in the context of a simultaneously injured brain, thus prompting the present study.

There are several possible explanations for the present negative findings. The authors' hypothesis that AKI-related circulating uremic toxins would worsen the outcome from cerebral ischemia is supported by a report that intraventricular injection

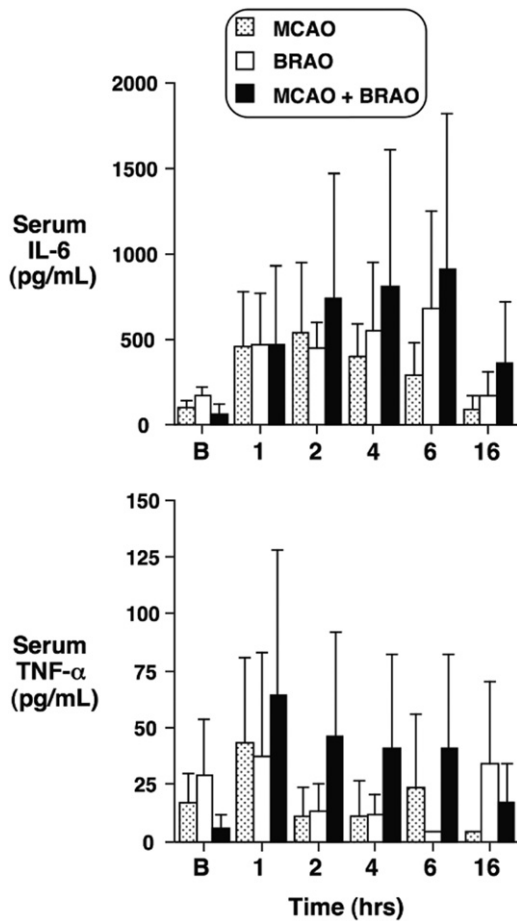
of TNF- $\alpha$  increased cerebral infarct size in a rat stroke model, whereas TNF- $\alpha$  antagonism decreased infarct size.<sup>44</sup> Increases of circulating TNF- $\alpha$  and other cytokines (eg, IL-6, IL-1 $\beta$ ) associated with renal ischemia/reperfusion may result in cerebral concentrations lower than those associated with an intracerebroventricular TNF- $\alpha$  injection. This is supported by the fact that the authors did not detect a substantial effect of tandem injury on circulating TNF- $\alpha$ . Further, although IL-6 presents a proinflammatory effect on other organs,<sup>45</sup> it improves focal cerebral ischemic outcome by modulating endogenous antiapoptotic and antioxidant defenses.<sup>46,47</sup>

Other studies reporting an association between renal ischemia and adverse effects in distant organs (eg, brain, lungs, and liver) have not evaluated tandem ischemia/reperfusion in those organs, as was completed in this study. The presence of cytokines in the systemic circulation may be inconsequential in the context of independent end-organ ischemic injury. Stated another way, the systemic release of inflammatory mediators from an ischemic kidney injury of the order of magnitude used in this investigation may have an inconsequential effect on the brain when the brain has endured a severe ischemic insult and an endogenous inflammatory response.

This raises the question of whether an interaction between AKI and a lesser degree of cerebral ischemia could occur that could be measured through a study of cognitive deficits.



**Fig 4.** Linear regression analysis in rats with middle cerebral artery occlusion plus bilateral renal artery occlusion using 48-hour serum creatinine as an independent variable and (A) total infarct volume or (B) 7 day neurologic scores as dependent factors. There was no association for either dependent factor (total infarct volume =  $155.8 - 14 \times 48\text{-h creatinine}$ ;  $R^2 = 0.09$ ,  $p = 0.71$ ; neurologic score =  $13.5 + 0.6 \times 48\text{-h creatinine}$ ;  $R^2 = 0.008$ ,  $p = 0.71$ ). Open circles represent values for individual rats. Best-fit lines are provided.



**Fig 5.** Serum interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) concentrations measured at baseline (B) and 1, 2, 4, 6, and 16 hours after bilateral renal artery occlusion (BRAO), middle cerebral artery occlusion (MCAO), or bilateral renal artery occlusion plus middle cerebral artery occlusion ( $n = 4-5$  per group). For interleukin-6, a main effect was present for the reperfusion interval ( $p \leq 0.0001$ ) but not for group ( $p = 0.41$ ). Statistical significance was not observed for tumor necrosis factor- $\alpha$  values as a function of time ( $p = 0.08$ ) or group ( $p = 0.36$ ). Values are presented as mean  $\pm$  standard deviation.

There is considerable evidence that renal failure alters mental status.<sup>31</sup> Only a direct investigation can answer this question. The magnitude of cerebral ischemia used in the present experiment repeatedly has been shown to be responsive to different physiologic and pharmacologic interventions, including inflammatory events associated with cardiopulmonary bypass.<sup>26,48-52</sup> This suggests a high grade of sensitivity of the lesion used in the present experiment to exogenous factors. If AKI and brain ischemia interact, the

present data would predict the effect to be small and perhaps better assessed in global ischemic insults, which can be titrated in severity to induce only selective neuronal necrosis, where cognitive function can be assessed independently of motor deficits.

The authors also may have missed an effect present with greater magnitudes of renal injury. As described in Methods, pilot work defined the maximal durations of combined MCAO and BRAO compatible with 1-week survival in the rat. Despite expert husbandry, these animals were not subjected to a management regimen approaching clinical critical care, when more severe injuries in either organ might be supported and plausibly show systematic interactions in injury evolution. To explore the possibility that an effect of AKI on ischemic brain may depend on the severity of kidney dysfunction, the authors performed a post hoc regression analysis to look for an effect of a relative increase in serum creatinine on cerebral infarct size and neurologic score in the tandem occlusion animals. No association was found.

This study may have been confounded by the use of isoflurane anesthesia. Isoflurane has been reported to inconsistently provide protective effects in the context of experimental renal and brain ischemia.<sup>50,53,54</sup> Of more interest, however, is the recent report that isoflurane can serve to decrease remote organ dysfunction (liver and intestine) resulting from renal ischemia by inducing the sphingosine kinase-1/sphingosine-1 phosphate pathway that serves to attenuate proinflammatory cytokine production and apoptosis.<sup>55</sup> The nature of the invasive surgical procedures used in this study required an anesthetic. Isoflurane was chosen because its impact on MCAO outcome is better understood than that of alternative anesthetics.

Finally, the experiments were conducted devoid of cardiopulmonary bypass. Cardiopulmonary bypass is recognized widely as a potent stimulus for inflammatory responses potentially associated with organ dysfunction<sup>56,57</sup> and has been shown to exacerbate the outcome from MCAO.<sup>51</sup> A result different from that obtained in the present study could occur in the context of experimental cardiopulmonary bypass. The present study allows an opportunity for such an investigation through the development of a recovery from a tandem BRAO and MCAO insult.

The acute and substantial effects of AKI on normal brain and other organs have been described widely. However, under the conditions of the brain ischemia model used in this study, AKI did not alter neurologic dysfunction or cerebral infarct size. Although different magnitudes of AKI or types of cerebral ischemic insult may yield a different result, these data indicate that the remote effects of AKI reported for normal organs may be of lesser importance in the context of organs recovering from simultaneous injury.

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