Journal of Cardiothoracic-Renal Research (2006) 1, 141—145

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# DNA damage and repair in human spinal cord following ischemia—reperfusion injury

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Received 19 May 2006; accepted 19 May 2006

**KEYWORDS**
Spinal cord ischemia;
DNA damage;
DNA repair;
Reactive oxygen species (ROS)

**Summary** Spinal cord ischemia leading to paraplegia is a rare, sporadic, but devastating complication of surgery on the thoracoabdominal aorta. Our patient, a 69-year-old man, succumbed from a stroke on the third hospital day following surgical repair. He also had bilateral leg paralysis. At autopsy done 4 h after death there were remarkable differences between the thoracic or normally perfused spinal cord and the lumbar potentially ischemia or reperfused spinal cord. The measurements of injury were small in the thoracic spinal cord and extensive in the lumbar spinal cord DNA D/R. Apoptotic cell numbers and apoptosis-related enzymes such as caspase-3 were increased in the lumbar spinal cord. These findings duplicated those we reported in the rabbit subjected to 30 min of aortic occlusion and reperfusion injury. This is the first report in humans documenting DNA oxidative injury and apoptosis in ischemia—reperfusion injury of the spinal cord.


## Introduction

During operations on extensive thoracoabdominal aneurysms, clamping of the aorta is likely to interrupt essential intercostal arterial blood supply to the spinal cord at least temporarily, leading to ischemia. This insult coupled with the generation of reactive oxygen species (ROS) on reperfusion is likely the cause of post-operative spinal cord dysfunction which remains a devastating complication [1—4]. Animal models have been helpful in aiding our understanding of the molecular events attending ischemia and reperfusion of the spinal cord but questions always reside as to their relevance to man [5]. In this report, a post-mortem examination after a tragic clinical outcome provided the opportunity to perform studies on the human spinal cord duplicating those we have reported in the rabbit.

## Patient information

The patient is a 69-year-old man presented with documented expansion of a descending thoracic aortic aneurysm

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1574-0668/$ — see front matter 
doi:10.1016/j.jccr.2006.05.004

to 6.0 cm. He also had a 4.5 cm infra-renal aneurysm. Past history includes a coronary artery bypass. His left ventricular ejection fraction was 30%, but a stress thalium test was negative. There was a suggestion that T-10 provided a very small branch to the spinal cord, but this was not conclusive. A drain was placed in the lumbar sub arachnoid space to sustain a spinal fluid pressure of 10 cm of water. The patient underwent thoracotomy aided by atrial femoral by-pass using a heat exchanger to cool to 32 °C. Immediately upon clamping the aorta inferior to the left subclavian artery the patient became hypotensive. Flow rates were adjusted and after a 5-min period, the patient became stable hemodynamically and remained so for the duration of the operation. The aorta was clamped just inferior to the left subclavian artery and in themid chest. After completion of the proximal anastomosis, the distal clamp was moved to the aorta at the diaphragm and coincidentally motor evoked potentials were lost. We determined that the largest pair of intercostal arteries was at T-7 and we decided to connect this pair to the graft and to include the pair at T-10 in the distal anastomosis. Sensory potentials returned but motor evoked potentials did not.

On the first post-operative day the patient was intermittently restless and moved his arms spontaneously but not his legs. He made no purposeful movements to commands. A CT scan showed extensive old and new infarcts in the watershed area without hemorrhage. On the second post-operative day he had decerebrate posturing. His wife agreed to withdraw support and he died on the ventilator 60 h post-operatively.

A post-mortem examination was done which disclosed widespread athero-embolism to the gut, pancreas, kidneys and even the left ventricle. His brain was notable for widespread hypoxic injury and cholesterol emboli were found in the right parietal and occipital lobes. Sections of the spinal cord were unremarkable. Because the emboli were present in the heart and the brain we reasoned they came from the arch associated with our proximal clamp which also explained the episode of hypotension immediately after clamping. The entire spinal cord was removed by laminectomy and portions from the thoracic and lumbar areas frozen in liquid nitrogen or fixed in formalin. These tissues were processed by our laboratory as approved by Johns Hopkins University Institutional Review Board.

## Methods

### Histochemistry

Formalin-fixed, paraffin-embedded spinal cord sections from the thoracic and lumbar zones were deparaffinated and examined as described [5]. Briefly all sections were incubated with 3% hydrogen peroxide to block endogenous peroxidases, and 10% goat serum for 2 h to block non-specific binding. Primary antibodies were obtained from commercial sources anti-8-hydroxygnanosine (anti-8-oxoG) (Ncogene Research, San Diego, CA); MYH anti-caspase-3, OGG1 and $MSH_2$ (Novus Biologicals, Inc.); and diluted 1:100 in a PBS solution containing 0.3% Triton-X for overnight incubation. Following two rinses secondary peroxidase conjugated antibody at 1:100 (Amersham Biosciences UK Limited, Little Chalfont Buckinghamshire, England) was added and incubated for 1 h.

In the case of 8-oxoG, the final reaction was obtained by incubating sections with freshly prepared 3-amino-9-ethylcarbazole. Otherwise the sections were developed with DAB (Dako Corporation, Carpinteria, CA). Counterstaining was obtained using either hematoxylin or methyl green. Sample origins were concealed during evaluation by two trained observers. Controls consisted of substituting normal rabbit serum for the second antibody.

### Western blot analysis

Western blot analysis was performed as we previously described [5]. Tissue samples were homogenized in a protein lysis buffer (10 mm PMSF NaCl, 20 mm Tris—HCl, pH 7.5, 2 mmol/L EDTA; and 0.1% chaps, 0.25 m sucrose and 50 mm 2-mercaptoethanol), and then the homogenates were centrifuged at 13,000$\times$ *g* for 15 min at 4 °C. Supernatants were used as protein samples. SDS-PAGE was performed in a 10% polyacrylamide gel. In brief, protein samples were boiled at 100 °C in 2$\times$ SDS loading buffer, and lysates equivalent to 20 $\mu$g of protein from each sample were run on the gel for 90 min at 20 mA together with a size marker (Invitrogen, Carlsbad, CA). After reacting with the primary and secondary antibodies, the membrane was subjected to the enhanced chemiluminescence analysis system from Amersham. Polyclonal antibodies against the DNA repair enzymes MYH, OGG1 and MSH2 were obtained from Novus Biological. Monoclonal antibody against actin (Ab-1) (Oncogene) was used as a control for equal protein loading. To ascertain specific binding of the antibody for the protein, another membrane was studied without the primary antibody.

### Immunohistochemical staining

Immunohistochemical staining was performed as we previously described [5]. Briefly, after samples deparaffinized, sections was rinsed in 0.1 mol/L PBS for 20 min and blocked in 2% normal horse serum for 2 h. The sections then were incubated with primary MYH, OGG1 or MSH2 antibody (1:100) in 10% normal horse serum or 10% normal rabbit serum and 0.3% Triton-X 100 for 20 h at 4 °C. After endogenous peroxidase activity was quenched by exposure of the slides to 0.3% $H_2O_2$ and 10% methanol for 20 min, the sections were washed in PBS and incubated with secondary antibody—horseradish peroxidase conjugate (Amersham Biosciences UK Limited). The final reaction was achieved by incubating the sections with freshly prepared reagent containing 3-amino-9-ethylcarbazole (Sigma) dissolved in dimethyl-formamide and sodium acetate. The sections were counterstained with hematoxylin, mounted and reviewed with an Olympus microscope. Two trained independent observers reviewed these sections. Sample identities were concealed during evaluation. The specificity of positive staining was further confirmed by substitution of normal rabbit serum (NRS) instead of primary antiserum.