

The effect of adrenergic β_2 receptor agonist on paraplegia following clamping of abdominal aorta

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Abstract

Introduction: Surgical repair of an aortic aneurysm might be complicated by spinal cord injury and paraplegia. Since β -adrenoreceptor agonists showed neuroprotective effects, the study was designed to investigate the effect of clenbuterol on post-aortic clamping paraplegia and to identify if there is hyperemia associated with paraplegia.

Material and methods: Thirty rabbits were divided into two groups: 15 control and 15 experimental (given clenbuterol 9 mg in drinking water 24 h prior to surgery). All the animals were subjected to laparotomy whereas the abdominal aorta was identified. Using a vascular clamp, the abdominal aorta was clamped just distal to the renal arteries. Abdominal aortic blood flow was recorded with a transonic flow meter. The neurological assessment was made according to Tarlov's Neurological Scale upon recovering from anesthesia. Anal sphincter tonus and bladder sphincter function were also checked.

Results: Four rabbits (2 control and 2 experimental) developed complete paraplegia within 30 min of cross-clamping of the aorta. Of the 13 controls, 77% developed paraplegia, and of the 13 experimental rabbits administered clenbuterol 24 h prior to surgery with 22 min of aortic cross-clamping, 38% developed paraplegia. The rabbits which did not develop paraplegia had a minimal increase in aortic blood flow, whereas the rabbits which developed paraplegia had a significant increase in aortic blood flow measurements after aortic decamping.

Conclusions: Post-aortic clamping paraplegia is associated with hyperemia and clenbuterol has a significant neuroprotective effect, obviously by preventing an increase in aortic blood flow following unclamping.

Key words: aortic clamping, hyperemia, clenbuterol, paraplegia.

Introduction

After the surgical repair of an aortic aneurysm, spinal cord injury with paraplegia is among the most devastating and unpredictable complications. The incidence of spinal cord injury as a complication of aortic aneurysm repair is 3.0% to 18.0% [1-6]. Spinal cord injury can occur not only during extensive thoracoabdominal aneurysm repair but also postoperatively, causing delayed-onset paraplegia. Spinal cord ischemia is caused by aortic cross-clamping and interruption of blood flow to the spinal cord via critical intercostal arteries. Basically, various causes of spinal cord ischemia have been suggested, including interference with pelvic blood supply, prolonged aortic cross-clamping, prolonged suprarenal clamping, intraoperative hypotension, thromboembolic phenomena and interference with a low-

origin arteria radicularis magna [3]. Moreover, the causation of spinal cord ischemia during abdominal aortic repair is thought to depend on clamping time, reperfusion injury, and hemodynamics [1].

Cerebrospinal fluid drainage, hypothermia, monitoring of somatosensory and motor-evoked potentials, intercostal artery reattachment, distal aortic perfusion, and direct spinal cord cooling have all been used to protect against spinal cord ischemia [7-11]. However, this complication remains unpredictable and unpreventable [12-14].

In reviewing 609 patients who underwent surgical descending thoracic or thoracoabdominal aortic aneurysm repair, it was found that the extent of segmental artery sacrifice is the most powerful predictor of paraplegia risk [15]. Fasudil has neuroprotective effects against ischemic spinal cord injury in rabbits by reducing the number of infiltrating cells in the ventral horn and prolonging the expression of eNOS [16]. Selective infusion of sivelestat sodium hydrate (ONO-5046) directly into the spinal cord via the lumbar arteries significantly attenuated functional and morphological ischemia-induced spinal cord injury [17]. The mitochondrial K-ATP channel opener diazoxide improves neurological function after spinal cord ischemia and reperfusion by diminishing levels of reactive oxygen species, decreasing DNA oxidative damage, and inhibiting caspase-dependent and -independent apoptotic pathways while preserving mitochondrial structure [18]. Excitatory amino acid neurotoxicity through the N-methyl-D-aspartate (NMDA) receptor is no doubt the pathologic hallmark of ischemic and postischemic spinal cord injury. The segmental infusion of noncompetitive NMDA receptor antagonist as an intraoperative spinoplegia could have a protective effect on the spinal cord neurons against excitotoxic neuronal injury *in vivo* [19].

Studies suggest that cyclooxygenase contributes to ischemic neuronal damage and that cyclooxygenase inhibitors may reduce injury. Intrathecal ketorolac may be of therapeutic potential for preventing spinal cord ischemic injury during

thoracoabdominal aortic surgery [20]. The combined use of CSF drainage and naloxone offers significant protection against neurological deficits in patients undergoing thoracoabdominal and thoracic aortic replacement [21]. Tumor necrosis factor- α (TNF- α) is one of the important contributing factors to ischemia-induced spinal cord injury which can induce necrosis and apoptosis of cells [22].

Additional damage to the spinal cord may occur during the reperfusion period [14]. The β_2 -adrenoreceptor agonist clenbuterol is known to act as a neuroprotective substance in the central nervous system, and also reduces muscle atrophy after denervation. In addition, β -adrenoreceptor agonists provide neuroprotective effects after focal cerebral ischemia in experimental settings, and improve neurological and histological outcomes after transient focal cerebral ischemia in rats independently of administration route [23]. Clenbuterol caused substantial enhancement of recovery of locomotor function at severe levels of injury of spinal cord [24]. Clenbuterol (0.01-0.5 mg/kg) reduced the cortical infarct volume in Long-Evans rats as measured 7 days after permanent occlusion of the middle cerebral artery [25].

This study was conducted to investigate the possible protective effect of clenbuterol on post-aortic clamping paraplegia and to identify if there is hyperemia following aortic clamping, and whether this hyperemia has any role in pathogenesis of paraplegia.

Material and methods

Thirty New Zealand rabbits weighing between 4 kg and 6 kg were used for experimentation. The animals were divided into two groups: 15 control and 15 experimental (given clenbuterol 9 mg in drinking water 24 h prior to surgery). All the animals were anesthetized with atropine 0.005 mg/kg subcutaneously followed by ketamine 20 mg/kg intramuscularly and xylazine 3 mg/kg intramuscularly. Abdominal wall fur was clipped pre-operatively to expose the abdomen wall skin. All rabbits were given heparin at the dose of 70 units/kg 5 min prior to surgery. Additional doses of ketamine and xylazine were given during the procedure intravenously as needed and there was no need for endotracheal intubation or mechanical ventilation. All the rabbits were kept on 100% oxygen during the procedure.

Following the preparation of the abdominal skin with Betadine solution, a vertical midline incision measuring 5 cm was made. The abdominal aorta was identified. A transonic flow meter probe (Transonic Systems, Ithaca, NY) was placed 1 cm below the renal arteries and direct blood flow measurements were recorded at the infrarenal aorta before and after aortic clamping (Figure 1). Normal saline was used as an acoustic medium between

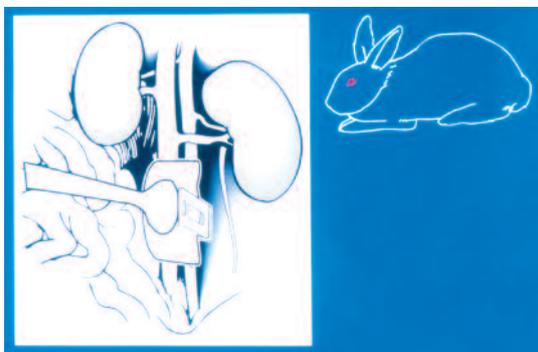


Figure 1. The rabbit as an experimental model is depicted, along with the Transonic Flowmeter probe *in situ*, positioned for the infrarenal aortic flow measurement

the aorta and the probe. Flow measurements were continuously recorded using a 1BM386 micro-computer connected to the flow meter in conjunction with the appropriate software program.

The principle of the transonic flow meter is dependent upon transit time ultrasound technology (Figure 2). The data processor is shown in Figure 3. The degree of reproducible ischemic injury was established by cross-clamping the infrarenal aorta for 22 min. Using a vascular clamp, the abdominal aorta was clamped just distal to the renal arteries for the specified time interval. Confirmation of aortic occlusion was obtained by a zero reading of the transonic flow meter. Abdominal aortic blood flow was recorded at the time of declamping of the aorta until the blood flow reading peaked and returned to baseline. Following the experiment, the abdominal wall was closed with 3-0 Dexon absorbable sutures for fascial closure and 3-0 silk sutures for skin closure.

All rabbits were observed for 24 h. The neurological assessment was made according to Tarlov's Neurological Scale (Table I) upon recovering from anesthesia. Anal sphincter tonus and bladder sphincter function were also checked. All the animals were euthanized at the completion of the study. Animal handling, care and disposal met the guidelines in the Principle of Laboratory Animal Care and Guide for the Care of Laboratory Animals (National Institute of Health Publication No. 86-23, revised 1983).

Results

Assessment of degree of paralysis of the hind limbs was recorded according to Tarlov's Neurological Scale (Table I). Four rabbits (2 control and 2 experimental) developed complete paraplegia within 30 min of cross-clamping of the aorta. Of the 13 controls, 77% developed paraplegia (Tarlov 0, $n = 9$; Tarlov 1, $n = 1$) and 23% did not develop paraplegia (Tarlov 3, $n = 1$; Tarlov 4, $n = 2$). Of the 13 experimental rabbits administered clenbuterol 24 h prior to surgery with 22 min of aortic cross-clamping, 38% developed paraplegia (Tarlov 0, $n = 3$; Tarlov 1, $n = 2$), and 62% did not develop paraplegia (Tarlov 3, $n = 2$; Tarlov 4, $n = 6$) (Figure 4). The mean Tarlov score for the experimental group was 2.461 ± 1.761 and for the control group 0.923 ± 1.605 ($p < 0.05$, p value 0.0147). The rabbits which did not develop paraplegia had a minimal increase in aortic blood flow (Figure 5), whereas the rabbits which developed paraplegia had a significant increase in aortic blood flow measurements after aortic declamping (Figure 6).

Discussion

The aortic flow measurements in the rabbits with paraplegia showed a significant increase in aortic flow following unclamping. Those rabbits with

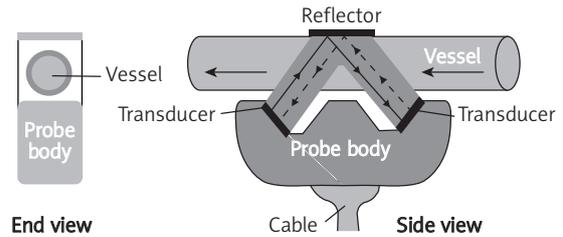


Figure 2. Transit time ultrasound technology (the principle of Transonic Flowmeter). Using wide beam illumination, 2 transducers pass ultrasound signals back and forth, intersecting the flow in upstream and downstream directions. The flowmeter derives a measure of the "transit time" it takes for the waves of ultrasound to travel from one transducer to the other. The difference between the upstream and downstream transit time is the measure of the volume flow

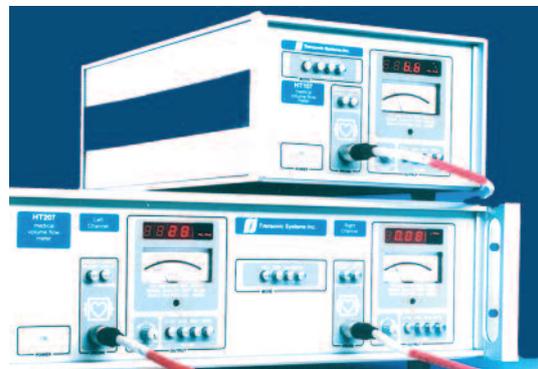


Figure 3. Transonic Flowmeter data processor

Table I. Tarlov's Neurological Scale

0 = Paraplegia, no movement of the lower limb
1 = Paraplegia, slight movement of the lower limb
2 = Good movement of the lower limbs but unable to stand
3 = Able to stand, but not able to walk normally
4 = Complete recovery

no paraplegia had minimal increase in aortic flow. There is a statistical difference between the control and the experimental group regarding Tarlov's score. A higher score was found in the experimental group as compared to the control group. It is clear that: 1) hyperemia and increased blood flow after the procedure were evident in animals who showed paraplegia, supporting the view that reperfusion injury might play a role in the pathogenesis of paraplegia and 2) clenbuterol could protect against paraplegia partly by attenuation of post-ischemic hyperemia injury.

The exact mechanism of postoperative paraplegia is not entirely clear. However, variation in aortic flow following unclamping may play a role

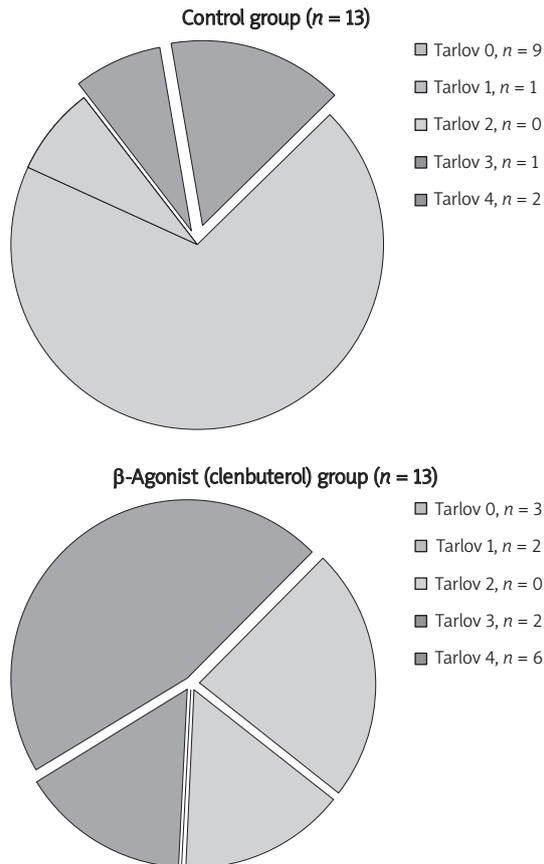


Figure 4. Neurological outcome of 22-min infrarenal aortic clamping and declamping. The pie chart above shows the control group ($n = 13$), in which 77% rabbits developed paraplegia (Tarlov 0 and 1, $n = 10$). The pie chart below shows the β -agonist (clenbuterol) group. In contrast to the control group, only half as many rabbits (38%) developed paraplegia (Tarlov 0 and 1, $n = 5$) in this group

in development of paraplegia. The studies in this area may be able to help clarify the causes of paraplegia development in thoracoabdominal aortic repairs. The rabbit model is an excellent model because the segmental distribution of spinal cord blood supply is similar to that in man.

A significant increase in lower thoracic spinal cord blood flow in dogs was found compared to the baseline flow after 30 min of thoracic aortic occlusion [26]. In addition, a significantly greater degree of hyperemia was noted in those dogs with neurological deficits compared with that seen in neurologically intact dogs. Other authors have correlated a reduction in spinal cord injury with a reduced hyperemic response during reperfusion [27]. Oxygen free radicals play a role in the ischemia/reperfusion-induced tissue injury by stimulating monocytes to produce TNF- α [28]. On the other hand, a likely mechanism of neurodegeneration following spinal cord injury involves generation of reactive oxygen species, which

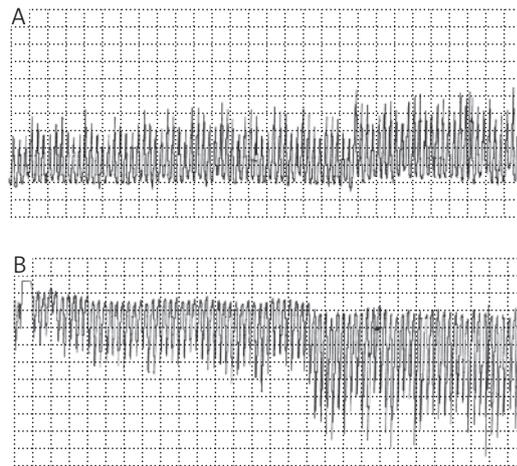


Figure 5. Transonic Flowmeter real-time recording of infrarenal aortic arterial blood flow. Figure 5 A shows baseline aortic blood flow before clamping. Figure 5 B shows aortic blood flow recording after declamping. Note that there is a minimal increase in post-declamping aortic flow. This rabbit showed complete postoperative recovery

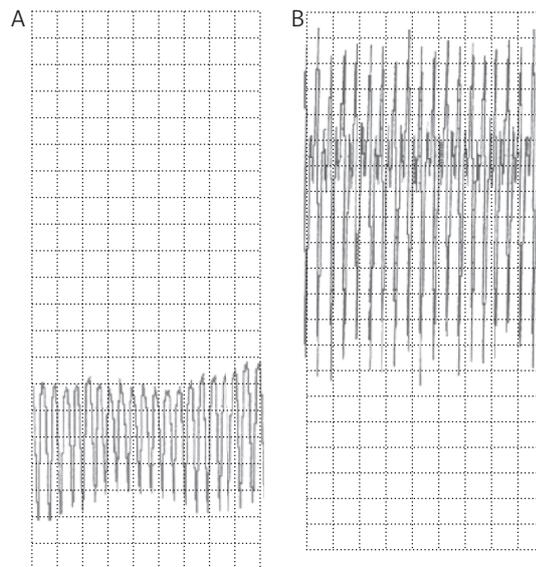


Figure 6. Transonic Flowmeter real-time recording of infrarenal aortic arterial blood flow. Figure 6 A shows baseline aortic blood flow before clamping. Figure 6 B shows aortic blood flow recording after declamping. Note that there is a marked increase in post-declamping aortic blood flow. Experimental animals with this kind of post-declamping hyperperfusion showed complete postoperative paraplegia

overwhelm endogenous antioxidants. In a review of 858 thoracoabdominal aneurysm repairs (June 1990-June 2006), it was suggested that paraplegia can result from inadequate postoperative spinal cord perfusion and delayed paraplegia can perhaps be prevented with better hemodynamic and fluid management [7]. Controlled low-pressure perfusion

at the beginning of reperfusion can attenuate neurological injury of the spinal cord after transient ischemia, and reperfusion initiated with low-pressure perfusion exerts neuroprotective effects on the spinal cord against ischemia/reperfusion injury [29].

Cerebrospinal fluid IL-8 levels are significantly elevated in thoracoabdominal aortic operation, and persistent elevation of CSF IL-8 levels may be predictive of further development of neurological deficits [30]. Oxidative stress induces cyclooxygenase-2 (COX-2) activity in neurons after various CNS insults, including global ischemia [31]. The COX-2 inhibitors SC-58125 and NS-398 have been shown to prevent delayed death of hippocampal neurons and to reduce infarct size after global ischemia [32, 33].

The importance of reperfusion injury in spinal cord dysfunction has been suggested, especially as pertains to delayed-onset paraplegia [34, 35]. The prevalence of paraplegia after descending thoracic aorta cross-clamping in a dog model was reduced by administration of the enzymatic antioxidant superoxide dismutase, implicating oxidants as one cause of the injury [36]. Ischemic reperfusion injury of the spinal cord occurs due to temporary interruption of the blood supply to the spinal cord. This may result in irreversible vascular injuries with subsequent paraplegia or other neurological deficits [37]. Oxidative stress with overproduction of reactive oxygen species, such as free radicals and peroxides, is incriminated in neurological vascular injuries [38]. Activated protein C (APC) reduced the ischemia/reperfusion-induced spinal cord injury by inhibiting neutrophil activation. The therapeutic mechanisms of APC might depend on its inhibitory effect on the production of TNF- α , which is a potent activator of neutrophils [39].

Neuroprotection by clenbuterol has been shown *in vivo* and *in vitro* [40-42]. Clenbuterol caused an increase in glutathione reductase activity; this suggests that activation of β_2 -adrenoreceptors during the acute phase of injury stimulates glutathione-dependent antioxidative processes that lead to reduced oxidative damage and greater locomotor function as the injury evolves during the subacute and chronic phases [43]. It has been found that clenbuterol plays a key role in the cardiac protection against myocardium I/R injury [44]. Moreover, clenbuterol induces nerve growth factor synthesis in cultured hippocampal cells and protects hippocampal neurons against excitotoxic damage. The neuroprotective activity of clenbuterol is also demonstrated *in vivo* in two rodent models of cerebral ischemia. There is evidence that the neuroprotective activity of clenbuterol is caused by activation of β -adrenergic receptors and the subsequent increased expression of nerve growth

factor [45]. In addition, clenbuterol has been shown to have neuroprotective activity after transient forebrain ischemia through increasing nerve growth factor expression [46-48]. Thus, the stimulation of the β_2 -adrenoreceptor seems to play an important role in activating nerve growth factor against neural damage. Clenbuterol has been shown to spare spinal cord tissue and enhance locomotor recovery in an experimental model of spinal cord contusion injury.

It has been shown that nerve growth factor mediated the neuroprotective effects of the β_2 -adrenoreceptor agonist clenbuterol *in vitro* and *in vivo* [41, 49, 50]. The increase in the ratio of Bcl-2 and Bax may contribute to the anti-apoptotic effect of clenbuterol [51]. Clenbuterol has anti-inflammatory and neurotrophic actions and elicits a neuroprotective effect in the kainic acid (KA) model of neurodegeneration [52]. Clenbuterol treatment activated cAMP response element binding protein within retrogradely traced neurons, which has been associated with axonal regrowth. Chondroitinase ABC and clenbuterol can act synergistically to promote recovery of locomotor function [53].

Clenbuterol suppressed LPS-induced expression of the inflammatory cytokines TNF- α and IL-6, the inflammatory chemokines RANTES and IP-10, and the co-stimulatory molecules CD40 and ICAM-1. Thus overall, clenbuterol suppresses the innate inflammatory response in rat brain [54]. It was shown that a novel mechanism of action of β_2 -adrenoreceptor agonists and suggested that increased formation of the endogenous glutamate receptor antagonist kynurenic acid could partially contribute to their neuroprotective activity [55].

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