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The Effect of Distal Aortic Pressure on Spinal Cord Perfusion in Rats

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Background: Aortic cross clamping is associated with spinal cord ischemia. This study used a rat spinal cord ischemia model to investigate the effect of distal aortic pressure on spinal cord perfusion. ***Materials and Methods:*** Male Sprague-Dawley rats (n=12) were divided into three groups. In group A (n=4), the aorta was not occluded. In groups B (n=4) and C (n=4), the aorta was occluded. In group B the distal aortic pressures dropped to around 20 mmHg. In group C, the distal aortic pressure was decreased to near zero. The carotid artery and tail artery were cannulated to monitor the proximal aortic pressure and the distal aortic pressure. Fluorescent microspheres were used to measure the regional blood flow in the spinal cord. ***Results:*** After aortic occlusion, blood flow to the cervical spinal cord showed no significant difference among the three groups. In groups B and C, the thoracic and lumbar spinal cord and renal blood flow decreased. No microspheres were detected in the thoracic and lumbar spinal cord of group C. ***Conclusion:*** The spinal cord blood flow is dependent on the distal aortic pressure after thoracic aortic occlusion.

Key words: 1. Animal model
2. Aorta
3. Spinal cord ischemia
4. Neurologic injury

INTRODUCTION

Paraplegia is one of the most feared complications after thoracoabdominal aortic surgery. The incidence is reported to be 5% to 40% in patients who have undergone thoracoabdominal aortic surgery [1].

Obstruction of the Adamkiewicz artery, reperfusion injury, collateral network concept and the steal phenomenon have all been suggested as causes of the paraplegia [1,2]. Among them, occlusion of the Adamkiewicz artery has long been believed to be the most important mechanism of spinal cord injury after thoracic or thoracoabdominal aortic surgery and most surgeons try to maintain blood flow to the Adamkiewicz artery during and after surgery [3].

Recently, endovascular aneurysm repair (EVAR) has been developed for the treatment of descending aortic disease and has become a popular procedure. Because there is no way to reestablish blood flow to the Adamkiewicz artery when it is occluded by a stent graft after EVAR, the debate over the role of thr Adamkiewicz artery on the mechanism of spinal

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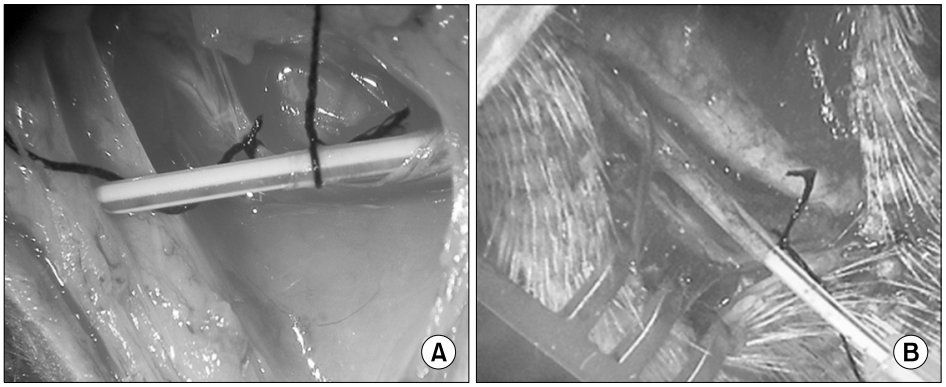

Fig. 1. Cannulation of the carotid artery (A) and tail artery (B) to monitor proximal and distal aortic pressure.

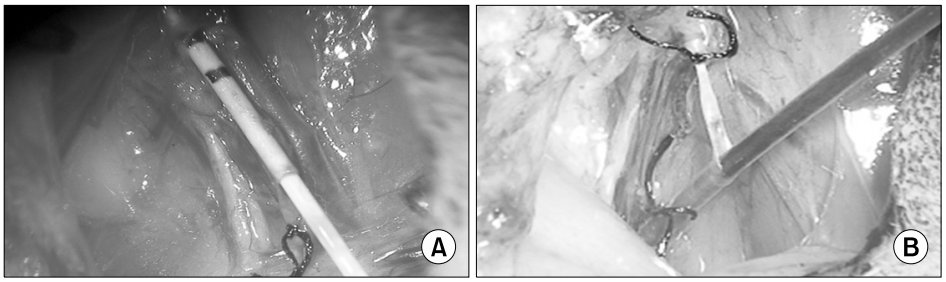

Fig. 2. (A) Fogarty catheter (2 French) insertion through the right femoral artery. (B) Blood draining from the left femoral artery catheter.

cord ischemia has resurfaced again.

In one study using a swine model, all the segmental arteries were ligated but no paraplegia was observed [4]. A clinical report from the same group, in which the segmental arteries were ligated or clipped before opening the descending aorta during aortic surgery, showed a low rate of postoperative paraplegia [5]. The basis of this procedure is the collateral network concept [5]. This is the theory that there are sufficient collaterals around the paravertebral area and spinal muscles to provide blood supply to the spinal cord even if the segemental arteries are occluded. This implies that, during thoracoabdominal aortic surgery, the artery of Adamkiewicz may function as a channel for the blood flow away from the spinal cord, the steal phenomenon. This study was designed to observe the steal phenomenon in a rat aortic occlusion model simulating thoracoabdominal aortic surgery.

MATERIALS AND METHODS

The experimental procedure described in this study was approved by the Institutional Animal Care and Use Committee of the Kangwon National University. Male Sprague-Dawley rats (n=12; weight, 350－400 g) were used in this study. For induction anesthesia, the animals were first placed in an acrylic plastic box perfused with 4% isoflurane and oxygen. After induction, they were each intubated with a 16-gauge catheter and mechanically ventilated with a tidal volume of 2.5 mL and a respiratory rate of 75 breaths/min. The inhalation anesthesia was maintained with 3% isofluorane in oxygen. The right common carotid artery and the tail artery were cannulated with a 22 gauge catheter for monitoring of the proximal and distal aortic pressure (Fig. 1). A 2 French Fogarty catheter was introduced through the right femoral artery and its tip was located just distal to the left subclavian artery (Fig. 2A). All animals were injected with 200 IU of heparin to prevent thrombosis. The chest was opened vertically and the left atrium was exposed by tearing the pericardium. The left atrium was cannulated with a small tube (internal diameter=1 mm) and connected to the microsphere loaded syringe (Fig. 3A).

The animals were divided into three groups. In group A (n=4), all the catheters were inserted but the aorta was not occluded. In group B (n=4), the aorta was occluded with inflation of the Fogarty catheter balloon with 0.15 mL of air.

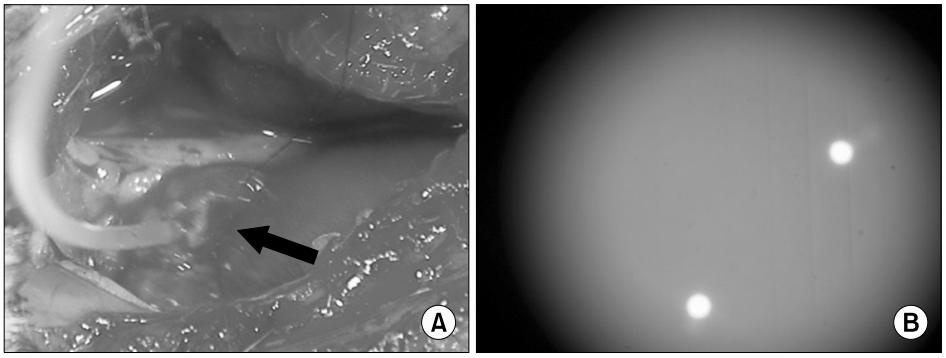

Fig. 3. (A) A catheter inserted in the left atrium (arrow) for microsphere injection. (B) Microspheres under a fluorescence microscope (×400).

The distal and proximal aortic pressures were allowed to fall or rise and become stabilized. However, in group C (n=4), the left femoral artery was cannulated with a 22-gauge catheter and the blood was drained to decrease the distal aortic pressure down to near zero (Fig. 2B). The drained blood was infused back into the animal with a mini-peristaltic pump through another catheter (22 gauge) placed in the left or right femoral vein.

After occlusion of the aorta with inflation of the balloon (groups B and C) and draining of the distal aortic blood (group C), the proximal and distal aortic pressures were allowed to stabilize. The changes in blood pressures were recorded in a laptop computer with a digital-analog converter (Compact DAQ; National Instruments, Austin, TX, USA) and software (Labview 8.5, National Instruments). In all the groups, after recording the blood pressure, reference blood sampling by withdrawing blood from the right carotid artery at the rate of 0.566 mL/min was begun. Then 0.1 mL of fluorescent microspheres (Fluo-Spheres, 15 μm, 1.0×10^6/mL; Molecular Probes Inc., Eugene, OR, USA) was infused into the left atrium. Reference blood sampling was continued until 2 minutes after the microsphere injection was over. Because the diameter of the microsphere was larger than the diameter of a capillary, the tissue was embolized with the injected microspheres and the number of the embolized microspheres found in the tissue correlates with the regional blood flow of the tissue.

Both kidneys and the spinal cord were removed. Each spinal cord was divided into cervical (C1－8), thoracic (T1－13) and lumbar (L1－6) segments. In order to calculate the regional blood flow, the microspheres were recovered from each of the tissue and blood samples that had been removed. To isolate the microspheres from the tissue, the sedimentation method was used as previously reported [6]. Briefly, the harvested organs were put in 4N KOH solution for 48 hours at room temperature. The samples were centrifuged 20 minutes at 2,000 g. Removing all but 1.5 mL supernatant, 8 mL 1% Triton X-100 was added and centrifuged at the same settings. The supernatant was discarded leaving 1 mL of the sample. Seven milliliters of distilled water was added and the sample centrifuged again. 0.15 mL of sample was left and the number of microspheres was counted using a cell count slide (C-chip; Digital Bio Technology Co. Ltd., Seoul, Korea) and fluorescence microscopy (BX53; Olympus, Tokyo, Japan) (Fig. 3B).

Calculation of the regional blood flow was performed using following formula:

$Q=([FR\times CT])/[CR\times VT]$

Where Q is the flow in mL/min/g; FR is the flow rate of the reference sample (mL/min); CT is the microsphere count in the tissue; CR is the microsphere count in the reference sample; and VT is the tissue weight (g).

Data are expressed as mean±standard deviation. To evaluate the statistically significant difference between the groups, the results were analyzed by Kruskal-Wallis one-way analysis of variance followed by Tukey's post hoc test using statistical analysis software SPSS ver. 11.0 (SPSS Inc., Chicago, IL, USA). A p-value less than 0.05 was considered to be significant.

RESULTS

Before aortic occlusion, the mean proximal aortic pressure (PAP) was about 90 mmHg and there was no significant dif-

ference among the groups (Fig. 4). The mean distal aortic pressure (DAP) also did not show any statistical difference between the groups (Fig. 4). The group in which the descending thoracic aorta was occluded without distal arterial drainage (group B) showed elevation of the PAP to 123±17 mmHg (Fig. 5). Also in group C, the PAP was elevated after aortic occlusion and the elevated PAP of groups B and C showed no difference. The DAP dropped to 21±1.3 mmHg in group B (Fig. 5). In group C the DAP decreased almost to zero after aortic occlusion and femoral artery drainage. The DAP was significantly different between group B and group C (Fig. 5).

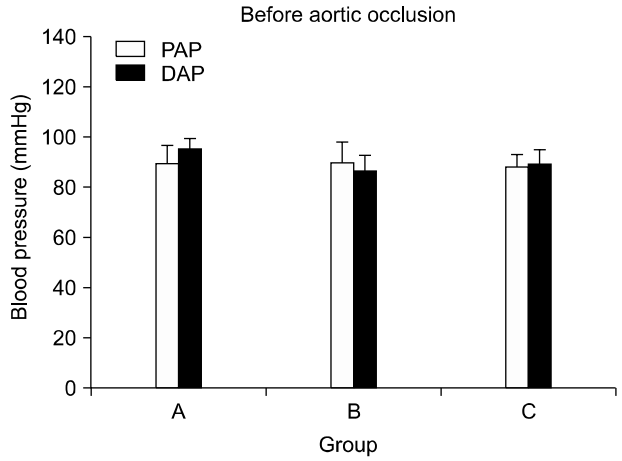

Fig. 4. Arterial pressure before occluding the aorta. No statistical difference was observed among the groups (Kruskal-Wallis one-way analysis of variance). PAP=proximal aortic pressure; DAP=distal aortic pressure.

The regional blood flow was calculated from the microsphere count and tissue weight using the formula above (Table 1). The microsphere counts in the carotid arteries were used as reference microsphere counts. The blood flow to the cervical spinal cord showed no significant differences among all three groups. However, all the three groups showed statistically significant differences in the renal blood flow, and thoracic and lumbar spinal cord blood flow (Fig. 6). The blood flow to the thoracic spinal cord and lumbar spinal cord in group B were 0.4±0.1 mL/min/g and 0.2±0.1 mL/min/g, respectively (Table 1). In group C there were no microspheres detected in the thoracic or lumbar spinal cord, indicating no blood flow to the lumbar spinal cord. The differences in the thoracic and lumbar spinal cord blood flow between group B and group C were statistically significant (Fig. 6).

DISCUSSION

Occlusion of the aorta just distal to the subclavian artery generates conditions similar to those of thoracoabdominal aortic surgery. Examining the distribution of microspheres showed no microspheres in the lumbar and thoracic levels of

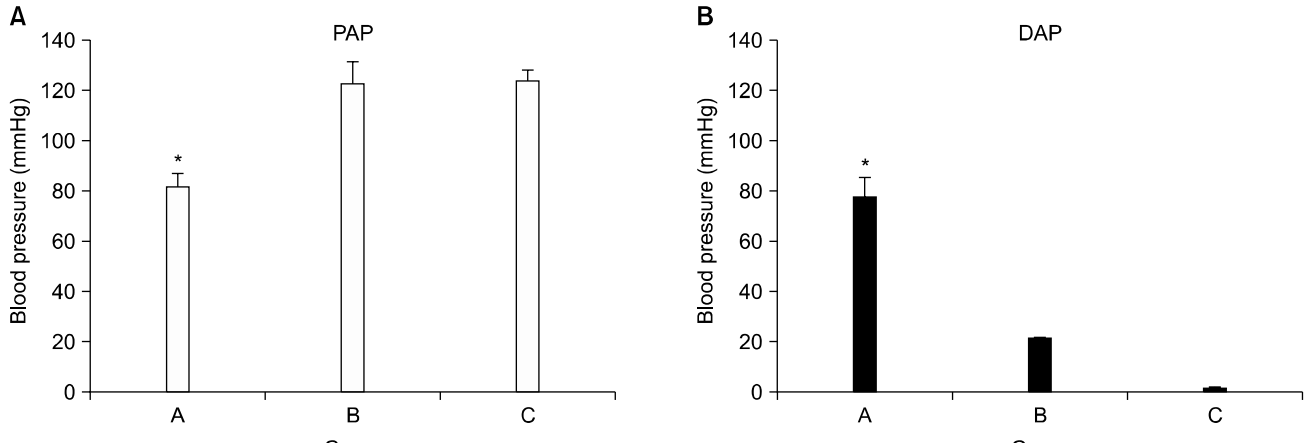

Fig. 5. The proximal aortic pressure (PAP) and distal aortic pressure (DAP) after aortic occlusion. Between groups B and C, the PAP showed no statistical difference (A). DAP was significantly different between the group B and C (B) (Kruskal-Wallis one-way analysis of variance followed by Tukey's post hoc test, p-value cutoff of 0.05). *aorta was not occluded in group A.

Table 1. Calculated regional blood flow

Region	Group	Blood flow (mL/min/g)
RBF	A	3.9±0.3
	B	0.8±0.2
	C	0.05±0.03
SCBF C	A	1.4±0.3
	B	1.5±0.5
	C	1.6±0.3
SCBF T	A	1.1±0.3
	B	0.4±0.1
	C	0
SCBF L	A	1.0±0.3
	B	0.2±0.1
	C	0

Values are presented as mean±standard deviation.
RBF=renal blood flow; SCBF C=cervical spinal cord blood flow; SCBF T=thoracic spinal cord blood flow; SCBF L=lumbar spinal cord blood flow.

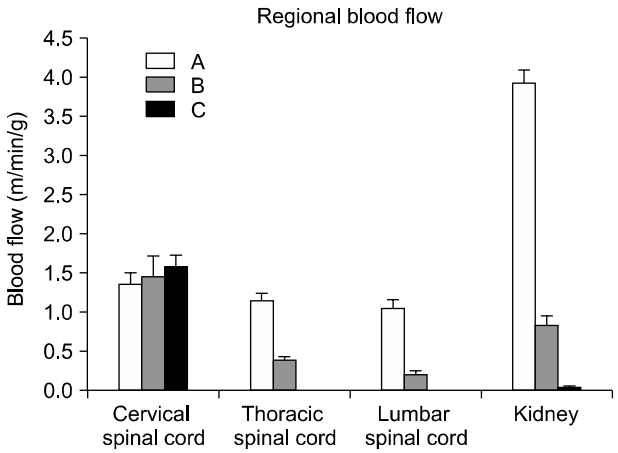

Fig. 6. The calculated regional blood flow during aortic occlusion. The blood flow to the cervical spinal cord showed no statistical difference among the three groups but the regional blood flow of other tissues of group B and group C differed significantly (Kruskal-Wallis one-way analysis of variance followed by Tukey's post hoc test, p-value cutoff of 0.05).

the spinal cord when the distal aortic pressure was kept near zero. In contrast, though small in amount, microspheres were found throughout the lower spinal cord in group B, in which the distal aortic pressures were maintained around 20 mmHg. Two conclusions can be drawn from these results. One is that there are collaterals supplying the spinal cord so that the blood flow is maintained even after occlusion of the descending thoracic aorta. The other is that the spinal cord blood flow from the collateral is affected by the distal aortic pressure and lowering the distal aortic pressure causes "stealing" of blood flow from the spinal cord. Although we could not observe how the spinal cord blood flow changes when the distal aortic pressure is increased to a higher level, the results of our experiment indicate that the distal aortic pressure is an important factor influencing the spinal cord blood flow.

Several mechanisms of spinal cord injury after aortic surgery have been suggested [1,2]. One of the oldest and most strongly believed mechanisms is Adamkiewicz artery occlusion during aortic surgery. During thoracoabdominal aortic replacement surgery, most surgeons routinely save segmental branches at the level of T10 to L2 where the Adamkiewicz artery is believed to originate [3]. However, this theory lacks confirmative evidence, and there are several studies showing that the blood supply from the Adamkiewicz artery is not essential for preventing spinal cord ischemic injury [4]. The so-called collateral network concept suggest that there exist abundant paravertebral collateral arteries that supply sufficient blood to the spinal cord and therefore segmental branches of the descending aorta can be ligated safely without causing paraplegia [4,5]. The other mechanism of spinal cord injury is the steal phenomenon. During aortic surgery, when the distal aorta is opened after clamping of the descending aorta, a considerable amount of blood flows back from the segmental arteries. This can cause blood flow away from the spinal cord during surgery. Indirect evidence the steal phenomenon as a cause of spinal cord ischemic injury has been presented by a study in which the descending thoracic aorta was double clamped some distance apart [7]. There was no spinal cord ischemia at the level between the two clamps suggesting no steal phenomenon and no spinal cord ischemia. Since the distal spinal cord blood flow was dependent on the DAP, we believe that the results of our experiment show evidence of the steal phenomenon and collateral blood flow.

The proximal aortic pressure is also an important factor influencing spinal cord ischemia after aortic clamping. One study found that a proximal aortic pressure below 40 mmHg resulted in ischemic spinal cord injury in rats when the thoracic aorta was occluded [8]. In that experiment, the distal aortic pressure decreased in accordance with proximal aortic pres-

sure, and was almost zero when the proximal aortic pressure was 40 mmHg. In our study, the same animal model and technique was employed to monitor the proximal and distal aortic pressure and to occlude the aorta, but the proximal aortic pressure did not change. Instead, only the distal aortic pressure changed. Since the results showed changes in blood flow to the spinal cord, we can infer that the distal aortic pressure is in direct relationship to the spinal cord blood flow.

It is interesting that no microspheres were found in the lower spinal cord when the distal aortic pressure was around 0 mmHg. This means that there was no blood flow to the tissue capillary bed. This can be explained by the difference between the arterial perfusion pressure and capillary pressure. Since the capillary pressure is known to be around 12 to 32 mmHg, there would be no flow to the tissue when the perfusion pressure was below that level.

Extrapolating the results from animal experiments to humans is always controversial, especially if they are small animals like rats. Because of their small size, the metabolism and distribution of substances in rats are different from humans. The spinal injury rat model is also notorious for its poor reproducibility of paraplegia. However, there are many previous studies using a rat spinal cord ischemia model because of its low cost and effectiveness. Although small in size, the hemodynamics and vascular structures of rats are similar to those of humans. The internal thoracic artery, anterior spinal artery, posterior spinal artery and paravertebral anastomosis are also found in rats [8,9]. In this study, we observed the distribution of microspheres to observe the hemodynamic response rather than observing paraplegia. We believe this obviates the influence of the size discrepancy.

There exist limitations in this study. It does not prove that extensive segmental artery occlusion is safe. The results indicate that aortic occlusion causes a distal aortic pressure drop and decreased spinal cord blood flow. However, we do not know how extensive the collateral blood flow would be if all segmental branches were occluded. Because there are collaterals to the spinal cord from the arteries distal to the descending aorta, the collateral blood flow when the aorta is occluded must be different from the blood flow when all the segmental arteries are occluded. For the same reason, we cannot conclude that the collateral blood flow is insufficient to prevent spinal cord ischemia. One other limitation is that paraplegia was excluded from the experimental parameters. Besides the difficulty of keeping the animal alive with paraplegia, death is inevitable immediately after microsphere injection. Even if the animal could survive microsphere embolization, the microsphere emboli could influence the results, making them difficult to interpret. Consequently, we cannot directly identify the steal phenomenon as a cause of paraplegia. Furthermore, other influencing factors like central venous pressure and the parameters of reperfusion injury were not evaluated.

Despite all these limitations, this study provides insight into the collateral blood flow of the spinal cord and the importance of distal aortic pressure as a factor in spinal cord ischemia that might cause paraplegia during aortic surgery.

CONCLUSION

There is collateral blood flow to the spinal cord after thoracic aortic occlusion. This collateral blood flow is influenced by distal aortic pressure. Decreasing the distal aortic pressure to zero leads the blood flow away from the spinal cord. Low distal aortic blood pressure or low perfusion pressure of the spinal cord causes the steal phenomenon, which might be one of the mechanisms of spinal cord ischemic injury and paraplegia after aortic surgery.

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