Excessively high systemic blood pressure in early phase of reperfusion exacerbates early-onset paraplegia in rabbit aortic surgery

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【 学位論文題目 】

Excessively high systemic blood pressure in early phase of reperfusion exacerbates early-onset paraplegia in rabbit aortic surgery(過高血圧による再貫流は兎の対麻痺を誘発する研究)

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学 位 論 文 の 内 容 要 旨

Excessively high systemic blood pressure in early phase of reperfusion exacerbates early-onset paraplegia in rabbit aortic surgery

脊髄虚血再灌流時の全身血圧上昇が大動脈手術後対麻痺に及ぼす影響に関する検討

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Introduction

Neurological complications such as paraplegia or paraparesis are still major concerns associated with repairs of thoracoabdominal aorta. Different measures are now, but definite strategies to prevent the intractable complications with high mortality and morbidity can not be established. Spinal cord ischemia (SCI) is of primary importance for the development of paraplegia or paraparesis after aortic surgery. Recently, we have demonstrated that augmentation of systemic blood pressure (BP) during the SCI can protect the spinal cord and prevent paraplegia after aortic surgery in an experimental model.

Early spinal cord reperfusion (SCR) with sufficient blood flow is important to reduce ischemic injury, but the SCR itself could bring spinal cord cell damage, known as "reperfusion injury". The aim of the present study was to elucidate the effect of high BP during the SCR on reperfusion injury in aortic surgery.

Materials and method

Animals and Surgical procedure

36 Japanese white rabbits weighing 2.5 to 3.0 kg were used to establish spinal cord ischemia-reperfusion model. To establish the SCI, the balloon of catheter was fully inflated 0.5-1.5cm distal to the left renal artery for 15 minutes. The mean BP during the SCI was medically kept

at around 120 mmHg for a minimal ischemic injury. After the 15-minute SCI, SCR was performed with an indicated BP controlled medically in its early phase of 15 minutes, followed by the natural recovery with no medication until each end-point.

Experimental Groups

Animals were randomly divided into two groups: high BP group (HR group); the mean BP was maintained approximately at 120 mmHg by an intravenous phenylephrine, control BP group (CR group); the BP was not medically intervened and mean BP recorded was approximately 80 mmHg.

Neurological assessment

Serial assessments of motor function of the hind limbs in all animals were performed at 3, 24, and 48 hours of reperfusion using the modified Tarlov scale (MTS).

Measurement of transcranial motor evoked potentials and pathologic Outcome

Transcranial motor evoked potentials (tc-MEPs) were recorded during 15 minutes of ischemia and 30 minutes of reperfusion, and recovery ratio was measured and analyzed. The spinal cord sections between L3 and L4 were harvested at 3, 24, and 48 hours of reperfusion, and stained with hematoxylin-eosin for histopathologic observation, such as motor neuronal viability, perivascular edema and gray matter vacuolation. To detect DNA fragmentation in cell nuclei, TUNEL staining was performed.

Western blot analysis

Immunoblotting assay was performed with the primary antibody as mouse anti-rabbit caspase 3 antibody and the secondary antibody used was goat anti-mouse immunoglobulin antibody. The signals were quantified by an image analyzer.

Vascular permeability, Myeloperoxidase (MPO) and superoxide generation in the spinal cord at 3 hours of reperfusion was assessed, as previously described with some modification.

Database management and statistical analysis were performed with the Statview version 5.0 software. All values are expressed as means ± SEM.

Results

Intraoperative blood pressure status

The intraoperative BP is shown in **Figure 1**. There were no statistical differences of the mean BP before and during the SCI between the HR group and the CR group (before, 81.5 ± 6.6 vs. 82.3 ± 3.6 mmHg; during 122.4 ± 1.6 vs. 122.5 ± 2.8 mmHg). The mean BP in early phase of SCR was adjusted at 121 ± 1.3 mmHg in the HR group, whereas it was 75 ± 9.1 mmHg naturally in the CR group, with a significant difference according to their definition ($P<0.0001$).

Tc-MEP recovery

The tc-MEPs disappeared immediately after aortic occlusion, and reappeared after balloon deflation. The recovery time of tc-MEP in the HR group was 17.3 ± 4.2 minutes, whereas 10.0 ± 3.1 minutes in the CR group. There was a tendency of longer recovery time in the HR group than that in the CR group although statistical significance was not reached **(Fig. 2A)**. The recovery ratio of tc-MEP amplitude at 30 minutes of reperfusion in the HR group was significantly lower than that in the CR group (P=0.008, **Fig. 2B)**.

Neurological outcomes

The modified Tarlov scale at 3, 24 and 48 hours of reperfusion are shown in **Figure 2C**. The neurological score deteriorated with time and were significantly different between the HR group and the CR group. (3hours, P=0.0005; 24 hours, P=0.0032; 48 hours, P<0.0001).

Histological assessment

At 3 and 48 hours of reperfusion, the number of viable neuron cells in the HR group was significantly fewer than that in the CR group (P=0.0400 and P=0.0005, respectively, **Fig. 3A and 3B)**, and the degree of perivascular edema and gray matter vacuolation in the HR group were significantly larger than those in the CR group (edema, P=0.0100 and P<0.0001, respectively, **Fig. 3A and 3C**; vacuoles, P=0.0030 and P<0.0001, respectively, **Fig. 3A and 3D**).

Spinal cord apoptosis

At 48 hours of reperfusion, the number of TUNEL-positive neuron cells in the HR group was significantly more than that in the CR group (P<0.0001, **Fig.4A and 4B).** Compared with the CR group, the protein expression of caspase 3 was significantly upregulated in the HR group (P=0.0002, **Fig.4C and 4D).**

Early reperfusion injury

At 3 hours of reperfusion, both Evan's blue level and MPO activity in the spinal cord tissues were significantly increased in the HR group, compared with the CR group (Evan's blue, 1.97 ± 0.19 vs. 0.66 ± 0.12 OD/g wet tissue, P=0.0012; MPO activity, 0.15 ± 0.26 vs. 0.008 ± 0.006 ΔAbs/min/g wet tissue, P=0.0021; **Fig. 5A and 5B)**. At 3 hours, superoxide level in the HR group was significantly higher than that in CR group (P<0.0001; **Fig. 5C and 5D).**

Discussion

All organ tissues are susceptible to reperfusion injury, but this susceptibility varies among tissues. Given the delicate nature of arterial supply to the anterior spinal cord, motor neuron cells of the spinal cord are very sensitive and vulnerable to any degree of ischemic

insult. In a field of aortic surgery, major intraoperative causes of spinal cord injury are the occurrence of one or more of the following events: (i) the duration and degree of ischemia, (ii) failure to re-establish blood flow to the spinal cord by surgical repair, and (iii) the degree of postischemic reperfusion injury. Focused on the intraoperative management during the SCI, we have currently demonstrated that systemic BP augmentation during the SCI could protect the spinal cord and prevent postoperative paraplegia after aortic surgery in rabbits.

Our recent study has shown that rabbits with a BP of 120 mmHg during the 15-minute SCI had reduced ischemic insult, resulted in less sign of neuronal damage. Using this model with the same ischemic conditions, we evaluated the effect of similar high BP (120 mmHg) during the SCR on simple reperfusion injury under minimal ischemic insult.

Interestingly, this study demonstrated that high mean BP of 120 mmHg (approximately more than 1.5 times the normal) in early phase of reperfusion has disadvantageous effects on the spinal cord, in contrast with beneficial effects of high BP during the SCI in our previous study.

In histological assessment, there were fewer viable neuron cells and more TUNEL-positive cells in the anterior horn of the spinal cord in the HR group, compared with the CR group. To further evaluate spinal cord apoptosis, we performed the western blotting of caspase 3. Ischemia by itself can trigger apoptosis and reperfusion accelerates the process,

and apoptosis has been shown to be an important mode of earlier neuronal damage in the spinal cord after ischemic insults. This study shows that neurological score at 48 hours was worst than at 3 hours and apoptosis was significantly severer at 48 hours suggesting that acceleration of spinal cord injury might have been mainly due to apoptosis.

The present study has shown that the high BP in early phase of SCR promoted an oxidative inflammatory cascade involving enhancement of vascular permeability, MPO activity and superoxide generation. These findings suggest that increased oxidative stress by the BP augmentation in early phase of SCR could contribute to the mechanism of early-onset paraplegia.

The first limitation of the present study was that the rabbit SCI model had less ischemic injury with a high BP during the SCI, based on our previous study. The second limitation is that there are some differences in vascular anatomy and clinical response for the SCI and SCR between rabbits and human. In rabbits, there are some collaterals from pial anastomoses via posterior spinal artery to lumbar cord, but the caudal blood flow is mainly from the segmental arteries. The third limitation is that we did not completely assess delayed-onset neurological deficit in the present study.

In conclusion, excessively high BP in an early phase of SCR increased reperfusion injury in the spinal cord, leading to exacerbation of early-onset paraplegia. Avoidance of SCR with high BP could be one of management strategies in thoracoabdominal aortic surgery.

Figure legend

Figure 1. Systemic blood pressure (BP) during surgery. There were no significant differences of the BP during ischemia between the HR group and the CR group. In early phase of reperfusion, the BP in the HR group was significantly higher than that in the CR group. *P<0.05.

Figure 2. Intraoperative and postoperative neurologic assessment. **(A)** Representative tc-MEPs complex. **(B)** Recovery ratio of tc-MEP amplitude at 10, 20 and 30 minutes of reperfusion. n=18 in each group. (C) Modified Tarlov score at 3, 24 and 48 hours of reperfusion. n=18 at 3 hours, n=6 at 24 and 48 hours of reperfusion in each group. All data are expressed as means±SEM. *P<0.05.

Figure 3. Postoperative histological assessment. **(A)** Hematoxylin and eosin (HE) staining in the ventral gray matter of spinal cord at 3 and 48 hours of reperfusion. Photomicrographs of sections show viable neuron cells (white arrows), perivascular edema (black arrows) and gray matter vacuoles (black arrowheads). Bar=200 μm. **(B)** Quantitative analyses of viable neuron cells, **(C)** perivascular edema and **(D)** gray matter vacuoles. *P<0.05. All data are expressed as means±SEM for n=6 rabbits. *P<0.05.

Figure 4. Postoperative evaluation of spinal cord apoptosis. (**A**) TUNEL staining of the ventral gray matter of spinal cord at 48 hours of reperfusion. Photomicrographs of sections show TUNEL-positive cells (brown). Bar=200 μm. (**B**) Quantitative analysis of TUNEL-positive neuron cells at 48 hours of reperfusion. (**C**) Western blot analysis of Caspase 3. (**D**) Relative optical density of caspase 3 in each group. All data are expressed as means±SEM for n=6 rabbits. **P*<0.05.

Figure 5. Early reperfusion injury in the spinal cord at 3 hours of reperfusion. (**A**) Vascular permeability. OD, optical density. (**B**) Myeloperoxidase (MPO) activity. ΔAbs indicates a change in absorbance. (**C**) *In situ* detection of superoxide generation (red fluorescence). Bar=200 μm. (**D**) Semi-quantitative analysis of the superoxide generation. FU, fluorescence unit. All data are expressed as means±SEM for n=6 rabbits. **P*<0.05.

神戸大学大学院医学系研究科（博士課程）

論文審査の結果の要旨			
受付番号	甲 第2082号	氏　名	Bishow Pokhrel
論文題目 Title of Dissertation	**Excessively high systemic blood pressure in early phase of reperfusion exacerbates early-onset paraplegia in rabbit aortic surgery** 脊髄虚血再灌流時の全身血圧上昇が大動脈手術後対麻痺におよぼす影響に関する検討.		
審査委員 Examiner	主　査 Chief Examiner 黒坂昌弘 副　査 Vice-examiner 伊村英二 副　査 Vice-examiner 平田健一		

（要旨は１，０００字～２，０００字程度）

Excessively high systemic blood pressure in early phase of reperfusion exacerbates early-onset paraplegia in rabbit aortic surgery

Introduction

Neurological complications such as paraplegia or paraparesis are still major concerns associated with repairs of thoracoabdominal aorta. Spinal cord ischemia (SCI) is of primary importance for the development of paraplegia or paraparesis after aortic surgery. Early spinal cord reperfusion (SCR) with sufficient blood flow is important to reduce ischemic injury, but the SCR itself could bring spinal cord cell damage, known as "reperfusion injury". The aim of this study was to elucidate the effect of high BP during the SCR on reperfusion injury in aortic surgery.

Animals and Surgical procedure and groups

Thirty-six Japanese white rabbits weighing 2.5 to 3.0 kg were used to establish spinal cord ischemia-reperfusion model. To establish the SCI, the balloon of catheter was fully inflated 0.5-1.5cm distal to the left renal artery for 15 minutes. The mean BP during the SCI was medically kept at around 120 mmHg for a minimal ischemic injury. After the 15-minute SCI, SCR was performed with an indicated BP controlled medically in its early phase of 15 minutes, followed by the natural recovery with no medication until each end-point. Animals were randomly divided into two groups: high BP group (HR group); the mean BP was maintained approximately at 120 mmHg by an intravenous phenylephrine, control BP group (CR group); the BP was not medically intervened and mean BP recorded was approximately 80 mmHg.

Functional, histological and biochemical assessments

Serial assessments of motor function of the hind limbs in all animals were performed at 3, 24, and 48 hours of reperfusion using the modified Tarlov scale (MTS). Transcranial motor evoked potentials (tc-MEPs) were recorded and recovery ratio was measured and analyzed. The spinal cord sections between L3 and L4 were harvested at 3, 24, and 48 hours of reperfusion, and stained with hematoxylin-eosin for histopathologic observation, such as motor neuronal viability, perivascular edema and gray matter vacuolation. To detect DNA fragmentation in cell nuclei, TUNEL staining and Immunoblotting assay was performed. Vascular permeability, Myeloperoxidase (MPO) and superoxide generation in the spinal cord at 3 hours of reperfusion was assessed.

Results

The mean BP in early phase of SCR was adjusted at 121 ± 1.3 mmHg in the HR group, whereas it was 75 ± 9.1 mmHg naturally in the CR group, (P<0.0001). The recovery time of

tc-MEP in the HR group was 17.3 ± 4.2 minutes, whereas 10.0 ± 3.1 minutes in the CR group. The recovery ratio of tc-MEP amplitude at 30 minutes of reperfusion in the HR group was significantly lower than that in the CR group (P=0.008). The neurological score deteriorated with time and were significantly different between the HR group and the CR group. (3hours, P=0.0005; 24 hours, P=0.0032; 48 hours, P<0.0001). At 3 and 48 hours of reperfusion, the number of viable neuron cells in the HR group was significantly fewer than that in the CR group (P=0.0400 and P=0.0005, respectively, and the degree of perivascular edema and gray matter vacuolation in the HR group were significantly larger than those in the CR group. At 48 hours of reperfusion, the number of TUNEL-positive neuron cells in the HR group and the protein expression of caspase 3 was significantly upregulated in the HR group. At 3 hours of reperfusion, Evan's blue, MPO activity and superoxide generation in the spinal cord tissues were significantly increased in the HR group (P<0.0001).

Summary

This study demonstrated that high mean BP of 120 mmHg (approximately more than 1.5 times the normal) in early phase of reperfusion has disadvantageous effects on the spinal cord. The effects such as, less neurological function, histological and immunoblotting findings in HR group clearly evident the reperfusion injury. Also the high BP in early phase of SCR promoted an oxidative inflammatory cascade involving enhancement of vascular permeability, MPO activity and superoxide generation. These findings suggest that increased oxidative stress by the BP augmentation in early phase of SCR could contribute to the mechanism of early-onset paraplegia.

In conclusion, excessively high BP in an early phase of SCR increased reperfusion injury in the spinal cord, leading to exacerbation of early-onset paraplegia. Avoidance of SCR with high BP could be one of management strategies in thoracoabdominal aortic surgery.

The candidate, having completed studies on **"Excessively high systemic blood pressure in early phase of reperfusion exacerbates early-onset paraplegia in rabbit aortic surgery"**, with a speciality in cardiovascular surgery, and having advanced the field of knowledge in the area of spinal cord protection during thoracoabdominal aortic surgery, is hereby recognized as having qualified for the degree of Ph.D. (Medicine).