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【 Original Article 】

Protective Effects of Recombinant Human Soluble Thrombomodulin on Ischemia Reperfusion Injury of the Spinal Cord in Rabbits

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Abstract

OBJECTIVES: Paraplegia is a well-known severe complication of ischemic spinal injury that occurs during surgery for descending thoracic and thoracoabdominal aortic aneurysm. Although several surgical procedures and medications have been used to prevent paraplegia, the strategy for preventing paraplegia has not yet been established. Thrombomodulin (TM), expressed on the plasma membrane of endothelial cells, has been considered to exert cytoprotective effects against ischemia reperfusion injury (IRI). The protective effect of recombinant human soluble (rhs) TM against IRI in the liver and kidneys has been reported; we investigated whether rhsTM can prevent paraplegia in a rabbit ischemic spinal injury model. Moreover, we examined whether rhsTM protects rat pheochromocytoma cell line, PC12, from hypoxia-reoxygenation damage.
METHODS: Twenty-two New Zealand white rabbits were intravenously injected with isotonic saline (group C; n = 11) or isotonic saline containing rhsTM (group T; n = 11) before clamping of the aorta just below the branching of the renal artery for 30 minutes. Hind limb motor function was assessed 48 hours after aortic declamping as per the modified Tarlov score. PC12 cells were pretreated without rhsTM (group N) or with 1 μg or 10 μg of rhsTM (group A1 or A2), oxygen and glucose were depleted for 210 minutes, and the cells were incubated for another 24 hours. The cell viability was assessed using the methyl thiazolyl tetrazolium (MTT) method.
RESULTS: Lower limb motor function was significantly better in group T as compared to that in group C *in vivo* experiment ($p < 0.05$). The cell viability of the PC12 cells in group A2 was higher than that in group N after the hypoxia-reoxygenation experiment ($p < 0.05$).
CONCLUSIONS: The results suggest that rhsTM may prevent paraplegia due to IRI of the spinal cord during surgical intervention for descending thoracic and thoracoabdominal aortic aneurysm.

Keywords: descending thoracic and thoracoabdominal aortic aneurysm, ischemia reperfusion injury, spinal cord, recombinant human soluble thrombomodulin

Introduction

Paraplegia is a severe complication of ischemia reperfusion injury (IRI) in the spinal cord that occurs during surgery for descending thoracic and thoracoabdominal aortic aneurysm. In spite of the various surgical procedures and medications that have been used for preventing paraplegia, the strategy for preventing paraplegia needs to be improved. The surgical procedures to prevent paraplegia include peripheral perfusion, controlled hypothermia, segmental clamping, intercostal artery reconstruction, and cerebrospinal fluid drainage[1,2]. In addition to these surgical methods, the effectiveness of many pharmaceutical spine-protecting drugs, such as free radical scavengers, steroids, sodium channel blockers, opioid antagonists, and vasodilators have been experimentally proved[3].

The surgical methods for preventing paraplegia are insufficient because of several critical limitations. Controlled hypothermia promotes the tendency of bleeding and prolongs the extracorporeal circulation. Segmental clamping cannot be performed in case of very large aneurysms or severe calcification. Moreover, cerebrospinal fluid drainage cannot be performed in emergency situations. Thus, spinal cord protection with suitable drugs is extremely useful because it can be performed irrespective of the patient's condition and the surgical procedure.

In recent years, inflammatory response has been identified as an important cause of ischemic injury of the spinal cord[3]. Recombinant human soluble thrombomodulin (rhsTM) reportedly exerts anti-inflammatory effects against IRI in the liver and kidney[4-7]. Here, we investigated the protective effect of rhsTM on spinal IRI using rabbit model in vivo and confirmed its protective effect by using an *in vitro* PC12 cell model.

Methods

Materials: Recombinant human soluble thrombomodulin (rhsTM) was supplied by the Asahi Kasei Pharma Corporation (Tokyo, Japan). Ketamine was purchased from Daiichi Sankyo Co., Ltd (Tokyo Japan), and medetomidine hydrochloride was procured from Nippon Zenyaku Kogyo Co., Ltd (Fukushima, Japan). A 3-[4,5-di-methylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was purchased from Chemical Dojin Co., ltd (Kumamoto, Japan).

***In vivo* experiments (Animal study):** New Zealand white rabbits were purchased from KBT Oriental Co., Ltd. (Saga, Japan). Twenty-two New Zealand white male rabbits (body weight, 2.97-3.57 kg) were equally divided into two groups. All the rabbits were allowed free access to food and water before and after the experiment. Animal care and all other procedures were performed as per the "Guidelines for proper conduct of Animal Experiments" of Science Council of Japan. The *in vivo* experimental procedure has been shown in Figure 1. The rabbits were premedicated with an intramuscular injection of ketamine (25 mg/kg) and medetomidine hydrochloride (0.5 mg/kg). During the procedure, a continuous drip infusion of isotonic saline was administered at 20 mL/h via the left ear marginal vein for about 3 hours. A 24-gauge catheter was inserted into the right common carotid artery to monitor the systemic blood pressure. Without intubation, 0.5 L/min of oxygen was administered using a mask, and spontaneous respiration was managed without mechanical ventilation. The heart rate was monitored using electrocardiogram, and rectal temperature was measured continuously. Under sterile conditions, laparotomy was performed via abdominal midline incision. We administered 20 mL of isotonic saline (group C: n = 11) or rhsTM (6 mg/kg) + 20 mL of isotonic saline (group T: n = 11) intravenously for 30 minutes before aortic clamping, and 6 mg/kg of rhsTM was administrated in according to the report of Abeyama[8]. After exposing the aorta, 200 units/kg of heparin sodium of the rabbits was administered intravenously. Three minutes after the heparin sodium injection, clamping was performed just distal of the branching of the left renal artery and proximal of the aortic bifurcation and at the origin of the posterior mesenteric artery (PMA) for 30 minutes to induce ischemic spinal injury (Figure 1). During the procedure, invasive arterial blood pressure, electrocardiogram, heart rate, and rectal temperature

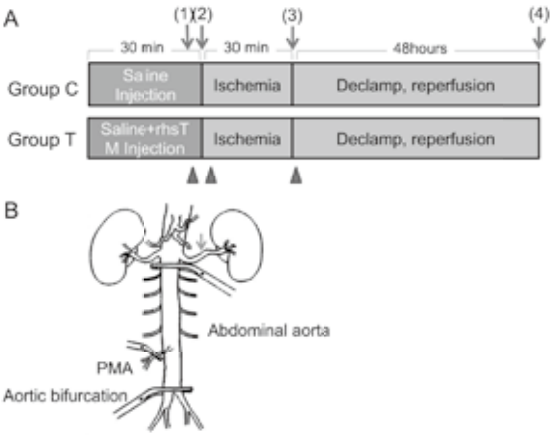

Figure 1. Experimental procedure *in vivo*. (A) Twenty-two rabbits were divided into two groups; group C and group T. Rabbits in Group C were injected with saline and those in Group T were injected with saline containing rhsTM before clamping of the aorta. Arrows show the timings of (1) heparin injection, (2) clamping aorta, (3) declamping aorta, and (4) neurological evaluation. Arrowheads represent three points of ABG measurement before and after clamping of the aorta and after declamping aorta. (B) Clamping was performed at the sites at the abdominal aorta just below the branching of the left renal artery (arrow), above the aortic bifurcation, and the origin of the posterior mesenteric artery (PMA).

were continuously monitored; arterial blood gas (ABG) data (pH, PaO_2 and $PaCO_2$) were measured at three time points. After removing the clamp, the abdominal wound was closed.

Neurological evaluation: Hind limb motor function was assessed 48 hours after aortic declamping with the modified Tarlov score as follows: 0 = absence of voluntary movement; 1 = spontaneous movement but inability to support weight; 2 = ability to support weight but no ability to walk; 3 = ability to walk but with marked spasticity or ataxia or both; 4 = ability to run but with mild spasticity or ataxia; 5 = normal motor function[9].

Cell culture: The rat pheochromocytoma cell line, PC12, was obtained from the American Type Cell Culture Collection (Manassas, VA) and maintained at the Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% horse serum, 5% fetal calf serum, 2.5 μg/mL amphotericin, and 100 U/mL penicillin at 37°C in a humidified 5% CO_2 atmosphere.

Cell treatment: The PC12 cells were seeded at a density of 4×10^5 cells per well in 96-well dishes and subjected to oxygen-glucose deprivation (OGD), as described previously[10]. Briefly, the cells were initially incubated with glucose-free RPMI 1640 medium, 2% horse serum, and 1% fetal calf serum without or with 1 μg/mL or 10 μg/mL of rhsTM (group N or A1 or A2). The cells were then placed in an anaerobic jar

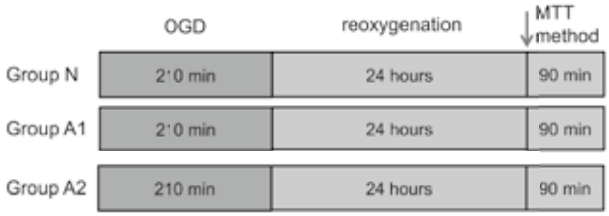

Figure 2. Experimental design *in vitro*. The PC12 cells were divided into the following three groups: Group N, Group A1, and Group A2 that were pretreated without, with 1 μg/mL and 10 μg/mL rhsTM.The three groups were incubated under hypoxic condition for 210 minutes. After the following 24 hours reoxygenation, MTT experiment was performed.

for 210 minutes. MTT assay was performed after the cells were moved back to normoxia and incubated for another 24 hours (Figure 2).

Cell viability: MTT assay was used to measure the cell viability, as described previously[11]. After the above-mentioned cell treatment, 2.5 mg/mL of MTT was added to each well, and the plate was incubated for 90 minutes thereafter. The formazan product was solubilized by adding 100 μL of dimethyl sulfoxide. The absorbance of the cell lysates was measured at a test wavelength of 570 nm, and the absorbance was measured at the reference wavelength of 630 nm using iMark Microplate Abosorbance Reader (Bio-Rad, Hercules, CA, USA). The values of each column were represented as percentage of control (% of control) (n = 12).

Statistical analyses: *In vivo*, the data were analyzed using the Microsoft Excel Statistical Package. Data are presented as the mean ± standard deviation values. Differences between the groups were evaluated using the Welch's t test. *In vitro*, all statistical analyses were performed using GraphPad version 8 (GraphPad Software, Inc.). Data are presented as the mean ± the standard error of the mean values. Differences between the groups were evaluated using one-way analysis of variance with a post-hoc Tukey's test. $P < 0.05$ was considered to indicate statistical significance.

Results

***In vivo* experiments:** The mean body weight of each rabbits group was comparable, 3.20 ± 0.17 kg in group C and 3.30 ± 0.16 kg in group T. Moreover, there was no significant difference between group C and T in terms of the hemodynamic variables (mean invasive arterial blood pressure and heart rate), rectal

Table 1. Comparison of blood pressure, heart rate and body temperature between the two groups at each timing.

		Group C (n=11)	Group T (n=11)	P
Blood Pressure (mmHg)	Before clamp	66 ± 9	62 ± 5	0.250
	After clamp	70 ± 9	69 ± 5	0.692
	After reperfusion	63 ± 10	62 ± 8	0.684
Heart rate (times/ minutes)	Before clamp	169 ± 27	166 ± 24	0.842
	After clamp	147 ± 20	142 ± 21	0.569
	After reperfusion	165 ± 16	155 ± 16	0.135
Rectal temperature (℃)	Before clamp	37.6 ± 0.6	37.6 ± 0.6	1.0
	After clamp	36.7 ± 1.0	36.3 ± 0.8	0.309
	After reperfusion	36.0 ± 0.9	35.4 ± 0.9	0.135

Table 2 . Comparison of pH, PaO_2 and $PaCO_2$ between the two groups at each timing.

		Group C (n=11)	Group T (n=11)	P
pH	Before clamp	7.29 ± 0.04	7.28 ± 0.04	0.689
	After clamp	7.28 ± 0.06	7.28 ± 0.03	0.928
	After reperfusion	7.26 ± 0.05	7.24 ± 0.04	0.314
PaO_2 (mmHg)	Before clamp	353 ± 64	349 ± 58	0.863
	After clamp	388 ± 72	421 ± 47	0.223
	After reperfusion	375 ± 110	394 ± 57	0.616
$PaCO_2$ (mmHg)	Before clamp	66 ± 7	63 ± 7	0.219
	After clamp	67 ± 9	60 ± 9	0.084
	After reperfusion	67 ± 12	62 ± 10	0.277

temperature, and arterial blood gas values (pH, PaO_2 and $PaCO_2$) at before and after aortic clamping and after reperfusion (Tables 1 and 2).

Neurological function: The hind limb motor function was evaluated after aortic operation with the modified Tarlov score (Figure 3). In group C, eight rabbits exhibited grade 0 paralysis, and three exhibited grade 5 normal neurological function. In group T, two rabbits had grade 0 paralysis, and nine had grade 5 normal neurological function. The average modified Tarlov score of group T was significantly higher than that of group C (4.09 ± 2.02 in group T vs. 1.36 ± 2.33 in group C; $p = 0.008$). This result suggests that pre-clamping administration of rhsTM in rabbit ischemic spinal injury model may prevent paraplegia.

***In vitro* experiments:** The cell viability of PC12 cells against OGD stress was assessed using the MTT method. The cell viability in group A2 was significantly higher than that in group N at 210 minutes of OGD [115.9 ± 4.7% in group A2 ($n = 12$) vs. 100 ± 5.0% in group N ($n = 12$) at 210 minutes; $p = 0.0476$). These results suggest that pretreated rhsTM has a protective effect on PC12 cells in a culture model of hypoxia-reoxygenation (Figure 4).

Discussion

Descending thoracic and thoracoabdominal aneurysms are currently treated with stent grafting, and the complication of paraplegia is considered rare (4-7% incidence); however, for anatomical reasons, stent grafting cannot be performed in all cases, and vascular grafting is still commonly performed. Among the possible complications, paraplegia is reportedly the most serious, with a prevalence of 8-28%, and it is mostly caused by ischemia reperfusion injury associated with aortic clamping and declamping[2].

Several studies have investigated and identified the mechanisms underlying ischemia reperfusion injury and the importance of inflammatory response in various organs, including the spinal cord[12]. The spinal-protecting effects of drugs acting on proteolytic enzymes and free radicals involved in inflammatory response in the endothelial cells, neutrophils, and macrophages have been reported. Iwamoto *et al*. showed that sivelestat sodium, a neutrophil elastase inhibitor, plays a major role in protecting the spinal cord by suppressing the inflammatory response in a rabbit ischemic spinal injury model[12]. Liu *et al*. reported that ulinastatin administration significantly improved postischemic neurologic function with concomitant reduction of apoptotic cell death in the spinal cord ischemia reperfusion rabbit model[13]. Nazil *et al*. investigated the protective effect of atorvastatin on ischemia-induced spinal cord injury in a rabbit model[14].

TM is a glycoprotein that presents on the membrane surface of endothelial cells, consisting of 5 domains; N-terminal lectin-like domain (D1), an EGF-like domain (D2), an O-glycosylation –rich domain (D3), transmembrane domain (D4) and cytoplasmic domain (D5). Thrombin binds EGF-like domain in TM that contributed to the anti-coagulant effect by activating protein C (APC). TM reportedly showed not only anti-coagulant, but also an anti-inflammatory effect[8].

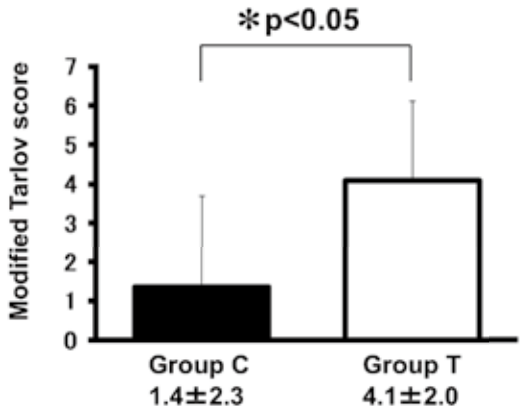

Figure 3. Evaluation of hind limb motor function. The modified Tarlov score in group T was significantly higher than that in group C ($p = 0.008$).

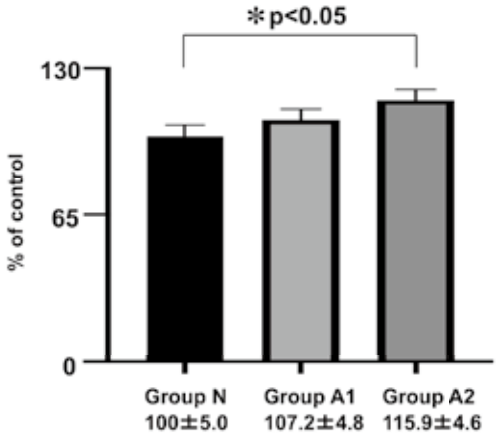

Figure 4. Evaluation of the viability in the PC12 cells. The viability of the PC12 cells in group A2 (n = 12) was higher than that in group N (n = 12) under OGD condition for 210 minutes ($p = 0.0476$).

Recently, rhsTM was clinically used for disseminated intravascular coagulation (DIC) owing to the anti-thrombin and anti-coagulation effects mediated by APC. Furthermore, anti-inflammatory and protective effects on ischemia-reperfusion injury of rhsTM in liver and kidney were reported[4-7].

Sharfuddin *et al*. reported that TM treatment prevented ischemia-induced renal dysfunction and improved survival in acute renal failure caused by clamping of the suprarenal aorta of rats. Moreover, the authors demonstrated that TM significantly improved microvascular erythrocyte flow rates and reduced microvascular endothelial leukocyte rolling and attachment[4]. Ozaki *et al*. reported the administration of rhsTM in rats with clamped aorta at the proximal and distal renal artery significantly improve renal function, independent of the coagulating factors[5]. The cytoprotective effects of rhsTM on ischemic liver have also been reported. Intravenous administration of rhsTM rescued the liver function in rats who underwent hepatectomy and liver transplantation[6,7]. Based on the above-mentioned reports on the kidneys and liver, we investigated and confirmed the protective effect of rhsTM on ischemic spinal cord model in rabbits and on hypoxia reoxygenation damage in PC12 cells. Previously, Kim *et al*. has shown the neuroprotective effect of ethyl pyruvate (EP), a pyruvate derivative, on primary microglial cells stimulated by OGD or hydrogen peroxide treatment by removing reactive oxygen species (ROS)[10]. Our study showed that rhsTM increased the cell viability in PC12 cells challenging by OGD. This *in vitro* data showing the protective effect of TM on PC12 cells might be caused by suppressing ROS in OGD-treated cells.

The present study has certain limitations. At first, the duration of sustained spinal cord ischemia *in vivo* was 30 minutes, this may be insufficient time for sustained ischemia in complicated cases, such as in the aortic aneurysm operation of Crawford types II and III. Secondly, the observation time was not sufficient for evaluating the delayed death of motor neurons. Thirdly, because of the differences in the species between humans and rabbits/rats in the experiments, it is unclear whether the administrated dose of rhsTM would be safe for humans. In fact, the dose was much higher than that used in clinical practice, however, there were no hemorrhagic complications in our rabbit model. To our knowledge, this is the first study to demonstrate that rhsTM protects against spinal cord IRI in rabbit model. Our findings suggest that rhsTM is a novel therapeutic agent against paraplegia caused by IRI in the spinal cord. In the future, rhsTM is expected to be used for preventing IRI with heparin sodium in aortic operation.

Conclusion

We demonstrated the beneficial effects of rhsTM *in vivo* and *in vitro*. The administration of rhsTM decreased IRI-induced paraplegia in rabbit model and the treatment of rhsTM suppressed OGD-induced PC12 damage. Our findings suggested that rhsTM is attributed to the protective effect against paraplegia caused by spinal cord IRI in aortic aneurysm surgery.

For its clinical application, the administered dose, timing of administration, and adverse effects require further investigation.

COI declaration

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References

1) Kawaharada N, Higami T. Open surgery of thoracoabdominal aortic aneurysm. Nihon Geka Gakkai Zasshi 2011;112:11-16. in Japanese
2) Sinha AC, Cheung AT. Spinal cord protection and thoracic aortic surgery. Current Opinion in Anaesthesiology 2010;23:95-102.
3) Nazli Y, Colak N, Namuslu M, et al. Cilostazol attenuates spinal cord ischemia-reperfusion injury in rabbits. J Cardio Vasc Anes 2015;29:351-359.
4) Sharfuddin AA, Sandoval RM, Berg DT, et al. Soluble thrombomodulin protects ischemic kidneys. J Am Soc Nephrol 2009;20:524-534.
5) Ozaki T, Anas C, Maruyama S, et al. Intrarenal administration of recombinant human soluble thrombomodulin ameliorates ischaemic acute renal failure. Nephrol Dial Transplant 2008;23:110-119.
6) Tanemura A, Kuriyama N, Azumi Y, et al. Thrombomodulin administration attenuates ischemia-reperfusion injury of the remnant liver after 70% hepatectomy in rats: simulated model of small-for-size graft in living donor liver transplantation. Transplantation Proceedings 2014;46:1107-1111.
7) Kashiwadate T, Miyagi S, Hara Y, et al. Recombinant human soluble thrombomodulin (ART-123) prevents warm ischemia-reperfusion injury in liver grafts from non-heart-beating donors. Transplantation Proceedings 2012;44:369-372.
8) Abeyama K, Stern DM, Ito Y, et al. The N-terminal domain of thrombomodulin sequesters high-mobility group–B1 protein, a novel anti-inflammatory mechanism. J Clin Invest 2005;115:1267-1274.
9) Hallenbeck JM, Jacobs TP, Faden AI. Combined PGI2, indomethacin, and heparin improves neurological recovery after spinal trauma in cats. J Neurosurg 1983;58:749-754
10) Kim JB, Yu YM, Kim SW, Lee JK. Anti-inflammatory mechanism is involved in ethyl pyruvate-mediated efficacious neuroprotection in the postischemic brain. Brain Res 2005;1060:188-192.
11) Kikuchi K, Kawahara K, Tancharoen S, et al. The free radical scavenger edaravone rescues rats from cerebral infarction by attenuating the release of high-mobility group box-1 in neuronal cells. J Pharmacol Exp Ther 2009; 329; 865-874
12) Iwamoto S, Higashi A, Ueno T, Goto M, Iguro Y, Sakata R. Protective effect of sivelestat sodium hydrate (ONO-5046) on ischemic spinal cord injury. Interact Cardiovasc Thorac Surg 2009;8:606-609.
13) Liu B, Huang W, Xiao X, Xu Y, Ma S, Xia Z. Neuroprotective effect of ulinastatin on spinal cord ischemia-reperfusion injury in rabbits. Oxid Med Cell Longev 2015;Article ID624819.
14) Nazli Y, Colak N, Alpay MF, Uysal S, Uzunlar AK, Cakir O. Neuroprotective effect of atorvastatin in spinal cord ischemia-reperfusion injury. Clinics 2015;70:52-60.

遺伝子組み換えトロンボモジュリンの虚血再灌流障害に対する脊髄保護効果

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和文要約

【背景】下行大動脈瘤や胸腹部大動脈瘤置換術において脊髄虚血による対麻痺は重篤な合併症の一つである．その予防として外科的，薬物的なさまざまな脊髄保護方法が試みられてきているが，未だ確立された方法はない．近年肝・腎の虚血再灌流障害に対する抗炎症作用を持つ遺伝子組み換えトロンボモジュリン（recombinant human soluble thrombomodulin：以下rhsTM）の保護効果が報告されている．我々は今回の研究で，ウサギの脊髄虚血モデルとPC12細胞を使って，脊髄の虚血再灌流障害に対するrhsTMの保護効果を*in vivo*及び*in vitro*実験で検討した．

【方法】*In vivo*実験：22羽のNew Zealand white rabbitsを等しく２グループに分けた．腹部正中切開で開腹し，左腎動脈直下と大動脈分岐部直上，後腸間膜動脈起始部を遮断し，30分間の脊髄虚血を作成した．大動脈遮断前30分間に経静脈的に生食20mL（C群: n = 11)またはrhsTM (6mg/kg) +生食/ 20mL (T群: n = 11)を投与した．大動脈遮断解除48時間後にmodified Tarlov scoreで後ろ脚の運動機能評価を行った．

*In vitro*実験：神経細胞モデルとしてラット副腎髄質由来親褐色性細胞腫由来の PC12 細胞を用いた．rhsTM非投与群（N群)と1, 10 μg/mL のrhsTM投与群（A1, A2群)とに分け，虚血病態を模したoxygen-glucose depriviation (OGD)条件下にプレートを210分間嫌気性培養装置アネロパックジャーに入れ，24時間後の細胞生存率を MTT 法で評価した．

【結果】*In vivo* 実験 : C群の8羽が麻痺のgrade 0, 3羽が正常のgrade 5であった．T群の2羽がgrade 0, 9羽がgrade 5であった．Modified Tarlov scoreの平均はT群で4.1 ± 2.0，C群で1.4 ± 2.3であり，有意にT群で高値であった($p < 0.05$)．ウサギ脊髄虚血モデルに対するrhsTMの遮断前投与による対麻痺の予防効果が示唆された．

In vitro 実験:細胞生存率はN群100 ± 5.0%に対して，A2群115.9 ± 4.7%であり，A2群がN群に比べて有意に高かった($p < 0.05$)．神経細胞モデルの虚血に対して，rhsTM投与による保護的作用が示唆された．

【結論】rhsTMの虚血前投与による虚血再灌流障害の軽減，脊髄保護効果が*in vivo*実験，*in vivo*実験でともに示唆された．大動脈瘤手術での虚血再灌流障害に対するrhsTMの脊髄保護効果が期待されるが，その臨床応用には投与量や投与時期，副作用などの更なる検討が必要である．