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Trans-vertebral Regional Cooling for Spinal Cord Protection during Thoracoabdominal Aortic Surgery: An Experimental Study

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ABSTRACT

We developed a simple cooling method for spinal cord protection against ischemic injury during aortic surgery. The neuroprotective effects of our method were investigated using an animal study.

Selective spinal hypothermia was produced by means of originally-designed cooling pads placed over the lower thoracic and lumbar vertebral column. Spinal cord ischemia was induced by cross-clamping the thoracic aorta for 60 min in beagle dogs. The neuroprotective effects were evaluated by a multi-modal study. The motor-evoked potentials of the spinal cord resulting from transcranial electric stimulation (MEPs) were recorded during both the ischemic and reperfusion periods. Hindlimb motor function was graded with the Tarlov score, and a histologic examination of the spinal cord injury was performed, at 24 hours after ischemia in animals undergoing hypothermia (hypothermia group: n = 7) or a sham (control group: n = 7).

The spinal cord temperatures at the lower thoracic (T10) and lumbar (L3) levels decreased by –9.1°C per hour and –8.1°C per hour, respectively. The amplitude of the MEPs decreased during ischemia in both groups of animals, and significantly recovered during the early phase of aortic reperfusion in the hypothermia group. The Tarlov scores in the hypothermia and control groups were 3.3 ± 1.0 and 1.1 ± 1.5 (mean ± SD, p = 0.015), respectively. Histopathologic study revealed that ischemic injury of the lumbar cord was reduced in the animals undergoing hypothermia.

Trans-vertebral regional cooling reduced ischemic spinal cord injury in a canine study. The current method is potentially feasible for clinical use, especially in view of its technical simplicity and few procedure-related complications.

Key words: *Spinal cord ischemia, Spinal cord protection, Cooling method, Thoracoabdominal aortic surgery*

Paraplegia caused by ischemic spinal cord injury is one of the most devastating complications related to the surgical repair of aneurysms involving the thoracic and thoracoabdominal aorta. Despite refinements in surgical technique and adjunctive measures for spinal cord protection, the risk of postoperative paraplegia and paraparesis remains significant, with its incidence ranging 2% to 21%[2,6,18].

Hypothermia has been proven to preserve the integrity of the spinal cord against an ischemic insult[1-5,8-10,12,13,16,17]. Cooling the ischemic region of the cord has been considered to be a potential measure for neurologic protection during thoracoabdominal aortic aneurysm (TAAA) repair. Although several methods for selective spinal cord hypothermia have been introduced[1-3,5,8,12,16,17], none has been prevalent for clinical application, because each method involves such problems as the complex procedure and the potential risk of procedure-related neuronal injury.

We have designed a unique concept for spinal cord protection in which a simple procedure promises substantial effects. Spinal cord sustaining blood flow insufficiency is preserved by cold pads which are placed over the vertebral structure within the body cavity. The purpose of the present experiment is to evaluate both the cooling efficiency and the neuroprotective effects of our method.

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MATERIALS AND METHODS

Animal Preparation

Animal care and all procedures were performed in compliance with the Guide for Care and Use of Laboratory Animals. This study protocol was approved by the Research Facilities for Laboratory Animal Science, Hiroshima University.

Twenty-five beagle dogs of either sex, weighing 9.0–11.0 kg, were studied. Each animal was immobilized with an intra-muscular injection of ketamine hydrochloride (50 mg/kg) after premedication with atropine sulfate (0.5 mg). After anesthetic induction, one intravenous line was introduced into an anterior limb vein. An inflatable cuffed endotracheal tube was inserted following the intravenous administration of thiamylal sodium (15 mg/kg) and pancronium bromide (0.2 mg/kg), and the animals were then ventilated with a volume-controlled ventilator delivering 1.0–3.0% isoflurane in a mixture of 33% O_2 in N_2O. The adequacy of the ventilation was confirmed by blood gas analysis at 37°C. The arterial blood pressure of the proximal and distal aorta was measured by catheters placed into the right femoral artery and the left interthoracic artery. Electrocardiograms and the arterial pressure were monitored continuously. The systolic arterial pressure of the proximal aorta was maintained at 100–140 mmHg throughout the experiment. Fluids were substituted by Ringers' lactate solution.

The animals were placed in the right lateral decubitus position. A thoracotomy was performed via the left 8th intercostal space. A left hypochondrial incision was made and extended to the left lateral abdomen. A laparotomy was performed following an incision of the muscles of the abdominal wall. The retroperitoneal space was dissected, exposing the left-side perivertebral muscles and anterior surface of the lumbar vertebral bodies at L1-L5.

Cooling Procedure

A bag (12 × 3 cm) made of polyvinyl chloride was packed with 16 ml of gel (ingredients: water, 98%; acrylate sodium polymer, 1%; propylene glycol, 1%) to prepare a cooling medium for use (Fig. 1-A). This pad was frozen at –20°C for at least 24 hours in a freezer, and was taken out just before use. We chose this temperature because the cooling material frozen at 0°C melted for only 10–15 min under the operative light. The cold pad was placed longitudinally along the vertebral column at the level of T8-T13, and was attached on the left side surface of these vertebral bodies and the left articulation of the head of the rib (Fig. 1-B). Another pad was simultaneously attached to the anterior surface of the lumbar vertebral bodies and the perivertebral muscles at the level of L1-L5 in a similar fashion. Visible changes caused by thermal injury were not evident on the surface of the vertebral and perivertebral structure. The pads were replaced by new ones every 30 min.

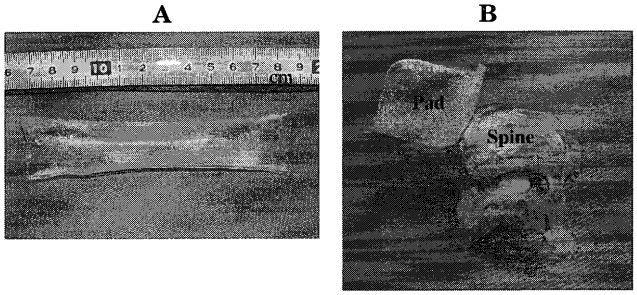

Fig. 1. Photographs showing a cooling pad (A) and the pad application in a cross section (B).

Spinal Cord Temperature Measurement

Eleven animals were used to determine the cooling efficiency of this method. To prepare for temperature measurement, a laminectomy at T10 and L3 was performed, and a needletip for recording the temperature was inserted into the central portion of the spinal cord with a puncture of the dura. The spinal cord temperature was recorded using a digital thermometer, PTW-100A (Unique Medical Co. Ltd., Tokyo, Japan), at the levels described above. The rectal temperature was simultaneously recorded. The thoracic aorta just distal to the left subclavian artery was cross-clamped for 60 min following the intravenous administration of heparin (100 units/kg). The temperatures were then recorded during aortic occlusion. These measurements were performed in animals undergoing the current cooling method (n = 6) or a sham procedure (n = 5).

Neurologic Studies

Other fourteen animals were used to evaluate the protective effects of our method against spinal cord ischemia. The schema of this neurologic study protocol is shown as Fig.2. In the animals undergoing hypothermia (n = 7: hypothermia group), the cooling procedure was initiated 15 min before the aortic cross-clamping and continued throughout the aortic occlusion for 60 min. The cold pad application was initiated before aortic occlusion, because we intended that the spinal cord temperature should decrease below 34°C and that the cord could be protected to some degree at the beginning of the ischemic challenge. The sham animals underwent the same protocol except for the cooling procedure in the control group (n = 7).

Before the thoracotomy, the preparation for electrophysiologic studies had been completed. As a recording electrode for the motor-evoked potential of the spinal cord resulting from transcranial electric stimulation (MEP), a catheter-type bipolar electrode (UKG-100-2PM; Unique Medical, Tokyo, Japan) was inserted into the epidural space at the L3 level with a 17 gauge Tuohy needle. The MEP

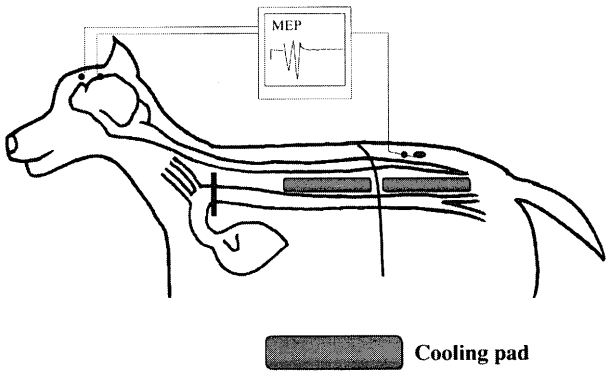

Fig. 2. Schematic demonstration of the neurologic study protocol. MEP: motor-evoked potential of the spinal cord resulting from transcranial electric stimulation.
A cross-clamp was placed on the proximal descending aorta. Two cold pads were simultaneously placed over the low thoracic and lumbar vertebral column. The MEPs were measured for monitoring spinal cord function.

was elicited by transcranial stimulation of the motor cortex. The stimuli (100mA, pulse duration of 0.5 ms, pulse rate of 4.0 per second) were applied to the bilateral temporal scalp using 2 needle type electrodes. Stimulation and recording were performed using a Nicolet Viking IV system (Nicolet Biomedical, Inc., Madison, WI). The recorded potentials were amplified and averaged for 100 impulses per recording. The amplitude of the MEP was confirmed as a peak-to-peak difference of the wavelet. The MEP amplitude recorded at 15 min before the ischemia served as the control value. The MEP measurement was taken every 15 min during both aortic clamping and reperfusion, and ended at 60 min after declamping. These amplitude data at each time point were expressed as a percentage of the corresponding control value.

At the end of aortic occlusion, the vascular clamp was removed and the heparin was neutralized with protamine (1 mg/kg). The chest and abdominal wounds were closed and all of the catheters were withdrawn after the last recording of the MEP. The animals were extubated when fully awaken. The neurologic outcome in each group was evaluated at 24 hours after the spinal cord ischemia. The motor function of the hindlimbs was graded using the Tarlov score, in which 0 indicates no movement of the hindlimbs, 1 indicates perceptible movement of the joints of the hindlimbs, 2 indicates good movement but the inability to stand, 3 indicates the ability to stand and walk, and 4 indicates a complete recovery[19].

Histologic Studies

After completion of the neurologic evaluation, anesthesia was introduced again with an intravenous administration of thiamylal sodium (15 mg/kg) following intramuscular injection of ketamine hydrochloride (1.5 ml/kg) and xilazine (0.2 ml/kg). The animals were sacrificed with an intravenous administration of kalium chloride (2.0 ml/kg), and the spinal cord was excised for histologic examination after removing all of the thoracic and lumbar vertebral arches. The spinal cord was sectioned from the low thoracic to lumbar levels, because our preliminary study revealed that the spinal cord distal to the low thoracic level was likely to be affected after cross-clamping the thoracic aorta. Among the sectioned specimens, the six levels at T 8, T10, T12, L1, L3 and L5 were selected. From each specimen, two 7-micro-meter sections were cut and stained with hematoxylin and eosin. An examiner blinded for group designation reviewed all of the slides and scored the extent of the ischemic changes based on the grading scale shown in Table 1.

Table 1. Histologic assessment score of ischemic spinal cord injury.

Histologic assessment	Score
No change	0
Perineural edema	1
Scattered one-cell necrosis	2
Necrosis of central-medial portion of aneterior horn	3
Necrosis of entire anterior horn	4

Statistical Analysis

All results except for the MEP data were expressed as means ± SD. The MEP amplitudes were expressed as means ± SE. The data were compared between the two groups using the Mann-Whitney U test and were determined to be significantly different when p was < 0.05.

RESULTS

Cooling Efficiency

The changes in the spinal cord temperature in the animals undergoing hypothermia and the controls are shown as Fig. 3. The spinal cord temperature at the T10 and L3 levels in dogs undergoing the cooling method gradually decreased from 36.3 ± 0.8°C and 36.2 ± 1.2°C to 27.2 ± 1.0°C and 28.2 ± 0.2°C at 60 min after initiating hypothermia, respectively. The rectal temperature in these animals decreased from 36.9 ± 0.7°C at initiation to 35.0 ± 1.1°C during the same period. The spinal and rectal temperature in the animals undergoing only aortic cross-clamping remained almost unchanged.

Recovery of the MEP Amplitude

The changes in the MEP amplitude of each group are shown in Fig. 4. The amplitude decreased during aortic occlusion in both groups.

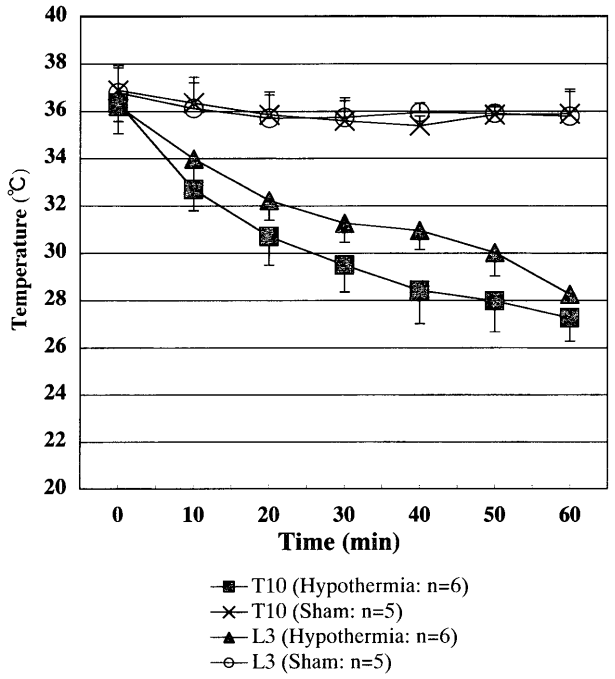

Fig. 3. Changes in the spinal cord temperature at T10 and L3.
The spinal cord temperature in animals not undergoing hypothermia remained almost unchanged throughout the protocol. The temperature at T10 and L3 in animals undergoing the cooling procedure decreased simultaneously and reached 27.2 ± 0.99°C and 28.2 ± 0.19°C, respectively after 60 min.

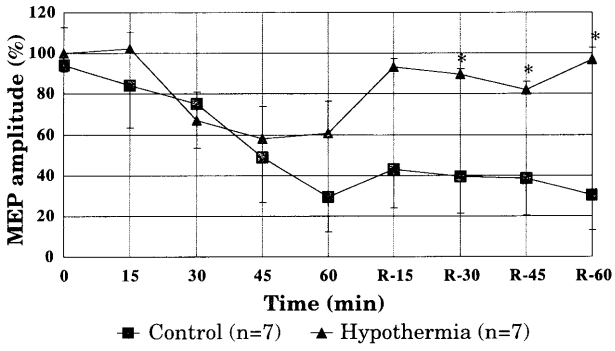

Fig. 4. Changes in the amplitude of the motor-evoked potential of the spinal cord resulting from transcranial electric stimulation (MEP) during the aortic occlusion and reperfusion period. *$p < 0.05$
The amplitude decreased during ischemia in both groups. The mean amplitudes of the hypothermia group were significantly larger than those of the control group at 30, 45 and 60 min during reperfusion.

There was no significant difference in the amplitude between these groups at any time point during ischemia. The amplitude recovered during the reperfusion period in the animals undergoing hypothermia. Significantly higher amplitudes were recorded at 30 ($p = 0.047$), 45 ($p = 0.034$) and 60 ($p = 0.025$) min after aortic declamping in the hypothermia group than in the controls.

Neurologic Outcome

Three animals in the control group were completely paraplegic, but none were paraplegic in the hypothermia group. Two of the control animals demonstrated a Tarlov score of 1, and the other two had scores of 2 or 4. Four animals undergoing hypothermia experienced complete functional recovery. Two animals had a Tarlov score of 3 and the other one 2. The scores of the control and the hypothermia group were 1.1 ± 1.5 and 3.3 ± 1.0 ($p = 0.015$), respectively.

Histologic Assessment

The typical histopathologic findings in the lumbar cord of animals in the hypothermia and control group are shown in Fig.5. The histologic assessment scores at different vertebral levels are shown in Fig.6. Necrosis of nearly the entire or central-medial portion of the gray matter of the lumbar cord was detected in a control animal presenting with a poor functional outcome. In the hypothermia group, no ischemic damage was

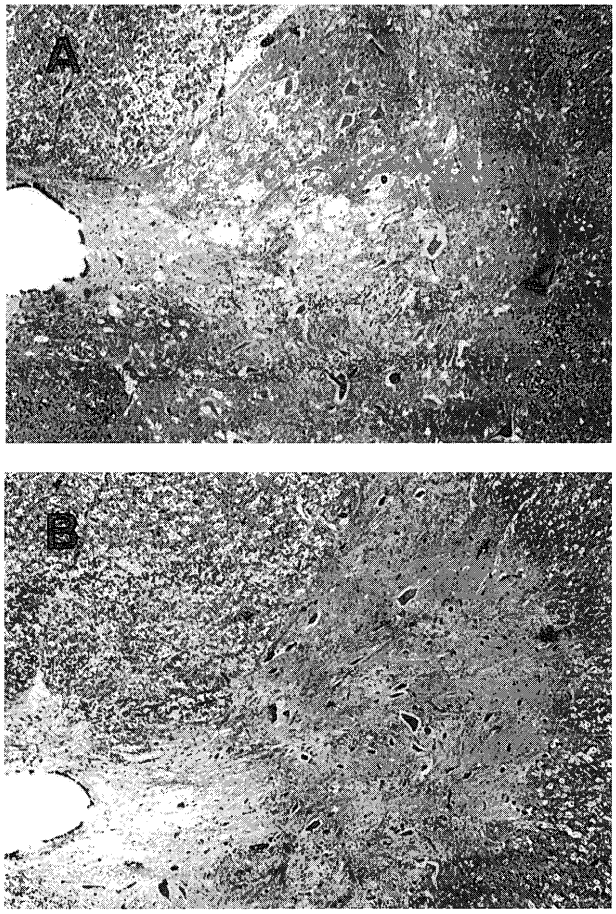

Fig. 5. Typical histopathologic findings of the spinal cord at the L3 level at 24 hours after ischemia (magnification × 40).
A: This animal did not undergo hypothermic protection. Necrosis in the central-medial portion of the anterior horn was detected. B: This animal underwent hypothermia. No histologic damage was detected in the spinal cord.

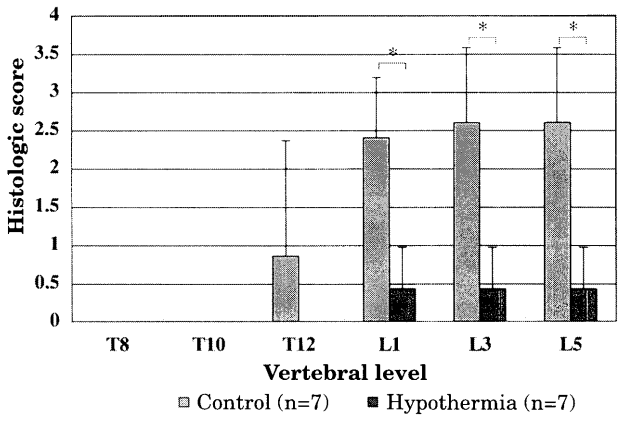

Fig. 6. Scores for the histologic assessment of different vertebral levels of the spinal cord. *p < 0.01
In the control animals, findings of ischemic injury were evident in the lumbar region. In the animals undergoing hypothermic protection, tissue injury was significantly reduced compared with the control.

detected in the animals with a full neurologic recovery. Animals with a Tarlov score of 2 or 3 had perineural edema in the lumbar cord. Significant differences in this assessment score were noted at L1 (p = 0.0023), L3 (p = 0.0025) and L5 (p = 0.0025), between the two groups.

DISCUSSION

Since Hufnagel's first report in 1945[9], both localized and systemic hypothermia have been shown to reduce ischemic injury of the spinal cord[1-5,8,10,12,13,16,17]. Profound systemic hypothermia (18–25°C) instituted with cardiopulmonary bypass clearly extended the window of spinal ischemic tolerance[16]. However, this is not commonly used for TAAA repair, because of the risk of myocardial irritability[14], coagulation defects[15] and an increased risk of wound infection[11]. On the other hand, a number of methods to cool the ischemic region have been proposed, such as epidural cooling (EC)[2,3,8], selective hypothermic perfusion with an individual pump[5], subdural cooling[1] and retrograde hypothermic perfusion via the hemiazygous vein[17]. Among these modalities, EC is the only one which was shown to be efficacious by clinical studies[2,3].

Cambria et al applied EC to 170 patients in which the cerebrospinal fluid (CSF) temperature was reduced to 26°C, with a core temperature of 35°C. The incidence of total paraplegia was reduced to 2%, and a favorable shift in the pattern and severity of lower-extremity neurologic deficits was evident[2]. However, there are several drawbacks to the use of EC. Hemodynamic instability usually renders EC preparation difficult for emergency cases, in which the incidence of paraplegia is relatively high. In addition, managing the infusion rate of the cold solution and the CSF pressure is not easy[1]. Moreover, an increase in the CSF pressure during infusion has the potential to cause some damage in the central nervous system.

In the context of this study, we have presented a very simple method for spinal cord protection. Although surface cooling of the back for spinal hypothermia has been previously introduced[12], the current concept of placing cooling materials over the vertebral structure in the body cavity has never been documented. Regarding the local temperature for reducing ischemic injury, mild hypothermia at 34°C was shown to produce motorneuronal protection in two previous studies using retrograde hypothermic perfusion[17] and percutaneous cooling[12]. In Cambria's experience, as described above, the spinal cord temperature would certainly be higher than 26°C because this value was recorded in the CSF temperature measurement. In the current experiment, our technique created a local hypothermia at 27°C. This value was nearly equivalent to the lowest temperature in clinical EC application.

The conus medullaris is located in the lower lumbar region in dogs, while its location in the human is at T12 or L1. In the current canine model, not only the thoracic but also the lumbar spine had to be simultaneously protected, because the spinal cord distal to the lower thoracic level was susceptible to ischemic insult in our preliminary investigations. If our method was applied in humans, even a single application in the thoracic cavity could protect almost the entire ischemic region of the cord.

The limitation of this experimental study lies primarily in an anatomic problem. Heat flow through the bone can be theoretically calculated from the equation, $Q = k_b A_b T_b / t_b$, where Q = the heat flow, k_b = the thermal conductivity of bone, A_b = the cross sectional area of heat flow, T_b = the temperature drop across the specimen and t_b = the thickness of the bone[7]. The amount of thermal flow is inversely proportional to bone thickness. Accordingly, differences in vertebral size between different species substantially affect the cooling efficiency of our method. We chose the vertebral arch as an index to determine the differences in vertebral size between different species, because the pedicle of the arch is considered to play some role in thermal conduction. The maximum thickness of the pedicle of the vertebral arch and the maximum distance between the central point of the spinal cord and the left lateral surface of the pedicle at T12 were measured in the sacrificed dogs; these values were 0.58 ± 0.07 and 1.05 ± 0.04 cm (n = 20: mean ± SD), respectively. The same measurements were made in humans, using CT scan images of Japanese males with body weights ranging from 55 to 60 kg (n = 20). The average values were 0.91 ± 0.13 and 1.73 ± 0.16 cm, respectively. Since this anatomic factor makes

it difficult to estimate the cooling potential in clinical use, further studies with larger animals or an examination of the CSF temperature in humans remains necessary.

Finally, our technique has potential problems for clinical use. During a number of TAAA repairs, there remains little space for placing a cold pad over the thoracic vertebrae behind a huge aneurysm and above the diaphragm. One possible solution is that a mechanical pump perfusion is used to perfuse the distal aortic segment with an institution of mild hypothermia (32–34°C), before aortic cross-clamping, and then cold pads are placed after opening the aneurysm. Thus, the spinal cord temperature can be reduced to 32°C before spinal ischemia. The cold pad application after incising the aneurysm is expected to produce spinal hypothermia of below 30°C, supplementing the neuroprotective effects of the hypothermic circulation. Another problem is the cooling rate of the current method. Since half an hour was required to produce local hypothermia at nearly 30°C in this canine experiment, it may take more time for our method to produce protective actions in humans. Nevertheless, our technique will have a certain degree of favorable effect on the ischemic spinal cord, especially during operations where a long cross-clamp time is necessary.

CONCLUSION

This experimental study showed that the transvertebral cooling method resulted in neuroprotective effects against ischemic spinal cord injury. Further studies remain necessary to assess its precise feasibility for TAAA surgery. Nevertheless, our method has potential for clinical use because of its technical simplicity.

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