The Consequences of Cerebral Venous Circulatory Disturbances Associated with Brain Ischemia

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Summary: The aim of this study was to test the effects of interference with venous return from the brain on the brain damage produced by occlusion of the middle cerebral artery (MCA-O). Tests were performed in anesthetized, artificially ventilated rats. The extent of the infarction produced in the hemisphere ipsilateral to the occluded middle cerebral artery (MCA) was less in rats in which cerebral venous return had been disrupted by bilateral ligation of the external and internal jugular veins. The blood flow of a sample cortical area ipsilateral to the MCA-O (measured by a laser Doppler flowmeter) decreased less, during a 45 min MCA-O, in the group with venous return interference. The blood concentration of the marker of CNS damage (the S-100 protein), measured 24 hrs after the end of the 45 min MCA-O, was lower in the rats with venous return interference. In order to be effective, the interference with venous return had to start before, and be concurrent with, MCA-O. When the interference with venous return started during the period of MCA-O, the brain damage was enhanced and resulted in death of the animals within 24 hrs post-occlusion. It is concluded that increasing brain venous pressure may, under some conditions, protect brain tissue against ischemic damage.

Key words cerebral infarction, middle cerebral artery occlusion, cerebral venous circulatory disturbance

INTRODUCTION

Negative outcomes are often observed after brain ischemia. In particular, brain ischemia can occur during surgery of the aortic arch and carotid artery. When cerebral infarction develops perioperatively, its size cannot be estimated accurately. Therefore, it is difficult to determine the factors, other than arterial ischemia, influencing the size of cerebral infarction. A cerebral venous circulatory disturbance (CVCD) can occur during cardiovascular surgery as a result of inappropriate venous drainage during cardiopulmonary bypass (CPB) or prolonged retrograde cerebral perfusion (RCP). CVCD and recirculation may have an influence on cerebral ischemia and they modify postischemic events in various ways. For example, CVCD during RCP prolongs the term of brain ischemia. We studied the consequences of CVCD in case of brain ischemia using a focal ischemic rat model consisting of unilateral middle cerebral artery (MCA) occlusion. In this study, we showed that CVCD affects brain ischemia.

MATERIALS AND METHODS

Rats were used in this study. The study protocol was approved by the Kurume University Ethics Committee for Animal Research. All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals.”

Received for publication December 5, 2003

Abbreviations: ACA, anterior cerebral artery; CBF, cerebral blood flow; CBV, cerebral blood volume; CPB, cardiopulmonary bypass; l-CBF, local cerebral blood flow; r-CBF, regional cerebral blood flow; CVCD, cerebral venous circulatory disturbance; FAM, formaldehyde: glacial acetic acid: methanol; HCA, hypothermic circulatory arrest; HE, hematoxylin and eosin; LDF, Laser-Doppler flowmetry; MCA, middle cerebral artery; MCA-O, middle cerebral artery occlusion; RCP, retrograde cerebral perfusion; SCP, selective cerebral perfusion; TTC, 2, 3, 5-triphenyltetrazolium chloride.
Twenty-three male Wistar rats weighing 250 to 310 g were used for this study. The rats were allowed free access to food and water before and after all procedures. Anesthesia was induced with inhalation of diethyl ether and intraperitoneal injection of sodium pentobarbital (50 mg/kg) after premedication with 0.5 mg atropine. Anesthesia was maintained with hourly intraperitoneal injections of sodium pentobarbital (25 mg/kg). After endotracheal intubation, the animals were placed in the supine position and maintained on positive pressure ventilation with 30% oxygen. The pump rate was set at 60 strokes/min. End-tidal carbon dioxide was kept within 35-45 mmHg. A polyethylene catheter was placed into the tail artery for continuous monitoring of arterial blood pressure. Rectal temperature was maintained at approximately 37°C by a heating pad. Local cerebral blood flow (l-CBF) was measured by Laser-Doppler flowmetry (LDF) using a 0.8 mm needle probe (ALF-21, Advance, Tokyo). l-CBF during the experiment was expressed as % change from the control, pre-experiment value. Using an operating microscope (Nikon, Tokyo), a cranial window (4.5 mm X 6 mm) was created over the right frontoparietal region using a high-speed drill. The drill tip was cooled during the craniotomy by continuous irrigation with physiological saline. The right frontoparietal cortex was exposed. The probe was mounted on a micro-manipulator and placed approximately 0.5 mm above the pial surface within the cranial window. Once a suitable placement was obtained, the probe was left at that site for the duration of the experiment.

The rats were divided into four experimental groups as follows.

Group 1 (n=6): middle cerebral artery occlusion (MCA-O) without CVCD.
Group 2 (n=6): CVCD without MCA-O.
Group 3 (n=6): CVCD was applied before MCA-O.
Group 4 (n=5): CVCDs was induced during MCA-O.

The mean weight of the rats was 281±22 g in Group 1, 277±31 g in Group 2, 280±27 g in Group 3, and 260±17 g in Group 4, with no significant differences among the groups. Twenty-four hours post-operatively, the rats were again anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Blood samples were taken for S-100 protein and lactic acid determinations. The rats were then sacrificed. The brains were removed and sliced into 1-mm thick sections. The sections were stained with 2, 3, 5-triphenyltetrazolium chloride (TTC). Infarcted brain regions did not convert TTC and remained unstained [1]. The unstained area was measured in each block by NIH image for Macintosh computers. The volume of infarction was the sum of the unstained areas times the sum of the slice’s thickness. Blood data and infarction volumes were compared among groups 1-3. Group 4 was used only as a comparison to Group 3 with respect to neuronal damage.

MCA-O procedures

With the rats in the supine position, after a median incision of the neck skin, the right common carotid artery was exposed and ligated with careful conservation of the vagus nerve. The right external carotid artery and the pterygopalatine artery were similarly exposed and ligated. We refer to Koizumi et al. [2] for the following procedure. The right MCA was occluded with a silicone rubber cylinder attached to a nylon surgical thread introduced from the bifurcation of the internal carotid artery [3]. The thread extended from the bifurcation of the internal carotid artery to the proximal portion of the anterior cerebral artery (ACA). The origins of the right MCA and of the posterior communicating artery were occluded by the silicone rubber cylinder (Fig. 1A). MCA-O was confirmed by LDF. The motor area of the frontoparietal cortex of the occluded side was supplied by the ipsilateral ACA via the anterior communicating artery from the contralateral internal carotid artery. MCA-O was continued for 45 min and then the thread was removed for recirculation. In this model, the ischemic area could be recirculated via the cerebral arterial circle (circle of Willis) through the contralateral carotid artery, basilar artery, and collateral circulation of the cortical branches of the cerebral arteries since the ipsilateral common and external carotid arteries had been ligated (Fig. 1B).

CVCD procedure

With the rats in the supine position, through the median incision of the neck, the internal and external jugular veins were carefully exposed bilaterally, ligated and cut. CVCD was performed before MCA-O in Group 3 and CVCD during MCA-O in Group 4.

Additional comparison between Group 3 and 4

All the experimental rats in Group 4 died within 24 hrs (n=5). Therefore, an experiment was performed on two additional sets of five rats each (treated as those in groups 3 and 4, respectively, and...
Fig. 1. Schematic representation of the position of the silicon rubber cylinder during occlusion of the right middle cerebral artery (MCA) (A) and after recirculation (B). ACA, anterior cerebral artery; PCA, posterior cerebral artery; ICA, internal carotid artery; ECA, external carotid artery; CCA, common carotid artery; PPA, pterygopalatine artery; BA, basilar artery; SCA, superior cerebellar artery.

Fig. 2. Anatomical regions of coronal section to assess neuronal damage. 1, frontoparietal cortex, somatosensory area, supplied by middle cerebral artery; 2, lateral segment of caudate putamen; 3, medial segment of caudate putamen; 4, frontoparietal cortex, motor area, supplied by anterior cerebral artery. Shaded area represents ischemic area.
henceforth referred to as groups 3 and 4, respectively). Recirculation continued for 120 min following 45 min of MCA-O. The brains were perfusion-fixed with 40% formaldehyde: glacial acetic acid: methanol (1:1:8, FAM) via the ascending aorta [4]. The brains were removed and stored in FAM until they were embedded in paraffin. Brain sections (5 µm) were stained with hematoxylin and eosin (HE staining). The sections were examined under a light microscope, and regional neuronal damage in four ipsilateral areas was graded according to the number of cells with morphological changes. The damage was graded as mild (+), moderate (++), or marked (+++). Mild involvement (+) indicated that the number of damaged neurons was less than 30% of the total number. Moderate involvement (++) meant that the number of damaged neurons was more than 30% but less than 60% of the total. Marked involvement (+++) corresponded to cases in which the number of damaged neurons was greater than 60% of the total. The regions studied included the anterior neocortex and caudate putamen, which were most frequently damaged in this ischemic model (Fig. 2).

Data analysis
All values shown are means values±SD. The pairwise comparisons of individual group means were conducted by means of the Student t test. Statistical analyses were performed with Statview for Macintosh version 5.0 (SAS Institute Inc, Cary, NC).

RESULTS
As regards arterial blood pressure during the

![Fig. 3. Color images of brain sections stained with 2, 3, 5-triphenyltetrazolium chloride (TTC) in rats. The colorless area was the infarcted brain region. While ischemic damage in Group 1 was common in the frontoparietal cortex (somatosensory area) and the lateral segment of the caudate putamen, the damage in Group 3 was observed in only the lateral segment of the caudate putamen. Group 2 had no infarction in either hemisphere. Group 1: middle cerebral artery occlusion (MCA-O) without cerebral venous circulatory disturbance (CVCD), Group 2: CVCD without MCA-O, Group 3: CVCD was applied before MCA-O was induced.](image-url)
BRAIN ISCHEMIA WITH VENOUS CONGESTION

Experiment, almost no changes were observed in any of the groups. Four of six rats in Group 1 exhibited neurologic deficits characterized by left hemiparesis and right Horner’s syndrome within 24 hrs after the experiment. Such symptoms were not observed in Groups 2 and 3. All experimental rats in Group 4 died within 24 hrs after surgery. In the additional Group 4 (see Methods), the only observation made was an estimate of neuronal damage using hematoxylin and eosin-stained brain slices and a comparison of this damage to that observed in the additional Group 3 (Table 4). Figure 3 shows the extent of the ischemic area in this model demonstrated by TTC staining. The colorless area was considered to correspond to the territory supplied by the occluded MCA. In the ipsilateral hemisphere of Group 1 and 3 there was a constant pattern of infarcted area. While ischemic damage in Group 1 was common in the frontoparietal cortex (somatosensory area) and the lateral segment of the caudate putamen, the damage in Group 3 was observed in only the lateral segment of the caudate putamen. There was no area of infarction in the contralateral hemisphere of Groups 1 and 3, and Group 2 had no infarction bilaterally.

The brain volume of the left hemisphere showed no significant differences among the three groups. However, the volume of the right hemisphere in Group 1 was significantly larger than in Group 2 and 3. Regarding the infarcted region, its volume was significantly larger in Group 1 than in Group 3 (Table 1).

Figure 4 shows the changes in LDF during the experiment. The low values observed immediately after MCA-O, were not significantly different between Groups 1 and 3. However, while the LDF values in Group 1 did not recover significantly during the 45 min of MCA-O until recirculation, the LDF values in Group 3 recovered from about 31.8% of control to about 52.3% after 5 min of MCA-O. After recirculation, the LDF values of Group 3 were back to control, whereas those of Group 1 were still below control 15 min after the start of recirculation (Table 2).

Regarding the S-100 protein, its level was significantly higher in Group 1 than Group 3 (Table 3). The values of lactic acid did not differ significantly

### TABLE 1.

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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<tbody>
<tr>
<td>Left hemisphere (mm³)</td>
<td>364.5± 21.9</td>
<td>373.3±18.2</td>
<td>360.7±5.4</td>
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<tr>
<td>Right hemisphere (mm³)</td>
<td>437.2± 22.7</td>
<td>282.8±17.2*</td>
<td>365.4±7.9*</td>
</tr>
<tr>
<td>Infarcted region (mm³)</td>
<td>183.7±103.2</td>
<td>0</td>
<td>23.9±8.4*</td>
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All values are the means ± SD.
* P<.05 vs Group 1.

Fig. 4. Changes in Laser-Doppler flowmetry (LDF) values during the experiment. The starting flow value was defined as 100%. The graph of Group 3 was qualitatively similar to that of Group 1, but the values of Group 3 were higher than those of Group 1 throughout the experiment. CCA, common carotid artery; ECA, external carotid artery; PPA, pterygopalatine artery. Group 1: middle cerebral artery occlusion (MCA-O) without cerebral venous circulatory disturbance (CVCD), Group 2: CVCD without MCA-O, Group 3: CVCD was applied before MCA-O.
TABLE 2.
Laser doppler flowmetry (LDF)

<table>
<thead>
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<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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<tbody>
<tr>
<td>CCA ligation (%)</td>
<td>62.7±17.4</td>
<td>—</td>
<td>89.2±2.5*</td>
</tr>
<tr>
<td>MCA occlusion (%)</td>
<td>20.9±8.3</td>
<td>—</td>
<td>31.8±8.9</td>
</tr>
<tr>
<td>Recirculation (%)</td>
<td>33.4±12.1</td>
<td>—</td>
<td>82.3±16.3*</td>
</tr>
<tr>
<td>Minimum (%)</td>
<td>16.5±10.5</td>
<td>98.9±8.4*</td>
<td>31.8±3.6**</td>
</tr>
</tbody>
</table>

All values are percentages of the starting value and are shown as means±SD.
CCA, Common carotid artery; MCA, middle cerebral artery.
* P<.05 vs Group 1.
† P<.05 Group 3 vs Group 2.
Except for the minimum, the values shown are those occurring immediately after the indicated event.

TABLE 3.
Laboratory data of blood samples

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-100 protein (ng/ml)</td>
<td>2.1±1.4</td>
<td>0.8±0.6</td>
<td>0.4±0.1*</td>
</tr>
<tr>
<td>Lactic acid (m mol/l)</td>
<td>3.3±0.5</td>
<td>3.5±0.7</td>
<td>3.8±1.1</td>
</tr>
</tbody>
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All values are shown as the means±SD.
* P<.05 Group 3 vs Group 2 and Group 1.

TABLE 4.
Grade of neuronal damage between Group 3 and 4

<table>
<thead>
<tr>
<th></th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>FrPaM</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>FrPaSS</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>CPu(L)</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>CPu(M)</td>
<td>+</td>
<td>++</td>
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FrPaM, Frontoparietal cortex, motor area, supplied by anterior cerebral artery; FrPaSS, frontoparietal cortex, somatosensory area, supplied by middle cerebral artery; Frontoparietal cortex, motor area, supplied by anterior cerebral artery; CPu(L), lateral segment of caudate putamen; CPu(M), medial segment of caudate putamen.

among the three groups.

Additional comparison between Group 3 and 4

Neuronal damage evaluated by HE staining was greater in Group 4 than in Group 3 in all ipsilateral regions (the frontoparietal cortex supplied by the ACA, the frontoparietal cortex supplied by the MCA, the lateral and the medial segments of the caudate putamen). CVCD applied during brain ischemia resulted in greater neuronal damage than CVCD applied before MCA-O. In Group 3, the infarcted area involved only in the lateral segment of the caudate putamen, as shown by TTC staining. However, in Group 3, neuronal damage evaluated by HE staining was observed even in the area which converted TTC; for example, the frontoparietal cortex supplied by the ACA, the frontoparietal cortex supplied by the MCA, and the medial segment of the caudate putamen. These results indicate that CVCD occurring during brain ischemia aggravates the effects of cerebral (Table 4).
DISCUSSION

The interaction of CVCD with the mechanisms of brain ischemia has been the topic of several experimental studies. It is thought that CVCD results in venous congestion and increase in volume of the cerebral vascular bed. The increased cerebral blood volume (CBV), vasogenic edema due to venous congestion with secondary cerebral ischemia, and subsequent cytotoxic edema make intracranial pressure rise. If cerebral perfusion pressure becomes insufficient as a result of the increase in intracranial pressure, brain edema becomes increasingly aggravated, due to vasogenic edema with secondary cerebral ischemia and also due to subsequent cytotoxic edema. Increased CBV, vasogenic edema, and subsequent cytotoxic edema have been considered to be the main pathophysiological mechanisms after CVCD in the brain [5-10]. On the other hand, other studies have suggested that in states of severe hypotension, an increase of cephalic venous pressure improves cerebral perfusion by preventing the leptomeningeal veins from collapsing, in spite of reducing the cerebral perfusion pressure [11].

In our study, the volume of the right hemisphere in Group 1 (MCA-O without CVCD) was larger than in Group 2 (CVCD without MCA-O) and Group 3 (CVCD just before MCA-O). Based on TTC staining the rats in Group 1 had the greatest neuronal damage among all groups, suggesting that brain ischemia was most severe in Group 1. Some degree of increase in cephalic venous pressure, without cerebral arterial ischemia, may not cause the risk of cerebral infarction. In dogs, cerebral perfusion was not impeded even when cephalic venous pressure was raised by 25 mmHg by constriction of the superior caval vein; rather, there was a slight increase of cerebral blood flow (CBF) in most animals [12]. Presumably, cephalic venous pressure in our CVCD model was not so high as to decrease cerebral perfusion. Instead, CVCD appeared to provide protection against brain ischemia, when applied before MCA-O, as evidenced by the fact that cerebral infarction was less severe in Group 3 than in Group 1.

The LDF values in Group 2 showed no significant changes during this experiment. Accordingly, evidence of brain ischemia was not observed in this group. The reason why LDF values of Group 3 during MCA-O were higher than those of Group 1 might have been that CVCD allowed the dimension of the vascular beds to increase, leading to a decrease in resistance to flow. Hence, the flow in collateral vessels could have increased above the values associated with MCA-O alone. LDF is based on the direct detection of the velocity and number of red blood cells flowing through microvessels [13-15]. Therefore, LDF values reflect both arterial and venous blood flow.

Ischemic neuronal damage and anaerobic glycolysis result from occlusion of the MCA. It has been reported that degree of neuronal damage and volume of cerebral infarction both correlate with the values of S-100 protein in the cerebrospinal fluid and serum [16-17]. In this study, the values of S-100 protein in blood were largest in Group 1. This result suggested that neuronal damage was greater in Group 1 than in the other groups. In cases in which anaerobic glycolysis takes place during brain ischemia, lactic acid will rise locally and spill into the blood. However, no significant differences in blood lactic acid levels were observed among the three groups. Because we took blood samples 24 hrs after completing the arterial occlusion experiments presumably, by that time the values of lactic acid had already returned to normal after recirculation.

The extent of neuronal damage was different depending on whether CVCD had been induced before (Group 3) or during (Group 4) MCA-O. Neuronal damage shown by HE staining was greater in Group 4 than in Group 3 in all ipsilateral regions (Table 4). On the other hand, the infarcted area shown by TTC staining involved only the lateral segment of the ipsilateral caudate putamen in Group 3. Neuronal damage shown by HE staining existed even in ipsilateral regions converting TTC. This type of neuronal damage may result from CVCD associated with brain ischemia. Comparison of the findings obtained in Group 3 and 4 indicate that CVCD during recirculation aggravates neuronal damage from brain ischemia. If the event during recirculation had been observed over a longer period of time, then the damage due directly to CVCD might have become more apparent.

There is compelling evidence that a rise of cephalic venous pressure by 10 mmHg does not impair cerebral blood flow [11]. In dogs, cerebral perfusion was not impeded even when the cephalic venous pressure was raised by 25 mm Hg by constriction of the superior caval vein; rather there was a slight increase of CBF in most animals [12]. However, CBV increases after sinus vein thrombosis or cortical vein occlusion. Intracranial hypertension
can develop as a result of brain edema, caused by the increased venous pressure, and this, in turn, can result in secondary reduction of regional cerebral blood flow (r-CBF), and in brain damage [7-10,18-20]. In the present study, the rats of Group 4 died within 24 hrs after recirculation; thus, brain damage might have been severe due to a steep increase in intracranial pressure after recirculation.

The cerebral ischemic area decreased when CVCD was applied before brain ischemia (Group 3). This finding seems to contrast with the fact that when intracranial pressure is increased, cerebral perfusion pressure would decrease. However, a reduction in cerebral perfusion pressure resulting from an increase in venous or intracranial pressure is likely to be compensated for by a proportionate dilatation of the cerebral vessels, which is thought to be mediated myogenically [21-23].

The leptomeningeal veins are known to be a site of flow resistance. Within the physiological pressure range, the leptomeningeal veins are prevented from collapsing by a valve-like mechanism which has been described at the entrance of the leptomeningeal veins into the dural sinuses [24,25]. The slit-like terminal segments of the leptomeningeal veins are more prone to compression than the circularly shaped leptomeningeal veins themselves. The flow resistance of these structures is thought to depend on the intracranial pressure value allowing the transmural pressure of the leptomeningeal veins to remain at a positive value [24-26]. Therefore, collapse of the leptomeningeal veins may occur during the hypotension produced by arterial occlusion, when intravascular pressure declines below intracranial pressure. In this study, MCA-O after CVCD before MCA-O in Group 3 may have prevented the leptomeningeal veins from collapsing. Therefore, in spite of the decrease in cerebral perfusion pressure, it was expected that vascular beds and collateral flow in the ischemic area supplied by the occluded MCA would increase by the compensatory mechanism. These events might result in cerebral protection. However, when intracranial pressure increased steeply as was presumably the case in Group 4, this mechanism was exhausted and its operation was probably further impeded by the functional anemia and the acidosis resulting from MCA-O [27]. Moreover, it is possible that CVCD preconditions the brain for ischemia. In our present study, neuronal damage was prevented by proportionate cephalic venous hypertension in Group 3, in which induced CVCD was induced before MCA-O, but was aggravated by the steep increase in cephalic venous pressure that might have occurred in the case of Group 4. Long-term observation after 45 min of MCA-O followed by recirculation will be necessary to determine the effects of CVCD.

In conclusion, our data suggest that in states of brain ischemia, a moderate increase in cephalic venous pressure improves cerebral perfusion by preventing the leptomeningeal veins from collapsing, even in cases in which the cerebral perfusion pressure is reduced. In cardiovascular surgery, a moderate increase in cephalic venous pressure before hypothermic circulatory arrest (HCA) may be effective at producing cerebral protection. However, CVCD during brain ischemia (cephalic venous hypertension and a steep increase in cephalic venous pressure immediately after reperfusion) negatively affected cerebral protection mechanisms. Therefore, regarding RCP, a moderate increase in cephalic venous pressure immediately before and during HCA will result in cerebral protection. However, during subsequent recirculation, venous pressure should be lower, maintained by efficient venous drainage of CPB. If selective cerebral perfusion (SCP) is applied as cerebral protection method, high-flow perfusion of the brain may interfere with cerebral protection similar to the situation of a steep increase in cephalic venous pressure during reperfusion after HCA.

CONCLUSIONS

In our present study, neuronal damage was prevented by proportionate cephalic venous hypertension. Therefore, ligation of the jugular veins may reduce the damage on brain ischemia. We will think that further studies on CVCD are necessary, for example long-term observation after 45 min of MCA-O followed by recirculation.

ACKNOWLEDGMENTS: The author would like to thank Prof. Aoyagi and also Dr. Akashi, Dr. Fujino and Dr. Tayama of the Department of Surgery, Dr. Harada of the Department of Anesthesiology, and Dr. Kutami of the Department of Pathology, Kurume University School of Medicine for their critical advices and suggestions.

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