Sympathetic Nervous Activity and Myocardial Damage Immediately After Subarachnoid Hemorrhage in a Unique Animal Model

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Background and Purpose—Obvious cardiac dysfunction, including ECG abnormalities and left ventricular asynergy, is known to develop after subarachnoid hemorrhage (SAH). To clarify the close relationship between myocardial damage and sympathetic nervous activity immediately after SAH, a novel experimental animal model was used.

Methods—SAH was provoked by perforation of the basilar artery with the use of a microcatheter inserted through the femoral artery in 18 beagle dogs. Hemodynamic changes were recorded, and plasma concentrations of noradrenaline, adrenaline, and 3-methoxy-4-hydroxy-phenylethylene glycol (MHPG) and serum levels of creatine kinase–MB (CK-MB) and troponin T were measured at 0, 5, 15, 30, 60, 120, and 180 minutes after SAH.

Results—Noradrenaline (pg/mL), adrenaline (pg/mL), and MHPG (ng/mL) increased abruptly from 120/70, 130/70, and 1.3/0.5 before SAH to 1700/1200, 5600/3500, and 3.2/1.2 at 5 minutes after SAH, respectively. Aortic pressure, left ventricular wall motion, and cardiac output increased by 60%, 40%, and 30%, respectively (P<0.001) at 5 minutes and then decreased by 50%, 55%, and 40%, respectively (P<0.001) >60 minutes after SAH compared with baseline values. The peak value of CK-MB correlated positively with the peak values of noradrenaline and adrenaline (r=0.730 and r=0.611, respectively). The peak value of troponin T also correlated positively with the peak values of noradrenaline and adrenaline (r=0.828 and r=0.792, respectively).

Conclusions—These results suggest that the elevated activity of the sympathetic nervous system observed in the acute phase of SAH induced myocardial damage and contributed to the development of cardiac dysfunction. (Stroke. 2002; 33:1671-1676.)

Key Words: cardiovascular diseases ■ catecholamines ■ subarachnoid hemorrhage ■ sympathetic nervous system ■ ventricular dysfunction ■ dogs

Subarachnoid hemorrhage (SAH) is known to be complicated by cardiopulmonary dysfunction, including ECG abnormalities and pulmonary edema. It has been considered that the cardiopulmonary dysfunction is induced by an excessive discharge of catecholamines following the marked activation of the sympathetic nervous system immediately after the onset of SAH. Recently, some clinical studies have shown wall motion abnormalities of the left ventricle that developed in the acute phase of SAH, and myocytolysis or contraction band of the myocardium was observed at the autopsy of those patients. Although several investigators have suggested that myocardial dysfunction associated with pathological damage is related to the development of ECG abnormalities and pulmonary edema in patients with SAH, the precise mechanisms underlying myocardial damage, presenting as a complication of SAH, are still unknown.

In the present study we used a unique animal model that simulated the rupture of a cerebral aneurysm and subsequent SAH to examine the existence of a correlation between sympathetic activation and myocardial damage immediately after SAH.

Materials and Methods

Animal and Experimental Preparation

The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85/23, revised 1996) and was approved by the animal committee of Kitasato University. Eighteen beagle dogs weighing from 13.5 to 17.5 kg (mean±SD weight, 15.5±1.2 kg) were anesthetized with sodium pentobarbital (25 mg/kg) administered intravenously, intubated, and ventilated with a mixture of oxygen (3 L/min) and nitrous oxide (2 L/min) with the use of a Harvard respirator.
A 6F plus sheath introducer (501-606U, Cordis) was placed in the abdominal aorta from the left femoral artery to measure aortic blood pressure. A 5.5F Swan-Ganz catheter (93-631H-5.5F, Baxter) was inserted into the pulmonary artery through the left femoral vein to measure pulmonary artery pressure (PAP), pulmonary capillary wedge pressure (PCWP), central venous pressure (CVP), and cardiac output (CO). Intracranial pressure (ICP) was continuously monitored throughout the experiment with a Camino pressure-monitoring catheter (model 110-4G, Camino Laboratory).

A pericardial cradle was placed after a left thoracotomy was performed at the fifth intercostal space. A pair of ultrasonic crystal transducers (45295, NEC Sanei) were fixed on the anterolateral wall of the left ventricle at a distance of 15 mm to assess left ventricular wall motion (LVWM). Left ventricular pressure (LVP) was measured with the use of a tip manometer (SPR-524, Miller Instruments) that was inserted into the left ventricle through an 18-gauge catheter (Instyle 3878188, Becton Dickinson Vascular Access) puncturing the LV free wall. The peak rate of LVP increase (peak positive dp/dt), as a parameter of isovolemic contractility, and the peak rate of LVP decline (peak negative dp/dt), as a parameter of myocardial relaxation, were measured by analog differentiation of the LVP. All pressures were recorded with the use of calibrated pressure transducers connected to an appropriate amplification system. The catheter (5.0F IC COBRA A, Toray Medical) for blood collection was inserted into the coronary sinus through the left external jugular vein.

**Procedure of Experimental SAH**

An angiocatheter (Graid Cath II, RF-WL 14010, Termo) was inserted into the right vertebral artery via the right femoral artery. After the circle of Willis was identified by angiography, a microcatheter (RF-SP 26137, Termo) was inserted into the basilar artery through the angiocatheter to provoke SAH (Figure 1a and 1b). We used the guidewire of the microcatheter to pierce the top of the basilar artery and cause SAH. Three thousand units of heparin were injected intravenously before this manipulation to promote bleeding that was inserted into the left ventricle through an 18-gauge catheter (Instyle 3878188, Becton Dickinson Vascular Access) puncturing the LV free wall. The peak rate of LVP increase (peak positive dp/dt), as a parameter of myocardial relaxation, were measured by analog differentiation of the LVP. All pressures were recorded with the use of calibrated pressure transducers connected to an appropriate amplification system. The catheter (5.0F IC COBRA A, Toray Medical) for blood collection was inserted into the coronary sinus through the left external jugular vein.

**Evaluation of Myocardial Damage**

The concentrations of creatine kinase-MB (CK-MB), troponin T, and myosin light chain in coronary sinus blood were measured as parameters of myocardial damage. To determine serum CK-MB, sera were subjected to chemiluminescent immunooassay with the use of an anti–CK-MB monoclonal antibody (Chemiluminescent ACS-CK-MB, Bayer Diagnostics). Serum troponin T was measured with a commercially available assay kit (Elecsys Troponin T III STAT, Roche Diagnostics) to detect the immunocomplex of biotinylated anti–troponin T enzyme marker antibody. Serum myosin light chain was measured by the radioimmunoassay fixation method (Myosin L.I. Kit Yamasa, Yamasa Ltd).

**Statistical Analysis**

All data are expressed as mean±SD. ANOVA was used for statistical comparison between the values obtained after SAH and the
baseline value for each parameter. Differences with a $P$ value of $<0.05$ were considered statistically significant.

**Results**

Representative recordings of ECG, aortic blood pressure, PAP, CVP, and ICP are shown in Figure 3. The ECG tracings showed ventricular premature contraction approximately 2 minutes after the onset of SAH. Aortic blood pressure, PAP, and mean CVP increased from 150/90, 23/8, and 4 mm Hg to 310/170, 41/20, and 16 mm Hg, respectively, 1 to 2 minutes after SAH; ICP increased from a baseline value of 14 mm Hg to 210 mm Hg immediately after SAH.

Figure 4 demonstrates the changes in plasma concentrations of noradrenaline, adrenaline, and MHPG after SAH. Noradrenaline and adrenaline increased significantly from 120 and 130 pg/mL at 0 minute to 1700 and 5600 pg/mL at 5 minutes ($P<0.01$ and $P<0.01$), respectively, and returned to baseline values 30 minutes after SAH. MHPG increased significantly from 1.3±0.5 ng/mL at 0 minute to 3.2±1.2 ng/mL at 15 minutes ($P<0.01$) and returned to the baseline value 30 minutes after SAH.

Hemodynamic changes after SAH are shown in Figures 5 and 6. HR was 140±20 bpm at 0 minute and significantly decreased by 15% to 20% >15 minutes after SAH ($P<0.05$ or $P<0.01$). Aortic blood pressure (systolic/diastolic pressure) increased significantly from 160±20/110±20 mm Hg at 0 minute to 260±60/170±30 mm Hg at 5 minutes ($P<0.001$) and then decreased significantly to 50% of baseline values >30 minutes after SAH ($P<0.001$). PAP also increased significantly from 23±7/12±3 mm Hg at 0 minute to 41±18/24±13 mm Hg at 5 minutes ($P<0.001$); the systolic PAP decreased significantly to values 30% to 40% of baseline value >30 minutes after SAH ($P<0.05$). PCP and CVP increased significantly from 8±3 and 3±2 mm Hg at 0 minute to 20±10 and 7±3 mm Hg at 5 minutes ($P<0.001$ and $P<0.001$), respectively; they returned to baseline values >30 minutes after SAH. Peak positive and negative dP/dt increased significantly from 3300±700 and 3100±600 mm Hg/s at 0 minute to 7600±1600 and 4700±1200 mm Hg/s at 5 minutes ($P<0.001$ and $P<0.001$), respectively; they decreased significantly to values 50% to 60% of the baseline values >30 minutes after SAH ($P<0.01$ or $P<0.001$). Both LVWM and CO increased significantly by 30% to 40% of baseline values at 5 minutes ($P<0.001$ and
P<0.001); they decreased significantly by 30% to 50% of their baseline values 30 minutes after SAH (P<0.01 or P<0.001).

Figure 7 shows the plasma concentrations of CK-MB, troponin T, and myosin light chain before and after SAH. CK-MB increased significantly from 6.2±2.0 ng/mL at 0 minute to 13.8±5.6 ng/mL at 5 minutes (P<0.05) and continued to increase gradually until 180 minutes after SAH. Troponin T also increased significantly from 0.28±0.15 ng/mL at 0 minute to 1.1±0.9 ng/mL at 15 minutes (P<0.01) and reached its peak value of 2.5±1.6 ng/mL at 120 minutes (P<0.001). Myosin light chain showed a significant increase 60 minutes after SAH compared with its baseline value. Figure 8 illustrates the relationship between the peak value of CK-MB and peak values of catecholamines as well as between the peak value of troponin T and peak values of catecholamines. Both CK-MB and troponin T correlated positively with noradrenaline and adrenaline (CK-MB versus noradrenaline, r=0.730; CK-MB versus adrenaline, r=0.611; troponin T versus noradrenaline, r=0.828; troponin T versus adrenaline, r=0.792). However, there were no significant correlations between myosin light chain and catecholamines, nor did CK-MB, troponin T, and myosin light chain show a significant correlation with MHPG (data not shown).

Discussion
The effects of SAH on hemodynamics have been examined in several animal models. In many of the former models, SAH was induced by injection of autologous blood into the subarachnoid space. However, these models do not reflect the true pathophysiological features of aneurysmal SAH because the elevation of ICP occurs early after the injection of autologous blood, and this unnecessarily affects cardiohemodynamic functions. Our new model is considered to overcome that shortcoming and to simulate more accurately the cardiohemodynamic changes immediately after SAH. In clinical cases, it is supposed that systemic blood pressure directly influences ICP after the rupture of the aneurysm and that the subsequent hemorrhagic changes, including an abrupt elevation of ICP and dysregulation of cerebral blood flow, result in cardiohemodynamic dysfunction. Therefore, we have devised a novel animal model that reproduces the hemody-
namic changes that take place immediately after SAH caused by the rupture of a cerebral aneurysm to understand the mechanisms underlying myocardial damage in clinical SAH. The experimental model proposed in the present study is considered to simulate clinically severe SAH because ICP rises to levels much higher than those observed in clinical practice. Consequently, we could observe sequential changes of sympathetic nervous activity and myocardial damage immediately after the onset of SAH.

Recently, some clinical studies described left ventricular asynergy on an echocardiogram and left ventriculogram performed in patients with SAH. Furthermore, it was reported that myocardial necrosis occurred in these patients, as evidenced by elevated serum concentrations of CK-MB, troponin T, or myosin light chain above the normal values within 2 to 3 days after the onset of SAH as well as the contraction band, myocardial fragmentation, and focal myocytolysis observed in the myocardium at autopsy. We investigated the pathogenesis of cardiopulmonary complications on the basis of the clinical observation of 717 cases in the acute phase of SAH. There was transient left ventricular asynergy in 9.6% (69/717) of the cases, which consisted of mechanical heart failure and myocardial necrosis. One of the reasons for the occurrence of myocardial damage after SAH is the intense activation of the sympathetic nervous system, characterized by massive secretion of catecholamines from the terminals of sympathetic nerves into the tissue. Matsuyama et al showed that plasma levels of noradrenaline and adrenaline were higher in SAH patients with left ventricular asynergy than in those without it. Moreover, myocytolysis has also been observed around the terminals of sympathetic nerves in the autopsied myocardium obtained from patients with SAH. These previously reported clinical studies have demonstrated in detail the clinical course of myocardial damage subsequent to SAH. However, its pathogenesis is still unclear because we cannot evaluate the state of the patients until they are admitted to the hospital.

Using our unique model, we demonstrated in the present study that plasma concentrations of catecholamines temporally rose 15- to 30-fold at 5 minutes after SAH compared with baseline values. The present study showed an extremely enhanced sympathetic activity and a massive release of catecholamines from the terminals of sympathetic nerves. It is suspected that only a part of the catecholamines secreted into the tissues from the sympathetic nerve terminals enters the systemic circulation; therefore, the concentration of catecholamines at tissue level would be more elevated. The high concentration of catecholamines in the myocardium would bring about a calcium overload of myocardial cells, which would primarily cause a reduction of myocardial contractility and would secondarily lead to an impairment of cardiac function due to the perfusion disturbance at the level of capillaries caused by an enhanced platelet aggregation. The main reason for the enhanced sympathetic activity is expected to be the sudden elevation of ICP. This phenomenon could be induced when massive bleeding into the subarachnoid space occurs in a short period or when the systemic blood pressure directly causes an elevation of ICP after the rupture of a cerebral artery. Neil-Dwyer et al demonstrated that the autopsied myocardium of patients with SAH who had been given α-blockers and β-blockers had significantly less damage, such as focal necrosis and inflammatory cell infiltration. Thus, additional studies should be planned with the use of this animal model not only to clarify the mechanism underlying cardiac complications of SAH but also to develop a treatment based on the protective effect of sympathetic nervous blockade on the myocardium.
In conclusion, the data obtained from our novel animal model of SAH suggest that the elevated activities of the sympathetic nervous system immediately after the onset of SAH induced myocardial damage, including dysfunction and necrosis.

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References

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