Caspase-1 Inhibitor Prevents Neurogenic Pulmonary Edema After Subarachnoid Hemorrhage in Mice

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Background and Purpose—We examined the effects of a caspase-1 inhibitor, N-Ac-Tyr-Val-Ala-Asp-chloromethyl ketone (Ac-YVAD-CMK), on neurogenic pulmonary edema in the endovascular perforation model of subarachnoid hemorrhage (SAH) in mice.

Methods—Ninety-seven mice were assigned to sham, SAH + vehicle, SAH + Ac-YVAD-CMK (6 or 10 mg/kg), and SAH + Z-Val-Ala-Asp-fluoromethylketone (Z-VD-FMK, 6 mg/kg) groups. Drugs were intraperitoneally injected 1 hour post-SAH. Pulmonary edema measurements, Western blot for interleukin-1β, interleukin-18, myeloperoxidase, matrix metalloproteinase (MMP)-2, MMP-9, cleaved caspase-3 and zona occludens-1, MMP zymography, terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling staining, and immunostaining were performed on the lung at 24 hours post-SAH.

Results—Ten- but not 6-mg/kg of Ac-YVAD-CMK significantly inhibited a post-SAH increase in the activation of interleukin-1β and caspase-3 and the number of terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling-positive pulmonary endothelial cells, preventing neurogenic pulmonary edema. Another antiapoptotic drug, Z-VD-FMK, also reduced neurogenic pulmonary edema. SAH did not change interleukin-18, myeloperoxidase, MMP-2, MMP-9, zona occludens-1 levels, or MMP activity.

Conclusions—We report for the first time that Ac-YVAD-CMK prevents lung cell apoptosis and neurogenic pulmonary edema after SAH in mice. (Stroke. 2009;40:00-00.)

Key Words: apoptosis ■ caspase-1 inhibitor ■ pulmonary edema ■ subarachnoid hemorrhage
ing, and immunostaining using the rabbit anti-CD34 (Abbiotec, San Diego, Calif) and goat antisurfactant-associated protein-C (SP-C; Santa Cruz Biotechnology, Santa Cruz, Calif) antibodies were performed on the lung in the sham, SAH+vehicle and SAH+Ac-YVAD-CMK-10 mg/kg groups (n=4, respectively). 

As a separate study, the effect of 10 mg/kg of Ac-YVAD-CMK on blood pressure was examined through the femoral artery in 4 SAH rats. Also, 7 SAH rats received another antiapoptotic drug, Z-Val-Ala-Asp-fluoromethylketone (Z-VAD-FMK, 6 mg/kg; Sigma-Aldrich, St Louis, Mo) and pulmonary edema was measured as described previously. 

All values were expressed as mean±SD. Paired t tests, χ² tests, and one-way analysis of variance with Scheffe correction were used as appropriate with P<0.05 considered statistically significant.

Results

Mortality

The mortality at 24 hours post-SAH was 37.5% (12 of 32 mice), 20.0% (5 of 25 mice), and 10.5% (2 of 19 mice), and the average survival time of dead animals was 11.5, 13.0, and 21.3 hours in the SAH+vehicle, SAH+Ac-YVAD-CMK-6 mg/kg, and SAH+Ac-YVAD-CMK-10 mg/kg groups, respectively: the difference in mortality was significant between the vehicle and high-dose groups (P<0.05). No sham-operated mice (n=10) died.

Neurogenic Pulmonary Edema

SAH-induced brain injury was similar in terms of neurological impairment and the severity of SAH among the vehicle- and Ac-YVAD-CMK-treated groups 24 hours post-SAH (Figure 1A–B). High-dose, but not low-dose, Ac-YVAD-CMK treatment significantly reduced NPE without acute changes in post-SAH blood pressure (Figure 1C–D). In the SAH+vehicle group, some alveoli were filled with fluid but only a few inflammatory cells were observed, whereas little or no alveolar edema was observed in the SAH+Ac-YVAD-CMK-10 mg/kg group (Figure 1E). Z-VAD-FMK also showed a low mortality (14.3% [one of 7 rats]) and significantly reduced NPE (Figure 1A–C).

Western Blot and MMP Zymography

High-dose Ac-YVAD-CMK significantly suppressed a post-SAH increase in pulmonary active IL-1β and caspase-3 levels (Figure 2A–B). Neither SAH nor Ac-YVAD-CMK affected...
IL-18, myeloperoxidase, MMP-2, MMP-9, their substrate zona occludens-1 levels, or MMP activity (Figure 2C–I).

**Histological Assessment**

SAH significantly increased the number of TUNEL-positive pulmonary endothelial cells, which were significantly inhibited by the high-dose Ac-YVAD-CMK treatment (Figure 3).

**Discussion**

Increased hydrostatic pressure in the pulmonary capillaries by an excessive release of catecholamines has been presumed to be the main pathogenetic factor for the development of NPE. The hydrostatic pressure-independent mechanism has also been suggested but remains unknown. Norepinephrine not only leads to hydrostatic pulmonary edema, but also causes activation of cytokines. Inflammatory reactions can cause pulmonary edema associated with recruitment of neutrophils, which release MMPs that damage the alveolar–capillary barrier. However, inflammation is considered not to develop NPE but potentially to maintain and aggravate the edema by increasing capillary permeability. Although norepinephrine also causes lung cell apoptosis, resulting in pulmonary edema, the possibility that apoptosis is involved in the pathogenesis of NPE has not been investigated.

Caspase-1 inhibitors prevent the release of active IL-1β and IL-18, which initiate inflammation, resulting in decreased pulmonary edema. Ac-YVAD-CMK also prevents apoptosis independent of its caspase-1 inhibitor activity in addition to an indirect decrease in apoptosis through the inflammatory cascade modulation.

This study showed that pulmonary endothelial cell apoptosis, but not neutrophil infiltration and MMP activation, contributes to the pathophysiology of post-SAH NPE. Ac-YVAD-CMK suppressed post-SAH IL-1β activation and inhibited apoptosis, preventing NPE. The effects of another antiapoptotic drug, Z-VAD-FMK, supported the involvement of apoptosis in the development of NPE. The alveolar–capillary barrier weakened by apoptosis would cause diminished capacity to withstand hydrostatic pressure, resulting in NPE.

This study has some limitations including no studies of the time course of plasma norepinephrine concentration, pulmonary hypertension, lung cell apoptosis, and NPE, and the relationship between the intracranial pressure and NPE as well as the effects of AC-YVAD-CMK on norepinephrine levels and pulmonary hypertension. Thus, further studies are needed.

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Disclosures

None.

References


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