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Research Report

Neuroprotective effect of suppression of astrocytic activation by arundic acid on brain injuries in rats with acute subdural hematomas

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ABSTRACT

Acute subdural hematoma (ASDH) can cause massive ischemic cerebral blood flow (CBF) underneath the hematoma, but early surgical evacuation of the mass reduces mortality. The aim of this study was to evaluate whether arundic acid improves the secondary ischemic damage induced by ASDH. Our results confirmed that arundic acid decreases the expression of S100 protein produced by activated astrocytes around ischemic lesions due to cytotoxic edema after ASDH as well as reducing infarction volumes and numbers of apoptotic cells around the ischemic lesions.

In this study, we also evaluate the relationship of brain edema and the expression of Aquaporin 4 (AQP4) in an ASDH model. The expression of AQP4 was decreased in the acute phase after ASDH. Cytotoxic edema, assumed to be the main cause of ASDH, could also cause ischemic lesions around the edema area. Arundic acid decreased the infarction volume and number of apoptotic cells via suppression of S100 protein expression in ischemic lesions without changing the expression of AQP4.

1. Introduction

Acute subdural hematoma (ASDH) is the most common mass lesion occurring after severe head injury. In spite of major advances in its management during the past two decades, ASDH still remains one of the most lethal of all intracranial injuries (Karabiyikoglu et al., 2005). Moreover, its underlying pathophysiology is still not clearly understood. Although a number of mechanisms have been proposed to account for the underlying brain injury that follows subdural hematoma, neuropathological studies have shown that the most important of these is ischemic neuronal death, which is related to secondary brain damage in patients who die after ASDH (Bullock et al., 1991). Animal models have shown that some mechanisms of this secondary brain damage, which clearly causes neuronal death, are brain edema, hypoperfusion, and hypoxia (Baechli et al., 2010).

ASDH can cause massive ischemic cerebral blood flow (CBF) underneath the hematoma, but early surgical evacuation of the mass reduces mortality, possibly by restoring CBF and energy supply (Seelig et al., 1981). This would imply that the extravasated volume of blood is the main cause of ASDH-induced brain damage and mortality. Conversely, extravasated blood with all its components may induce additional

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pathophysiological mechanisms that are detrimental to brain tissue. In addition, astrocytic activation and increased S-100β after cerebral ischemia and brain trauma are well-documented (Barone and Feuerstein, 1999).

Arundic acid is an astrocyte modulating agent that improves neurological outcome in experimental acute stroke models. Recently, arundic acid improved neurological deficits and decreased infarction volume in rats with stroke induced by middle cerebral artery occlusion (MCAO) (Asano et al., 2005; Mori et al., 2005). We focused on the effect of arundic acid on astrocytic activation and increased S-100β protein within the cerebral ischemic lesion after ASDH. We examined whether arundic acid attenuates brain injury induced by ASDH in rats.

2. Results

2.1. Physiological parameters

We detected no statistical differences in the mean arterial blood pressure (MABP), pH, partial pressure of arterial oxygen (PaO_2), partial pressure of arterial carbon dioxide ($PaCO_2$), blood glucose or hematocrit values of rats prior to and after ASDH induction (Table 1).

2.2. Histology, IHC, and TUNEL

As we illustrate in Fig. 1A, the volume of the infarction lesion tends to increase with time and to decrease with arundic acid in ASDH-induced rats (73.5±4.5 and 61.0±3.4 mm^3 (4 h after ASDH), 118.7±3.4 and 92.7±6.8 mm^3 (24 h), 170.6±3.4 and 117.5±5.7 mm^3 (48 h) ($P<0.05$), 175.5±6.8 and 121.0±4.5 mm^3 (168 h) ($P<0.05$), respectively). Hematoxylin and eosin (H and E) staining revealed morphological changes in neurons in both the infarction areas induced by ASDH and the surrounding penumbral area next to the infarction area. Further, cells near the penumbra areas within the cortex were undergoing apoptosis as revealed by their histological morphology, i.e., nuclear pyknosis, chromatin condensation or fragmentation, and cytoplasmic shrinkage (Fig. 2).

Numerous TUNEL-positive cells, with darkly stained nuclei or nuclear fragments and cytoplasmic halos characteristic of apoptotic cells, were recognized in and around the infarction area (Fig. 3). As shown in Fig. 1B, the expression of TUNEL-positive cells was more frequent and was reduced by arundic acid with time in ASDH-induced rats (9.29±0.5 and 4.5±0.6 cell counts/mm^2 (4 h after ASDH) ($P<0.05$), 16.6±0.4 and 8.84±0.3 cell counts/mm^2 (24 h) ($P<0.05$), 26.5±0.6 and 12.5±0.5 cell counts/mm^2 (48 h) ($P<0.05$), 11.3±0.7 and 7.8±0.6 cell counts/mm^2 (168 h) ($P<0.05$), respectively).

As shown in Figs. 1C and 3, the expression of Bax-positive cells was more frequent and was reduced by arundic acid with time in ASDH-induced rats (10.4±0.6 and 6.1±0.6 cell counts/mm^2 (4 hrs after ASDH) ($P<0.05$), 20.5±0.4 and 10.4±0.7 cell counts/mm^2 (24 hrs) ($P<0.05$), 27.7±0.4 and 16.5±0.6 cell counts/mm^2 (48 hrs) ($P<0.05$), 11.4±0.5 and 8.4±0.5 cell counts/mm^2 (168 hrs) ($P<0.05$), respectively).

As shown in Fig. 1D and Fig. 3, the expression of Bcl-2-positive cells was more frequent and was increased by arundic acid with time in ASDH-induced rats (2.0±0.5 and 4.1±0.6 cell counts/mm^2 (4 h after ASDH) ($P<0.05$), 10.2±0.4 and 12.8±0.5 cell counts/mm^2 (24 h) ($P<0.05$), 18.5±0.4 and 22.1±0.6 cell counts/mm^2 (48 h) ($P<0.05$), 23.3±0.3 and 27.8±0.5 cell counts/mm^2 (168 h) ($P<0.05$), respectively).

2.3. Water content, S100, and AQP4 expression

Fig. 4A shows the brain water content at 4, 24, 48 and 168 h after infusion of 0.4 mL autologous blood. The edema reached its maximum after 24 h, which corresponds to other models of brain damage.

Figs. 4B and 5 also show AQP4-positive cells around the ischemic lesion at 4, 24, 48 and 168 h after infusion of 0.4 mL autologous blood. In ASDH-induced rats, AQP4-positive cells

Table 1

	MABP (mmHg)	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH	Ht (%)	Glu (mg/dl)
Normal saline						
Pre ASDH	107.0±3.8	122.3±5.5	41.6±3.7	7.39±0.04	44.1±0.6	79.4±4.3
30 min after ASDH	110.9±2.9	120.2±3.5	41.7±1.8	7.39±0.09	44.3±1.1	78.4±2.4
60 min after ASDH	110.9±3.4	121.3±4.2	41.3±2.1	7.39±0.11	44.8±0.7	79.2±3.1
90 min after ASDH	111.5±5.5	122.1±3.4	41.1±1.5	7.40±0.14	44.2±0.6	80.4±2.4
2 h after ASDH	109.2±5.3	120.8±5.2	41.6±1.2	7.39±0.12	44.8±1.2	79.9±2.9
4 h after ASDH	106.2±5.9	122.0±3.5	41.9±2.1	7.39±0.27	44.3±0.6	80.2±5.3
Arundic acid						
Pre ASDH	107.6±3.5	122.1±3.5	41.8±3.1	7.39±0.07	44.8±1.1	79.4±6.3
30 min after ASDH	111.2±1.8	120.0±2.2	41.9±3.4	7.39±0.14	44.7±0.9	79.0±3.3
60 min after ASDH	111.2±3.1	121.1±2.3	41.1±4.1	7.39±0.21	44.3±0.6	79.2±5.3
90 min after ASDH	111.3±2.8	122.1±2.3	41.3±2.7	7.40±0.15	44.8±0.7	80.4±5.3
2 h after ASDH	110.1±2.7	120.1±2.2	41.2±1.9	7.39±0.24	44.4±0.6	79.4±4.2
4 h after ASDH	107.1±3.3	122.1±2.5	41.0±1.7	7.39±0.54	44.8±0.6	80.4±6.3

All data are expressed as the means±SEM.
Parameters remained roughly equivalent between the 2 groups.
ASDH—acute subdural hematoma; MABP—mean arterial blood pressure.
PaO_2—partial pressure of oxygen; $PaCO_2$—partial pressure of carbon dioxide.
Hct—hematocrit.