Protective Effects of KW-3635, a Thromboxane A2 Antagonist, on Arachidonic Acid-Induced Transient Cerebral Ischemia in Dogs

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ABSTRACT—We investigated the effect of KW-3635, a selective thromboxane (TX) A2-receptor antagonist, on the arachidonic acid (AA)-induced transient cerebral ischemia in anesthetized dogs. Intracarotid-arterial injection of AA (0.25–1 mg/kg) produced a transient and reversible decrease in electroencephalographic (EEG) activity. The reduction of EEG power was inhibited by the intravenous injection of KW-3635 or aspirin, a cyclooxygenase inhibitor. Local cortical perfusion (LCP) measured by a laser-doppler flow meter was also reduced concomitantly with the reduction of EEG power. Although KW-3635 at 1 and 3 mg/kg (i.v.) did not affect the maximum reduction of LCP, the duration of the reduction period of LCP was significantly shortened by KW-3635. On the other hand, aspirin at 1 and 3 mg/kg (i.v.) inhibited both the maximum and the delay of LCP reduction. The intravenous administration of KW-3635 or aspirin caused dose-dependent inhibition of ex vivo platelet aggregation stimulated by AA (150 nM) at the doses that improve the EEG activity. These data suggest that TXA2 is one of the important factors in the AA-induced transient reduction of EEG activity in anesthetized dogs. The TXA2-receptor antagonist may be useful for protection against the ischemic brain damage following transient ischemic attack.

Keywords: KW-3635, Arachidonate, Cerebral ischemia, Thromboxane A2, EEG

Focal cerebral transient ischemic attack (TIA) is a type of cerebral ischemia of clinical importance, since TIA is associated with a higher risk for subsequent development of cerebral infarction. TIA is produced by the transient reduction of the cerebral blood flow, which is assumed to be strongly related to the thrombus formation. Platelet aggregation has been shown to be a fundamental component of the thrombus formation in cerebral blood vessels, particularly in the arteries. Transient cerebral ischemia has experimentally been studied by producing intravascular platelet aggregation with adenosine diphosphate (1, 2), by producing embolism of the capillary bed with microspheres (3, 4), by arterial air embolism (5), or by temporally interrupting the blood supply through clamping extra-cerebral or intracerebral vessels (6–8). A rabbit model of arachidonic acid (AA)-induced platelet emboli in the brain confirmed small intraarterial platelet aggregates resulting in TIA (9). On the other hand, AA has also been demonstrated to produce cerebral infarction or stroke rather than TIA in rabbits and rats when injected into the carotid artery (10–12). However, there have been few studies about the effect of AA in the dog prior to the present report.

The antiplatelet agent is expected to manifest a prophylactic effect against TIA. In fact, aspirin, an antiplatelet agent, has already been demonstrated to be useful for the treatment of patients with TIA (13). Recently, Koudstaal et al. (14) have reported that the urinary thromboxane A2 (TXA2) level (measured as 11-dehydro-thromboxane B2) is increased in patients with TIA. Therefore, release or production of TXA2 is thought to play an important role in the development of TIA. KW-3635 is a selective TXA2-receptor antagonist that inhibits U-46619 (a TXA2 mimetic)-induced aggregation of platelets (15), blocks the TXA2 binding to its receptor in the platelet membrane (16) and, furthermore, suppresses the vascular constriction stimulated by U-46619 (17).

In the present study, we investigated the possible protective effect of KW-3635 against the development of transient cerebral ischemia induced by AA in anesthetized dogs by determining the change of electroencephalogram (EEG) power and local cortical perfusion (LCP).
MATERIALS AND METHODS

Preparation of the ischemic model

Extracranial carotid artery thrombosis was induced in anesthetized dogs with a modification of the procedure described by Passero et al. (9). Adult mongrel dogs of either sex, weighing 8–12 kg, were anesthetized with 40 mg/kg, i.v. of pentobarbital sodium and placed on a heated operating table. All dogs were then tracheotomized and artificially ventilated with room air. Polyethylene canulas were placed in the left femoral artery and vein for monitoring arterial blood pressure and drug administration, respectively. The chest was opened to expose the aortic arch. The right and left subclavian, right and left internal thoracic, right common carotid and left vertebral arteries were ligated to limit collateral circulation into the brain. A polyethylene cannula connected to a needle was placed just before the junction of the internal and external left carotid artery for the injection of AA. A transit-time flow probe (MFV-3100; Nihon Kohden, Tokyo) was placed on the left common carotid artery for measuring the blood flow. After the surgery, the dog was placed prone on a stereotaxic apparatus. The scalp was incised and the left parietal bone was exposed. Then a laser-doppler flow probe (ALF2100; Advance Co., Ltd., Tokyo) was attached just above the surface of the left cerebrum for monitoring local cortical microvascular perfusion (LCP). Stainless steel screws were implanted for recording the EEG power through a bioelectrical amplifier unit (AB621G, Nihon Kohden) connected with a polygraph system (RM6200, Nihon Kohden).

The dose of AA (0.25 to 0.75 mg/kg) was initially determined so that the lowering activity of EEG power continues for approximately 5 min. The response to AA was reproducible at least three times when AA was injected into the intracarotid artery (i.c.a.) at 40-min intervals. The dogs were divided into three groups, either treated with vehicle, KW-3635 or aspirin. After determining the dose of AA, AA was injected twice. The first application following the vehicle served as the control. The second application was performed after treatment with a drug. Drugs were intravenously administered 10 min before the second injection of AA.

Determination of platelet aggregation (ex vivo)

Fifteen minutes after the administration of a drug, a blood sample was collected from the dog, with 1/10 volume of 3.8% sodium citrate as anticoagulant, and then the platelet-rich plasma (PRP) was obtained by centrifugation at 100 × g for 5 min. The platelet aggregation of the PRP was determined by an aggregometer (C550; Chrono-Log, Havertown, PA, USA) according to the method of Born (18). PRP was incubated for 1 min with epinephrine (final concentration of 10 μM) and then stimulated by AA at 150 μM.

Statistical analyses

All data were expressed as means ± S.E.M. Significant differences between the first and second response to AA were examined by the paired t-test. P-values of less than 0.05 were considered statistically significant.

Drugs

KW-3635 (Sodium (E)-11-[2-(5,6-dimethyl-1-benzimidazolyl)ethylidene]-6,11-dihydro dibenz[b,e]oxepin-2-carboxylate monohydrate) (Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., Shizuoka) and aspirin (Wako Pure Chemical Industry Ltd., Osaka) were dissolved in 5% glucose containing 0.001 N potassium hydroxide. Arachidonic acid (99% pure, sodium salt: Sigma, St. Louis, MO, USA) was dissolved in saline.

RESULTS

In pentobarbital-anesthetized dogs, i.c.a. injection of AA caused a transient reduction of EEG amplitude and decreases of LCP (18.7±4.0 to 8.3±1.7 ml/min/100 g, n = 6), left common carotid arterial blood flow (CCBF) (49.3 ± 4.7 to 20.0 ± 3.7 ml/min, n = 6) and mean systemic blood pressure (mBP) (125.0±3.7 to 66.7±3.8 mmHg, n=6). The values of each parameter before the injection of AA were not significantly different among each group. The reductions of EEG amplitude, LCP, CCBF and mBP were recovered to the basal level within 5 min and were reproducible at least 3 times.

KW-3635 (1 and 3 mg/kg, i.v.) significantly shortened the recovery time of EEG power (Figs. 1 and 2). Aspirin also improved the flattening of EEG, and 1 and 3 mg/kg (i.v.) of this drug completely abolished the disappearance of EEG activity (Fig. 2). Both KW-3635 and aspirin inhibited the maximum reduction of CCBF and suppressed the duration of CCBF decrease in dose-dependent manners (Fig. 3). KW-3635 did not inhibit the maximum reduction of LCP, while aspirin dose-dependently suppressed the reduction of LCP (Fig. 4). Although KW-3635 did not change the maximum reduction of LCP, the duration of reduced LCP was significantly shortened (Fig. 4). Aspirin, but not KW-3635, inhibited the AA-induced hypotension (Fig. 5).

Ex vivo platelet aggregation in response to AA (150 μM) was also inhibited at 15 min after the administration of KW-3635 at 3 mg/kg or aspirin at 1 and 3 mg/kg (i.v.) (Fig. 6).
Fig. 1. Typical tracing of arachidonic acid (AA)-induced changes in blood pressure (BP), heart rate (HR), common carotid artery blood flow (CCBF), local cerebral perfusion (LCP) and electroencephalogram (EEG) in an anesthetized dog. AA was injected into the carotid artery (i.c.a.). Inserted square represents the EEG activity at 2 min after AA injection. (a) Control response, (b) effect of KW-3635 (3 mg/kg, i.v.).

DISCUSSION

Since Furlow et al. (11) have initially shown that AA can produce cerebral ischemia, several AA metabolites have been implicated to be important factors in the development of cerebral ischemia or infarction (2, 10, 19, 20). AA-induced cerebral infarction is concomitant with the attenuation of EEG amplitude in the rat (20). This attenuation of EEG is reported to appear within 1 min over the left hemisphere, which is the side of AA injection. Okamatsu et al. also reported that the EEG became flat by intracarotid injection of AA in rabbits (21). These EEG responses to AA did not return to the initial level during the experimental period, indicating that the AA-induced cerebral ischemia may be irreversible in the rat and the rabbit.
Fig. 2. Effects of KW-3635 and aspirin on the recovery time of electroencephalogram (EEG) amplitudes before (open column) and after (dotted column) intracarotid artery injection of AA in anesthetized dogs. Each value represents the mean ± S.E. (n=5–6). **P<0.01 vs. the value before the treatment of drugs.

Fig. 4. Effects of KW-3635 and aspirin on (a) the maximal change of local cortical perfusion (LCP) and (b) the recovery time of LCP before (open column) and after (dotted column) intracarotid artery injection of AA in anesthetized dogs. Each value represents the mean ± S.E. (n=5–6). *P<0.05, **P<0.01 vs. the value before the treatment of drugs.

Fig. 3. Effects of KW-3635 and aspirin on (a) the maximal change of common carotid arterial blood flow (CCBF) and (b) the recovery time of CCBF before (open column) and after (dotted column) intracarotid artery injection of AA in anesthetized dogs. Each value represents the mean ± S.E. (n=5–6). *P<0.05, **P<0.01 vs. the value before the treatment of drugs.

Fig. 5. Effects of KW-3635 and aspirin on the maximal changes of mean blood pressure before (open column) and after (dotted column) intracarotid arterial injection of AA in anesthetized dogs. Each value represents the mean ± S.E. (n=5–6). **P<0.01 vs. the value before the treatment of drugs.
In the present study using anesthetized dogs, AA (0.25–0.75 mg/kg, i.c.a.) attenuated the EEG power at the AA-injected side. In contrast to the results in the rat and the rabbit (20, 21), the EEG flattening was transient and reproducible in the dog. Prior to the reduction of EEG, CCBF and LCP decreased following the AA-injection. Therefore, the reduction of EEG is likely to be due to the focal ischemia in the hemisphere of the AA-injected side, resulting from the microemboli induced by AA. Fritz and Leven (22) suggested that a reproducible TIA-like phenomenon can be produced by liberation into the cerebral circulation of an agent that is known to stimulate intravascular platelet aggregation. Therefore, AA-induced dog cerebral ischemia seems to be mainly related to platelet aggregation, perhaps through the release or production of TXA2. However, although Cleemons et al. (23) have reported that AA directly stimulated the dog platelet aggregation, most of the other reports have shown the need for other substances, such as epinephrine or ADP, to elicit the response to TXA2 or AA (24). Thus, the mechanism of AA-induced TIA in dogs may be somewhat different from those in other species. On the other hand, the reduction of mBP occurred concomitantly with the reduction of LCP and CCBF following the injection of AA. As the hypotension induced by AA was blocked by aspirin, the response seems to be due to vasodilator prostaglandins, which were synthesized from AA escaped from the brain. Therefore, there is a possibility that the AA-induced hypotension is partially involved in the cerebral ischemia. However, further study is necessary to elucidate the precise mechanism for the AA-induced transient cerebral ischemia in the dog.

The present study demonstrated that KW-3635 suppresses the AA-induced lowering of the EEG activity and the CCBF and ameliorates the recovery of reduced LCP at the doses that inhibit the ex vivo platelet aggregation. These results suggest that the AA-induced reduction of EEG, CCBF and LCP is mediated via TXA2. Aspirin suppressed not only the neurophysiological and cerebral hemodynamic deterioration but also the hypotension. Thus, aspirin ameliorated these deteriorations without affecting the AA-induced hypotension. These observations support the notion that the AA-induced reduction of EEG power results from the cerebral ischemia introduced by platelet embolism rather than by the hypotension.

In addition to the platelet aggregation, both TXA2 (25) and AA (26) are known to produce the contraction of dog cerebral artery. The AA-induced contraction is assumed to be mediated by the formation of TXA2 (27) and prostaglandin H2, both of which are released from the aggregated platelets (28, 29) or vasculature (27). In fact, the TXA2 receptor has been regarded to be the same as the PGH2 receptor (30). Considering these observations, there is a possibility that the AA-induced cerebral injury is produced not only by platelet microemboli but also by contraction of the cerebral vasculature. Since KW-3635 has been shown to block not only the platelet aggregation but also the vascular contraction induced by U-46619, a stable TXA2 mimetic compound (15, 17), KW-3635 may ameliorate the response to AA also through the blockade of the vascular action of TXA2 and PGH2. Furthermore, the ability of KW-3635 to protect against AA-induced brain damage was similar to that of aspirin. This observation suggests that PGI2 and other prostaglandins do not play such important roles in the present model of AA-induced brain ischemia in the dog.

In conclusion, the present study demonstrated that the TXA2 blockade by KW-3635 suppressed the AA-induced transient cerebral ischemia in dogs. These results suggest that the TXA2-receptor antagonist may be useful for TIA therapy.

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