Effect of Ibudilast on Microcirculation Thrombosis in Rat Inner Ear

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ABSTRACT—The effect of ibudilast (0.1, 0.3 mg/kg), which has cerebral vasodilating and antiplatelet effects, was evaluated in two models of rat inner ear microcirculation thrombosis by using the photochemical reaction between green light (wave length: 540 nm) and intravenous injection of rose bengal. Furthermore, the inner ear blood flow was measured by a laser-Doppler flowmeter. In the hearing disturbance model, under anesthesia, the compound action potential of the cochlear nerve (AP) was measured by an electrocochleogram. The sound stimulus was an 8-kHz sine wave at 80 dB SPL. The AP was calculated 128 times. In the controls, the AP disappeared about 4 min after the intravenous injection of rose bengal (20 mg/kg). The time required to completely suppress the AP in the animals treated with ibudilast (0.1, 0.3 mg/kg) was significantly prolonged as compared with that in the controls. In the equilibrium dysfunction model, ibudilast (0.1, 0.3 mg/kg) reduced the time of abnormal swimming in the swimming test 24 hr after the completion of photo-illumination. Ibudilast (0.3 mg/kg) increased the inner ear blood flow during the 10-min observation period as compared with the controls, while it did not affect the mean blood pressure. In conclusion, ibudilast increased the inner ear blood flow and was effective in two models of rat inner ear microcirculation thrombosis.

Keywords: Thrombosis (inner ear microcirculation, anterior inferior cerebellar), Ibudilast, Hearing disturbance

Impairment of the inner ear microcirculation has been suspected to cause sudden onset of vertigo (1, 2), sudden deafness (1, 3, 4), Ménière’s disease and presbycusis (5). Furthermore, it was reported that an increase of platelet reactivity, whole blood viscosity or red cell filterability might be an important risk factors in microcirculation disorders of the inner ear (3, 6–9). In addition, vestibular vertigo and hearing impairment were often observed in patients with vertebro-basilar insufficiency (7). These findings suggest that vestibular vertigo and hearing impairment may be associated with hemorheological parameters, especially platelet functions. We, therefore, have established two models of hearing disturbance (10) and equilibrium dysfunction (11) due to inner ear microcirculation thrombosis.

Ibudilast has cerebral vasodilating and antiplatelet effects, probably through a mechanism linked to prostacyclin (12, 13). In the clinical situation, ibudilast was effective not only in patients with cerebral infarction, but also in those with vertigo and tinnitus. Our previous study showed that prostacyclin and prostaglandin E2 played an important role in blood flow regulation in the rat inner ear microcirculation (14). Therefore, we evaluated the effects of ibudilast in two models of hearing disturbance and equilibrium dysfunction and in the blood flow of inner ear with a laser-Doppler flowmeter.

MATERIALS AND METHODS

Animal preparation

Hearing disorders model: Wistar rats weighing 240–260 g were used. Hearing disorders due to inner ear microcirculation thrombosis has previously been described (10). The animals were anesthetized with pentobarbital-Na (50 mg/kg, i.p.). After tracheotomy, the animals were artificially ventilated with a respirator. A catheter was inserted into the femoral vein for the administration of rose bengal solution (20 mg/kg) or agents. The left middle ear was approached ventrolaterally and opened without disturbing the tympanic membrane and ossicles. A recording electrode was placed on the round window membrane, and an indifferent electrode was placed in the middle of the back of the head for measuring the electrocochleogram (Neuropack II, Nihon
The administration of rose bengal or agents. The left middle ear was exposed by the ventrolateral approach, and the tympanic membrane and ossicles (malleus and incus) were removed without damaging the inner ear. The photo-illumination was applied on the vestibule through the oval window for 15 min, while rose bengal (20 mg/kg) was injected intravenously. After the completion of the photo-illumination, the catheter was removed and the operative incision was closed. The swimming test for evaluating the degree of equilibrium dysfunction was performed 24 hr after the completion of photo-illumination. In the swimming test, the behavior of swimming animals in the water bath was observed for 60 sec. The time in the swimming test was calculated by the following formula:

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\begin{align*}
\text{[the duration (seconds) of normal swimming]} & + 1/2 \\
\times [\text{the duration (seconds) of abnormal swimming}].
\end{align*}
\]

When the animals sank in the water, rotating about their longitudinal axes, the observation was stopped and then the time was calculated. When rats swim with both ears above the water, the behavior of the swimming animals was assessed as normal swimming; on the other hand, when the rats swim with a unilateral ear under the water, that was considered to be abnormal swimming. In the experimental groups, various doses of ibudilast (0.1 or 0.3 mg/kg) or aspirin (10 mg/kg) were injected 10 min before the administration of rose bengal, while the controls were injected with saline. Each experiment for different doses or agents was performed in each of 7 animals.

**Measurement of inner ear blood flow**

The use of a laser-Doppler flowmeter for measurement of inner ear blood flow has been described in detail previously (12). In brief, under pentobarbital-Na (50 mg/kg, i.p.), the animals were artificially ventilated with a respirator (Harvard 683). A catheter was placed in the femoral artery and connected to a pressure transducer (Nihon Kohden, MPU-05, PR-5) for measuring the systemic blood pressure. Another catheter for the infusion of saline or ibudilast was placed in the femoral vein. The left middle ear was opened, and the mucosa on the lateral bony wall of the cochlea was removed gently. The probe of the laser-Doppler flowmeter (ALF 2100 Advance, Japan) was placed on the lateral bony wall of the cochlea with a micromanipulator. Varying doses of ibudilast (0.1 or 0.3 mg/kg) or saline was infused intravenously for 3 min at the volume of 0.75 ml, and the blood flow of the cochlea and systemic blood pressure were recorded for 10 min.

In a separate experiment, aspirin (10 mg/kg) was injected intravenously 10 min before the administration of ibudilast (0.3 mg/kg) to investigate the interaction between ibudilast and prostacyclin. Ibudilast was infused intravenously for 3 min at the volume of 0.75 ml.

**Statistical analyses**

Data are reported as the mean ± S.E.M. The groups were compared by analysis of variance. In a case of significant difference, a Dunnett's type of multiple comparison was done. Two-way ANOVA was used to compare the inner ear blood flow and mean blood pressure of the three groups. A P value < 0.05 was considered to indicate a significant difference.

**Agents**

Ibudilast, 3-isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine, was a gift from Kyorin Pharmaceutical Ltd., Japan. Aspirin was a gift from Green Cross Corporation, Japan.

**RESULTS**

**Inner ear microcirculation thrombosis model**

A typical tracing of the AP on the electrocochleogram is shown in Fig. 1. There was no significant difference in the latency and the amplitude of the AP before the administration of drugs among four groups (Table 1). The AP disappeared about 4 min after the injection of rose bengal in the control animals. Ibudilast at doses of 0.1 and 0.3 mg/kg significantly prolonged the time required for complete suppression of the AP (Fig. 2), but aspirin (10 mg/kg) did not significantly prolong the time. In the equilibrium dysfunction model, ibudilast (0.1 and 0.3 mg/kg) significantly reduced the time of abnormal swimming in the swimming test as compared with that in the controls (Fig. 3). However, aspirin did not significantly reduce the time.
There was no significant difference in inner ear blood flow before the administration of drugs among three groups (Table 2). In Fig. 4, ibudilast (0.3 mg/kg) significantly increased the inner ear blood flow as compared with that of the controls; moreover, increases in the blood flow continued during the 10-min observation period. The mean blood pressure was not affected by each dose of ibudilast or saline. In a separate experiment, aspirin injected before the administration of ibudilast inhibited the increase of inner ear blood flow by ibudilast.

**DISCUSSION**

We demonstrated the effect of ibudilast in rat inner ear microcirculation thrombosis and blood flow of the cochlear nerve. The effects of ibudilast on the inner ear were further confirmed by the electrocochleogram (Fig. 1) and the swimming test (Fig. 3).
Ibudilast significantly increased blood flow in the cochlea, prolonged the time of complete suppression of the AP and reduced the time of abnormal swimming in the swimming test. Ibudilast was reported to have cerebral vasodilating and antiplatelet effects, probably through a mechanism linked to prostacyclin (12, 13). In this study, ibudilast continued to increase the inner ear blood flow over a 10-min period after the administration; furthermore, aspirin injected before the administration of ibudilast inhibited the increase of inner ear blood flow by ibudilast, since aspirin inhibits cyclooxygenase activity and the production of prostacyclin. In a previous study, prostacyclin increased the blood flow in rat inner ear without changing the systemic blood pressure; however, the increasing blood flow by prostacyclin recovered to the baseline value within 3 min after the administration (14). These findings suggest that the increase of inner ear blood flow by ibudilast might be caused through a mechanism linked to prostacyclin and that ibudilast might enhance the vasodilating effect of prostacyclin.

Ibudilast at the dose of 0.1 mg/kg, intravenously did not significantly increase the inner ear blood flow. On the other hand, it could prolong the time of complete suppression of the AP and reduce the time of abnormal swimming in the swimming test. These findings suggest that the potency of ibudilast in these two models was greater as compared with its potency to increase the inner ear blood flow. A possible explanation for this discrepancy is that the effect of ibudilast in the two models was evaluated under a condition in which the endothelium in the vessels of the inner ear microcirculation was damaged by the photochemical reaction, while the inner ear blood flow was measured under different conditions. These results suggest that ibudilast might be more effective when the endothelial cells are damaged. These findings are supported by evidence that the production of prostacyclin in endothelial cells is enhanced by utilization of platelet-derived endoperoxides, when platelets are activated and platelet arachidonic acid metabolism is stimulated due to endothelial damage (15). In conclusion, ibudilast caused increases in the inner ear blood flow, prolonged the time of complete suppression of the AP and reduced the time of abnormal swimming in the swimming test. Furthermore, ibudilast was more effective when the endothelium was damaged and platelets were activated.

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