Vasodilator Effects of Ibudilast on Retinal Blood Vessels in Anesthetized Rats

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Ibudilast (3-isobutyryl-2-isopropylpyrazolo[1,5-α]pyridine) is clinically used as a cerebral vasodilator in Japan. However, the effects of ibudilast on retinal blood vessels have not been fully examined. The aim of this study, therefore, was to examine the effects of ibudilast on retinal blood vessels in rats in vivo. Male Wistar rats (8 to 10 weeks old) were anesthetized with thiobutabarbital (120 mg/kg, intraperitoneally (i.p.)). Retinal vascular images were captured with a fundus camera system for small animals, and the diameter of retinal blood vessels was measured. Ibudilast (0.1 and 1 mg/kg, intravenously (i.v.)) elicited a sustained increase in the diameter of retinal blood vessels and heart rate without altering systemic blood pressure. The effects of ibudilast were significantly reduced by treatment with the nonselective cyclooxygenase inhibitor indomethacin (5 mg/kg, i.p.). These results suggest that ibudilast dilates retinal blood vessels through cyclooxygenase-dependent mechanisms in rats in vivo.

Key words cyclooxygenase; ibudilast; prostanoid; retinal blood vessel

It has been proposed that the impairment of retinal circulation contributes to the pathogenesis of retinal diseases, such as diabetic retinopathy1,2) and glaucoma.3,4) Therefore, agents that improve the retinal circulation would become therapeutic candidates for preventing or delaying the progression of these diseases.

Because of the lack of autonomic innervation to retinal vasculature,5) circulating hormones and local factors released from endothelial cells6,5) and retinal tissues8,9) might play a key role in the regulation of retinal blood flow. Changes in these regulatory mechanisms might lead to abnormalities of retinal hemodynamics. The retinal vasculature anatomically and functionally resembles the cerebral vasculature. For example, 1) an autoregulatory mechanism for blood flow is present in the retinal and optic nerve head circulation as cerebral circulation10,11), and 2) retinal endothelial cells form a blood-retinal barrier with a similar function as the blood-brain barrier.10,12) Therefore, agents that dilate cerebral blood vessels may produce vasodilator effects on retinal blood vessels.

Ibudilast (3-isobutyryl-2-isopropylpyrazolo[1,5-α]pyridine) is clinically used as an antiasthmatic drug and as a cerebral vasodilator in Japan. The clinical uses are based on the ability of ibudilast to relax airway smooth muscle,13) attenuate allergic reaction,13—16) inhibit the aggregation of platelets,15,16) and increase cerebral blood flow17—20) mainly through inhibition of phosphodiesterases (PDEs).21,22) A previous study demonstrated that 2-week administration of ibudilast shortened the mean retinal circulation time in patients with diabetes mellitus.23) The results suggest that chronic treatment with ibudilast would exert beneficial effects on the retinal circulation. However, the mechanism underlying the effects remains to be established. The purpose of this study, therefore, was to determine whether ibudilast dilates retinal blood vessels in anesthetized rats. In addition, we sought to determine whether the retinal vascular responses to ibudilast are mediated by a mechanism that involves prostanoids, since the contribution of the cyclooxygenase (COX) pathway to ibudilast-induced vasodilation of cerebral blood vessels is strongly suggested.17,18) For this purpose, we examined effects of indomethacin, a nonselective inhibitor of COX, on the retinal vascular responses to ibudilast.

MATERIALS AND METHODS

Experimental Procedures The present study was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals adopted by the Committee on the Care and Use of Laboratory Animals of Kitasato University. Male Wistar rats (8 to 10 weeks old) were obtained from the Charles River Breeding Laboratories (Tokyo, Japan) and maintained in a room with constant temperature (22±2 °C), constant humidity (55±5%), and 12-h light/dark cycle and allowed free access to regular rat chow and tap water. Rats were anesthetized with thiobutabarbital (120 mg/kg, intraperitoneally (i.p.)). After disappearance of the corneal reflex, each animal was placed on a heating pad. A tracheotomy was performed and a catheter was inserted into the right femoral vein for administration of drugs. The left femoral artery was cannulated for measurement of arterial pressure, which was recorded on a thermal pen recorder (WT-645G, Nihon Kohden, Tokyo, Japan), via a pressure transducer (DX-360, Nihon Kohden) and a preamplifier (AP-610G, Nihon Kohden). Heart rate was measured with a cardiofotachometer (AT-601G, Nihon Kohden) triggered by the blood pressure pulse. Systemic blood pressure and heart rate were digitized at 1 Hz using Science Link II (Keisoku Giken, Utsunomiya, Japan) and stored on the hard disk of a personal computer (PowerBook 165C, Apple Japan, Tokyo, Japan). Indomethacin (5 mg/kg, i.p.) (Sigma, St. Louis, MO, U.S.A.) or vehicle (0.24% Na2CO3; 1 ml/kg, i.p.) was administered 20—30 min before injection of ibudilast (a kind gift from Kyorin Pharmaceutical, Tokyo, Japan). After hemodynamic parameters reached stable levels, ibudilast (0.1 or 1 mg/kg) or the vehicle [polyethylene glycol 400: saline (2:1); 1 ml/kg] was injected into the right femoral vein.

Measurement of Retinal Blood Vessel Diameter The
pupils were dilated with one drop of 1% atropine sulfate (Nihon Tenganyaku Institute, Nagoya, Japan). To protect the eye, 0.3% sodium hyalurate (Santen Pharmaceutical, Osaka, Japan) was dropped onto the cornea. The optic disc was centered and focused in the field of view. Sodium fluorescein (10% solution, 0.8 ml/kg, intravenously (i.v.)) and brilliant blue 6B (5% solution, 0.8 ml/kg, i.v.) were injected into the right femoral vein to enhance vessel contrast. Real-time retinal vascular images were obtained using a fundus camera system for small animals (Model VMS-170, Scalar, Tokyo, Japan), displayed on a 14-inch display (KV-14GP3, Sony, Tokyo, Japan), and recorded on a digital videotape recorder (WV-D10000, Sony, Tokyo, Japan) for off-line processing. The vascular images were captured from each recorded movie using an image capture system (Photo DV 1.1.1., Radius Tokyo, Tokyo, Japan) on a Power Macintosh G3-266DT (Apple Japan).

The diameter of retinal blood vessels was measured as described previously. Briefly, the full-color (RGB) images were processed using image processing software (Photoshop 5, Adobe, Systems Inc., San Jose, CA, U.S.A.) (Fig. 1A—C). We chose the green channel image, which provided the greatest contrast among three individual color channels, for further processing. After intensifying the contrast of the retinal blood vessels, the region (80×160 μm) including a retinal arteriole or a retinal venule in the fundus image (2560×3413 μm) was selected (Fig. 1B). Blood vessels were distinguished from the background by determining a certain threshold value for each image (Fig. 1C). The diameter of the vessel was calculated by dividing the area of the vessel by the length of the vessel in the selected area (NIH image 1.62., National Institutes of Health, Bethesda, MD, U.S.A.). The diameter of blood vessel in the same region was measured throughout the experiment.

**Statistical Analyses** The diameter of the retinal blood vessel, mean arterial pressure, and heart rate were expressed as percentages of the baseline values just before the injection of ibudilast or the vehicle. The significance of the difference between mean values was evaluated with GraphPad Prism (San Diego, CA, U.S.A.) by unpaired t-test. When comparing the responses between groups, two-way analysis of variance (ANOVA) was used. A p value of less than 0.05 was considered to represent a statistically significant difference. All values are presented as mean±S.E.M.

**RESULTS**

Baseline values of the diameter of retinal blood vessels, mean arterial pressure, and heart rate of vehicle- and indomethacin-treated rats were not significantly different (retinal arteriolar diameter, vehicle 59.8±2.1 μm, n=15 vs. indomethacin 63.8±2.5 μm, n=5; retinal venular diameter, vehicle 77.2±3.6 μm, n=15 vs. indomethacin 78.0±5.2 μm, n=5; mean arterial pressure, vehicle 99±3 mmHg, n=15 vs. indomethacin 90±4 mmHg, n=5; heart rate, vehicle 376±8 beats/min, n=15 vs. indomethacin 362±11 beats/min, n=5).

Representative fundus images before and after the injection of ibudilast (1 mg/kg, i.v.) or vehicle are shown in Figs. 1D—G. Ibudilast dilated retinal blood vessels, and vasodilator responses were prevented by the administration of indomethacin. The data are summarized in Figs. 2 and 3.

Ibudilast (0.1, 1.0 mg/kg) increased the diameter of retinal blood vessels (Figs. 2A, B) and heart rate (Fig. 2D) in a dose-dependent manner. The magnitude of vasodilation induced by ibudilast was not significantly different between retinal arterioles and venules (e.g., increase in diameters of retinal blood vessels induced by 1 mg/kg of ibudilast, arterioles 11.7±1.7% vs. venules 9.1±2.2%). In contrast, ibudilast had no significant effect on mean arterial pressure (Fig. 2C). The vasodilator responses of retinal blood vessels and positive chronotropic response to ibudilast were significantly attenuated by treatment with indomethacin (Fig. 3).

**DISCUSSION**

The present study demonstrates that ibudilast exhibits vasodilator effects on retinal arterioles and venules in anesthetized rats. The retinal vascular responses to ibudilast were significantly attenuated by the inhibition of COX with indomethacin. Therefore, it is likely that COX-dependent mechanisms contribute to the ibudilast-induced vasodilation of retinal blood vessels.

The actions of ibudilast are mediated mainly through the elevation of intracellular cAMP and/or cGMP levels due to
inhibition of PDEs.\textsuperscript{21,22}) If this is the case, ibudilast appears to enhance the effects of endogenous substances that increase cAMP (e.g., prostacyclin, circulating catecholamines, etc.) and cGMP (nitric oxide), leading to the vasodilation of retinal blood vessels. The present results with indomethacin strongly suggest that the vasodilatory prostanoids play an important role in the retinal vascular responses to ibudilast. Previously, we showed that both prostacyclin and prostaglandin \(E_2\) exhibit vasodilatory effects on retinal blood vessels in rats \textit{in vivo}.\textsuperscript{24}) Therefore, the retinal vascular responses to ibudilast seem to be due, at least in part, to the enhancement of the vasodilatory action of these prostanoids.

Recently, the inhibitory profile of ibudilast against a large number of PDE families has been systemically investigated.\textsuperscript{22}) According to one investigation, ibudilast exhibits potent inhibitory action on PDE3A (\(K_i\) value: 9.5 \(\mu\)M), PDE4 (\(K_i\) values: PDE4A4, 4.1 \(\mu\)M; PDE4B2, 3.3 \(\mu\)M; PDE4C2, 6.3 \(\mu\)M; PDE4D3, 3.7 \(\mu\)M), PDE10 (\(K_i\) value: PDE10A1, 2.2 \(\mu\)M), and PDE11 (\(K_i\) value: PDE11A1, 8.9 \(\mu\)M).\textsuperscript{22}) At present, the functional role of these PDEs in the retinal circulation is still not completely understood. However, the PDE3 inhibitor cilostazol\textsuperscript{26}) and the PDE4 inhibitors rolipram and Ro-20-1724\textsuperscript{27}) have been shown to dilate the retinal blood vessels in rats. Therefore, the inhibitory effects of ibudilast on PDE3A and/or PDE4 activity may contribute to the retinal vasodilator responses.

It is well known that PDE3 inhibitors cause positive chronotropic and inotropic effects. Sun \textit{et al.} demonstrated that the heart rate of PDE3A knockout (KO) mice was significantly higher than that of age-matched WT mice, while the heart rate of PDE3B KO mice was similar to that of WT mice.\textsuperscript{29}) Furthermore, they showed that the pharmacologic inhibition of PDE3 increased the heart rate and decreased systemic blood pressure in WT and PDE3B KO mice, but not in PDE3A KO mice. These results suggest that PDE3A plays an important role in the regulation of cardiovascular function. In contrast, PDE4 inhibitors were shown to exhibit a mild vasodilator effect on isolated peripheral blood vessels\textsuperscript{29–31}) and no or weak cardiac action,\textsuperscript{27,32}) whereas they produced potent vasodilator effects on cerebral\textsuperscript{27}) and retinal blood vessels.\textsuperscript{27}) The findings that ibudilast dilates the retinal blood vessels without altering systemic blood pressure resemble those obtained with PDE4 inhibitors.

Our previous study demonstrated that the effects of PDE4 inhibitors on retinal arterioles were greater than those on retinal venules.\textsuperscript{27}) However, there was no significant difference in the vasodilator response to ibudilast between arterioles and venules. In addition, unlike PDE4 inhibitors, ibudilast caused a positive chronotropic response. These differences may be related to the difference in selectivity against the PDE4 isoenzyme. Interestingly, the positive chronotropic responses to ibudilast were attenuated by indomethacin; therefore the COX-dependent pathway appears to be responsible for this response. However, the question of whether the positive chronotropic effect of ibudilast is due to PDE inhibition and/or unknown mechanisms independent of PDE remains to be clarified.
In this study, we evaluated the effects of intravenous injection of ibudilast on the retinal circulation by measuring changes in the diameter of retinal blood vessels and found that ibudilast produces an approximately 10% increase in the diameter. According to Poiseuille’s law, blood flow is proportional to the fourth power of vessel diameter; therefore small changes in the diameter of vessels would lead to significant alterations in blood flow. Ibudilast may thus have the potential to improve retinal blood flow.

In conclusion, we found that ibudilast dilates retinal blood vessels through the COX-dependent pathway in anesthetized rats. The present results suggest that the vasodilator effects of ibudilast on retinal blood vessels may have beneficial effects on the retinal circulation in patients with diabetic mellitus. Thus ibudilast is one candidate for a therapeutic agent to prevent the development of ocular diseases that are associated with impaired retinal circulation.

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