

Ibudilast: A Non-selective PDE Inhibitor with Multiple Actions on Blood Cells and the Vascular Wall

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ABSTRACT

Ibudilast (3-isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine) is a nonselective inhibitor of cyclic nucleotide phosphodiesterase (PDE). It is widely used in Japan for improving prognosis and relieving symptoms in patients suffering from ischemic stroke or bronchial asthma. These clinical applications are based on the properties of ibudilast that inhibit platelet aggregation, improve cerebral blood flow and attenuate allergic reactions. The inhibition of platelet aggregation and vasodilatation by ibudilast may be due to synergistic elevation of intracellular cyclic nucleotides and release of nitric oxide (NO) or prostacyclin from endothelium, rather than direct inhibition of PDE5 or PDE3. Another important property of ibudilast is its antiinflammatory activity possibly associated with potent inhibition of PDE4. Combined with its relaxing effects on bronchial smooth muscle, antiinflammatory activity of ibudilast could favorably influence pathophysiology of asthma by antagonizing chemical mediators triggering asthmatic attacks. Ibudilast was also reported to significantly attenuate inflammatory cell infiltration in the lumbar spinal cord in an animal model of encephalomyelitis. Future investigations should include effects of ibudilast on inflammatory reactions between endothelium and blood cells, which may initiate the development of atherosclerosis.

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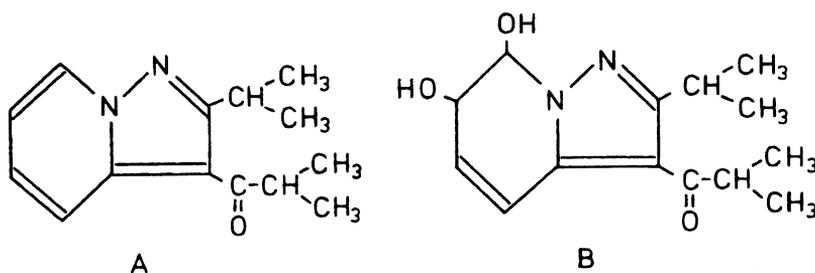


FIG. 1. Chemical structure of ibudilast (left) and diOH-ibudilast (right). Molecular formula: $C_{14}H_{18}N_2O$, molecular weight: 230.31.

INTRODUCTION

Ibudilast is an antithrombotic, antiasthmatic drug that is widely used in Japan for improving prognosis and relieving symptoms in patients suffering from ischemic stroke and for the treatment of bronchial asthma (14,28). The mechanism of action of ibudilast is likely to involve inhibition of cyclic nucleotide phosphodiesterase (PDE; EC 3.1.4.17). PDE consists of at least 11 families and more than 30 isozymes (39). Although PDE is ubiquitously expressed, PDE activity in any given tissue or cell type may be limited to a small number of isozymes (2). Thus, non-selective PDE inhibitors may provide multiple functions for various tissues. Recent studies have demonstrated that ibudilast exerts important effects on the interaction between platelets or other blood cells and endothelium (15,34), in addition to having a direct effect on vascular and bronchial smooth muscle cells (24,25). Of particular interest is the effect of this agent on the endothelium since endothelial function is extremely important for the preservation of normal vascular wall metabolism and protection from atherosclerosis and thrombosis (30).

In this review we focus on currently available chemical, pharmacological and clinical studies with ibudilast. We also describe possible therapeutic indications for ibudilast based on experimental results.

CHEMISTRY AND PHARMACODYNAMICS

Ibudilast, 3-isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine, (KC404) was originally synthesized at the Central Research Laboratories of Kyorin Pharmaceutical Co., Ltd. (Tochigi, Japan). The chemical structures of ibudilast and its dihydroxy derivative are shown in Fig. 1. Ibudilast is well absorbed after oral administration and is metabolized in the liver mainly to diOH-ibudilast, which has similar or even stronger pharmacological effects (26). Following a single oral administration of 10 mg of ibudilast to healthy male adults, the plasma concentration of the unchanged drug reached a maximum of 25 ng/ml at 4 h and declined slowly to 50% of the maximal concentration at 12 h (18). Sixty percent of the ingested drug is excreted in the urine as diOH-ibudilast or other metabolites within 72 h (37).

TABLE 1. IC_{50} values of ibudilast for major PDE isozymes

PDE isoenzymes	Enzyme source (reference)	IC_{50} , μ M
PDE1	Pig coronary SMC (17)	55
PDE2	Bovine aortic EC (16)	0.11
PDE3	Human platelets (15)	31
PDE4	Bovine aortic EC	0.08
PDE5	Human platelets	2.2

Enzyme activities were determined at a substrate concentration of 0.25 μ M for each cyclic nucleotide. PDE1 was assayed in the presence of 2 mM Ca^{2+} and 1.25 U/mL bovine brain calmodulin. PDE2 was assayed in the presence of 10 μ M cyclic GMP. IC_{50} values were determined from a plot of the percent inhibition of the enzyme activity vs. the log concentration of ibudilast.

PHARMACOLOGY

Mechanism of Action

Ibudilast is a non-selective PDE inhibitor with different IC_{50} values (the concentration of ibudilast for 50% inhibition of PDE activity) for the major isozymes (Table 1). These results are compatible with clinical benefits for patients with atherothrombotic cerebrovascular diseases and bronchial asthma since the major PDE isozymes are PDE3 and 5 for human platelets, PDE1, 3–5 for vascular smooth muscle cells, and PDE2–5 for bronchi (15,21). Ibudilast is a potent PDE4 inhibitor and inhibition of this PDE isozyme is associated with antiinflammatory and antiallergic effects (10,13). A definitive mechanism of its antiinflammatory action is yet to be established. However, inhibition of the release of inflammatory cytokines, inhibition of leukocyte activation, and inhibition of the expression of cell adhesion molecules have been proposed as likely mechanisms of action of ibudilast (35). Other reported pharmacological actions of ibudilast include inhibition of leukotriene D_4 -induced formation of inositol phosphates (6), antagonism of slow-reacting substance of anaphylaxis (SRS-A) (27), inhibition of tyrosine kinase in neutrophils (23), and inhibition of ATP-sensitive potassium channels (12).

Experimental Studies

Antithrombotic effects

The antithrombotic effects of ibudilast have been investigated almost exclusively in terms of its ability to inhibit aggregation of platelets, both *in vivo* (22,38) and *in vitro* (15,20,26). Nishimura et al. (22) examined the antiplatelet effects of ibudilast *in vivo* using a gerbil model of carotid artery thrombosis. Endothelial damage in a unilateral carotid artery of each animal was induced by tightly compressing the artery with surgical threads for 2 min. Ibudilast, or another antiplatelet drug, was injected intravenously 10 min before arterial compression. This procedure led to high incidence of thrombosis; 70 to 90% of non-treated animals reproducibly developed thrombus. Electron microscopic observation revealed that multi-layered activated platelets with prominent pseudopodia

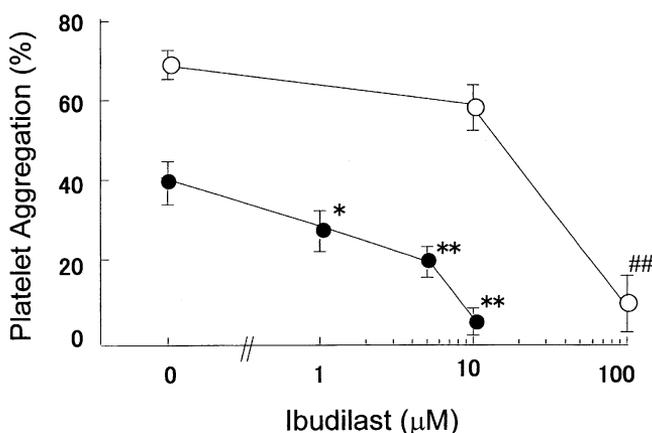
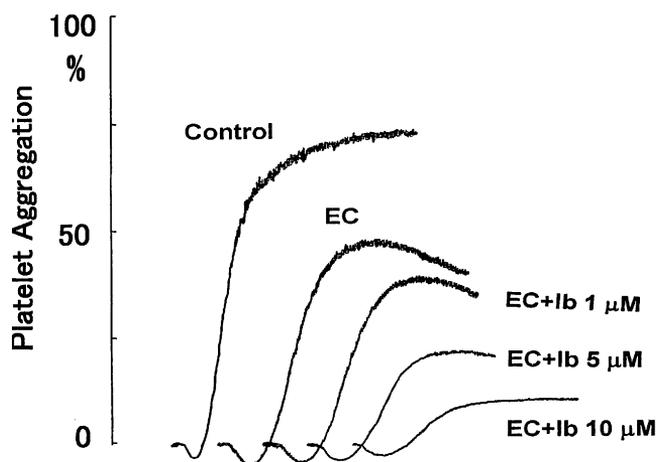


FIG. 2. The dose-inhibition relationship of 2 µg/mL collagen-induced platelet aggregation by ibudilast with (●) and without (○) EC. Washed platelets were preincubated with 5×10^4 EC/mL for 5 min at 37°C and, while agitating at 1000 rpm with a Teflon-coated stirrer, collagen was added to the cuvette. Platelet aggregation was monitored by standard optical densitometry. Values are means \pm S.E.M. ($n = 5$). ## $P < 0.01$ vs. control; * $P < 0.05$, ** $P < 0.01$ vs. control. Reproduced from ref. 15 with permission from Lippincott, Williams and Williams.

adhered to the vascular luminal surface of the injured area. Pretreatment with ibudilast at 0.3 and 1.0 mg/kg significantly reduced the incidence of thrombus formation, to 1/4 or 1/7 that of the control group. The antithrombotic effect of ibudilast was similar to that achieved by 3 mg/kg ticlopidine, an ADP receptor antagonist.

Inhibition of platelet aggregation by ibudilast was also demonstrated in *in vitro* studies using platelet-rich plasma or isolated platelets. However, the initial IC_{50} values for the antiplatelet effects of ibudilast (20,26) were too high to explain *in vivo* or clinical results, which showed favorable effects of the drug on the prognosis of patients with ischemic stroke. These findings led us to explore the effects of ibudilast on platelet-endothelium interaction (15). For this purpose, we measured platelet aggregation in the presence of endothelial cells (EC) using washed platelets. Fig. 2 shows the effects of ibudilast on platelet aggregation by 2 µg/mL collagen in the presence and absence of EC. A high concentration of ibudilast alone was needed to inhibit activation of platelets, while lower concentrations of ibudilast significantly augmented the effects of EC. A typical example of a platelet aggregation curve is shown in Fig. 3. Pretreatment of EC with L-NNA significantly reduced the ability of ibudilast to inhibit platelet aggregation, while pretreatment of EC with aspirin only slightly affected the antiplatelet activity of ibudilast (Table 2). These findings suggest that NO released from EC, rather than prostacyclin, plays a key role in the synergistic inhibition of platelet aggregation by ibudilast and EC. In accordance with the aggregation studies, ibudilast (at 1 µM or higher concentrations) significantly enhanced cyclic GMP accumulation in platelets co-cultured with EC. However, in the absence of EC, ibudilast increased platelet cyclic GMP levels only at high concentration (100 µM) (Fig. 4, left panel). Preincubation of EC with ibudilast had no effect on EC-induced cyclic GMP accumulation in platelets preincubated with 3-isobutyl-1-methylxanthine (IBMX), a potent non-selective inhibitor of PDE (Fig. 5), thus indicating no increase of NO release from EC.

FIG. 3. Representative tracings of platelet aggregation by collagen (2 µg/mL) with and without endothelial cells (5×10^4 /mL) and ibudilast. EC: endothelial cells, Ib: ibudilast. Reproduced from ref. 15 with permission.



In contrast, ibudilast enhanced cyclic AMP accumulation only at 100 µM, both with and without EC (Fig. 4, right panel), in line with the platelet aggregation study (Table 2). However, the role of a less potent effect of ibudilast on prostacyclin should not be ignored, since platelet inhibition in the presence of aspirin-pretreated EC was less than in the presence of non-treated cells (Table 2). This could be due to the enhancement of the effect of prostacyclin by ibudilast (1), because the elevation of cyclic GMP by ibudilast may suppress the degradation of cyclic AMP by PDE3 (cyclic GMP-inhibited PDE) in platelets, as described in the case of dipyridamole, a PDE5 inhibitor (3). Furthermore, the threshold concentration of diOH-ibudilast, a major metabolite of ibudilast, for potentiating prostacyclin action is 10 times lower than that of ibudilast (26). Thus, the synergistic interaction between prostacyclin and NO against platelet aggregation (4) can conceivably contribute to the anti-aggregation effect of ibudilast. The existence of the more potent metab-

TABLE 2. Effect of ibudilast (10 µM) on aggregation of washed platelets by collagen (2 µg/mL) in the presence of aspirin- or L-NNA-pretreated EC

	Control	Ibudilast	<i>P</i>
EC(-)	69 ± 4	58 ± 6	NS
EC (5×10^4 /mL)			
Pretreatment (-)	39 ± 6	5.4 ± 3.3	< 0.01
Aspirin (0.5 mM)	47 ± 4	22 ± 4	< 0.01
L-NNA (0.2 mM)	55 ± 4	51 ± 5	NS

NS, not significant. Aspirin-pretreated EC were prepared by incubating EC on 100 × 20-mm dishes with 0.5 mM aspirin for 3 h at 37°C. The surface of the cells was rinsed with solution B and cell suspension, prepared by trypsinization, was used for the aggregation experiment. L-NNA-pretreated cells were prepared by incubating an EC suspension with 0.2 mM L-NNA for 5 min at 37°C just before the assay. Values are the percentage of optical density relative to that of solution B (mean ± S.E.M., *n* = 3–5). Statistical analysis was done by the paired Student's *t*-test. Reproduced from ref. 15 with permission.

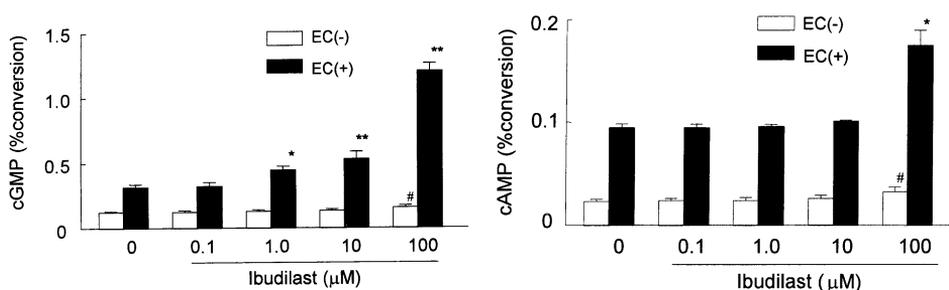


FIG. 4. Effects of ibudilast on cyclic GMP (left panel) and cyclic AMP (right panel) accumulation in platelets exposed to EC. [^3H]GTP- or [^3H]ATP-labeled platelet suspension was cultured in the presence of EC grown on $35 \times 10\text{-mm}$ dishes for 30 seconds and cyclic GMP (cGMP) or cyclic AMP (cAMP) accumulation in the platelets was determined. Values are cyclic GMP or cyclic AMP accumulation expressed as % conversion {(cyclic GMP formed/[^3H]guanine uptake by the platelets) $\times 100$ }. Statistical analysis was done by one-way analysis of variance (ANOVA) using Scheffé's test for multiple comparison. Values are mean \pm S.E.M., $n = 4$. * $P < 0.05$, ** $P < 0.01$ vs. control; # $P < 0.05$, vs. control. Reproduced from ref. 15 with permission.

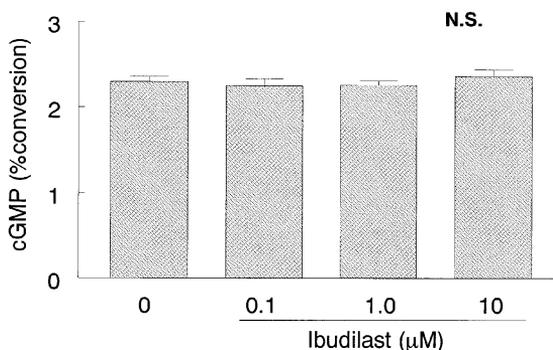


FIG. 5. Effect of ibudilast on NO release from EC as estimated by EC-induced cyclic GMP accumulation in platelets pretreated with IBMX. [^3H]GTP-loaded platelets were preincubated for 10 min with 1 mM IBMX. Aliquots of platelet suspension were added to an EC suspension (5×10^5 /tube) pretreated with 0.1–10 μM ibudilast for 5 min. After 30 sec, the reaction was terminated with 10% TCA, and cyclic GMP (cGMP) accumulation in the platelets was determined. Cyclic GMP accumulation in the platelets in the absence of EC was $0.063 \pm 0.006\%$ (mean \pm S.E.M.). Statistical analysis was done by one-way analysis of variance (ANOVA). Values are mean \pm S.E.M. of 4 samples. N.S.: not significant. Reproduced from ref. 15 with permission.

olite may also partially explain the apparent discrepancy between the ibudilast serum levels of patients (20 to 30 ng/mL, i.e., 0.08–0.13 μM) (8), and the 1.0 μM threshold concentration of ibudilast required to significantly inhibit platelet aggregation in the presence of EC.

Antiinflammatory effects

Pharmacological properties of ibudilast as an anti-asthma drug include bronchodilatory (27) and antiinflammatory actions (5,6). The antiinflammatory potency of ibudilast is particularly important, since it may be responsible for the favorable effects of ibudilast not

only on bronchial inflammation but also on certain types of encephalitis (8) and even on the development of atherosclerosis (30). Chihara et al. investigated the effect of ibudilast on β_2 integrin expression by a flow cytometry in EoL-1 cells, an eosinophilic cell line (5). They found that ibudilast inhibited CD11a, CD11b, and CD18 expression induced by platelet activating factor (PAF). PAF and LTD4 are chemical mediators for allergic reactions and inflammation, and other studies also confirmed that ibudilast had an antagonistic effect on these mediators (6,9,24,27). Inhibition of inflammatory disease in the central nervous system by ibudilast has been suggested as well. Fujimoto et al. examined the therapeutic efficacy of ibudilast on Dark August (DA) rat experimental autoimmune encephalomyelitis (EAE), which is commonly used as an experimental model of multiple sclerosis in humans (8). They reported that prophylactic oral treatment with ibudilast significantly attenuated the severity of acute EAE with less inflammatory cell infiltration in the lumbar spinal cord. Adjunct *in vitro* studies revealed that ibudilast suppressed MBP-induced proliferation of T cells in inguinal lymph nodes. Furthermore, it significantly ameliorated the secretion of interferon- γ from T cells activated by MBP and the secretion of TNF- α from macrophages in the peritoneal cavity of native DA rats. Another non-selective PDE inhibitor, pentoxifylline (29,31), and a selective PDE4 inhibitor, rolipram (32), shared these preventive effects on EAE. Thus, PDE4 inhibition may be associated with the antiinflammatory properties of ibudilast. However, other PDE isozymes or other mechanisms, independent of PDE, may be involved since a dose of rolipram much higher than clinical doses was administered for the prevention of EAE (32).

CLINICAL STUDIES

Cerebrovascular Disease

Ohtomo and co-investigators conducted a prospective, open label, multicenter study to evaluate the effects of ibudilast in secondary prevention of ischemic stroke (28). The study period was 38 months, from November 1991 to December 1994. A total of 932 patients (66.9 ± 10.2 years, mean \pm S.D.; M/F: 591/341) from 178 institutions in Japan participated. The patients were diagnosed as having ischemic stroke 1 to 12 months before enrollment, based on clinical symptoms and CT or MRI findings. Ibudilast was given for 2 years to all patients at a dosage of 10 mg t.i.d. None of the participants took other antiplatelet drugs, including aspirin. Of 932 patients, 271 did not return for the follow-up visits; the remaining 661 were subjected to statistical analysis. Relapse of cerebral infarction was documented in 59 cases; the annual relapse rate was 3.9%, 3.3% for symptomatic cerebral infarction, and 0.6% for asymptomatic ischemic stroke documented by brain CT or MRI during the follow-up period. Stratified analysis of the relapse rate of cerebral infarction showed that patient background factors, such as the gender (males), the age group (60–69 years old), the severity of motor paralysis, and presence of concomitant drugs and therapeutic drugs before the treatment were the factors leading to significantly poorer prognosis. Relapse rate was high in patients with concomitant ischemic heart disease (annual rate: 8.0%), hyperlipidemia (5.1%), hypertension (4.2%). Adverse reactions were observed in 22 cases (2.3%) and abnormal laboratory findings were observed in 34 cases (3.6%). No serious adverse effects were reported. Three patients developed non-fatal cerebral hemorrhage and 33 patients (3.5%) died from diseases unrelated to

TABLE 3. Cerebral blood flow changes after ibudilast loading

	CORN	CORL	BGGN	BGGL	THN	THL	CLN	CLL
Baseline								
Mean	33.01	30.15	36.15	38.39	42.24	37.22	37.77	39.41
S.D.	9.92	8.56	7.74	11.84	16.18	12.55	12.06	15.20
Loading								
Mean	37.30*	34.64*	43.61*	38.33	48.30	35.24	44.64	44.88
S.D.	9.65	8.90	9.52	7.12	27.38	9.49	10.54	14.81

*Statistically different from baseline CBF (ANOVA, $p < 0.05$). CORN, cortex non-lesion side; CORL, cortex lesion side; BGGN, basal ganglia non-lesion side; BGGL, basal ganglia lesion side; THN, thalamus non-lesion side; THL, thalamus lesion side; CLN, cerebellum non-lesion side; CLL, cerebellum lesion side. Reproduced from ref. 7 with permission of Forefront Publishing Group.

ibudilast, including respiratory infection (30.3%), heart failure (21.2%), malignant neoplasm (18.2%) or other diseases. This study was not placebo-controlled and the number of participants was relatively small. Despite these limitations, the authors assumed that long-term treatment with ibudilast resulted in fewer cerebrovascular events when these data were compared with the results of a number of similar clinical trials conducted inside and outside Japan (11,28,36).

Improvement in prognosis by ibudilast was supposed to be due to anti-platelet effects. Murashima et al. (19) conducted an ex vivo study on the effects of ibudilast on platelet aggregation in 35 patients (44–86 years of age, mean: 68 years; M/F: 19/18) with cerebrovascular diseases. After 8 weeks of ibudilast administration (10 mg t.i.d.), a significant reduction in platelet aggregation in response to 2 μ M ADP and 1 μ M PAF was observed. They also reported a decrease in plasma platelet factor 4 (PF4) and β -thromboglobulin (β -TG).

Ohtomo et al. and Murashima et al. also described symptomatic relief with ibudilast therapy and suggested that this might be due to the improvement of cerebral blood flow (19,28) associated with the cerebral vasodilator action of ibudilast (25). In fact, using positron emission tomography (PET), Fukuyama et al. examined the cerebral blood flow (CBF), oxygen extraction fraction (OEF) and cerebral metabolic rate of oxygen (CMRO₂) of five patients, all of whom had suffered strokes prior to the examination, and confirmed favorable effects of ibudilast on these parameters (7). Table 3 shows changes of CBF on the lesion side as well as the non-lesion side of cortex, basal ganglia, thalamus and cerebellum before and 30 min after 10 mg ibudilast per os. Mean values in both hemispheres and nonlesion side basal ganglia were significantly increased after ibudilast loading. Regional analysis of the cerebral cortex revealed statistically significant increases in CBF in the frontal, temporal, and occipital lesion side and in the temporal and occipital non-lesion side. Figure 6 depicts typical images of a 60-year-old patient with cerebral infarction in the left middle cerebral artery region. Remarkable improvement of CBF on the non-lesion side hemisphere and on the ischemic side hemisphere as well, was demonstrated after administration of ibudilast.

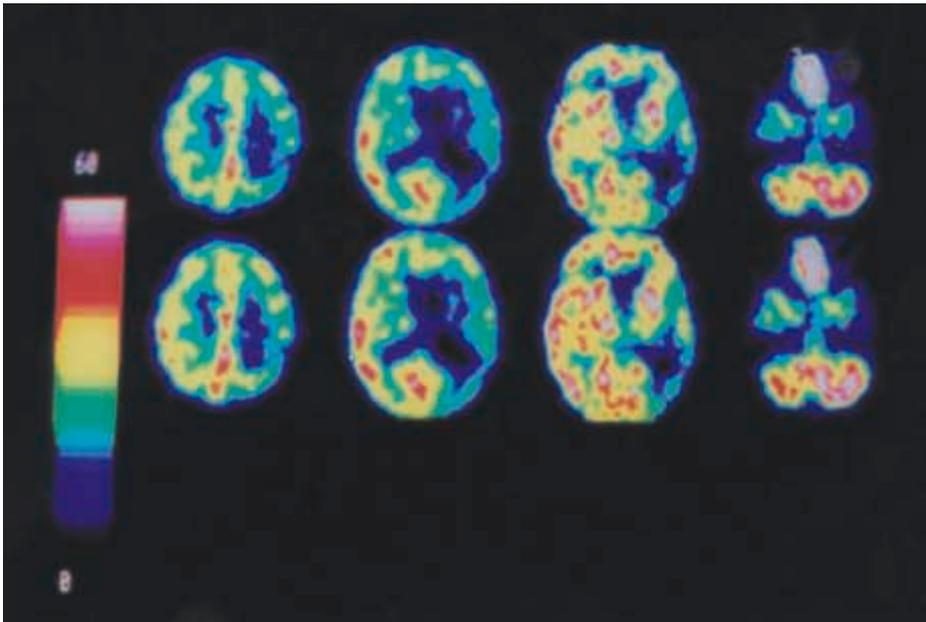


FIG. 6. CBF at baseline and 30 min after ibudilast loading in a 60-year old female patient with cerebral infarction (left middle cerebral artery region). Images in the upper row were CBF images at baseline and lower ones after loading. CBF increment was apparent after ibudilast loading. Reproduced from ref. 7 with permission.

Other Clinical Studies

As described earlier, ibudilast has antiallergic and vasodilator activity. Kawasaki et al. investigated the effect of ibudilast on airway hypersensitivity to histamine in 13 patients with bronchial asthma (14). Following the initial treatment with ibudilast (20 mg, b.i.d.), the PC₂₀ values improved significantly from 355.6 to 620.5 mg/mL at 3 months and further improved to 731.4 mg/mL at 6 months. The severity of asthma attacks was also reduced significantly during the course of treatment.

Sone et al. examined the effects of ibudilast on peripheral circulation in 41 patients with non-insulin-dependent diabetics using two-dimensional Doppler ultrasonography and laser Doppler flowmetry (33). One hour after oral administration of ibudilast, the cross-sectional area of the dorsal pedis artery, its blood flow index and dermal microcirculatory blood volume increased significantly as compared to the control group.

CONCLUDING REMARKS

As a non-selective PDE inhibitor, ibudilast has been shown to have multiple pharmacological and clinical effects. So far, clinical studies with ibudilast have been limited to cerebrovascular disease and bronchial asthma. These indications were based on the ability of ibudilast to inhibit platelet aggregation, improve cerebral blood flow and attenuate allergic reactions. In addition, ibudilast is a strong inhibitor of PDE2 and 4, which are major

isozymes of endothelial cells. Since endothelial function is extremely important for the maintenance of vascular integrity and since its dysfunction leads to atherosclerosis and vascular remodeling, the action of ibudilast on endothelial metabolism should be further investigated.

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