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BRAIN RESEARCH

Research Report

Ibudilast, a phosphodiesterase inhibitor with anti-inflammatory activity, protects against ischemic brain injury in rats

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ABSTRACT

Ibudilast, a non-selective phosphodiesterase inhibitor, is clinically used in patients with stroke or dizziness. However, whether the compound exerts a beneficial effect on acute ischemic stroke remains to be established. We used a rat model of transient focal cerebral ischemia using middle cerebral artery occlusion (MCAO) and reperfusion, and explored the effects of ibudilast on infarction size, brain edema, atrophy, and nerve cell death. Neurological outcomes (behavior and mortality) of rats were also assessed. An intravenous administration of ibudilast attenuated the size of cerebral infarction in a dose-dependent manner, with the most significant reduction achieved at the dose of 10 mg/kg. Ibudilast induced a significant reduction in infarct size when administered 30 min before MCAO or 0–2 h after reperfusion, with the largest reduction observed at 30 min before MCAO and 1 h after reperfusion. Ibudilast significantly attenuated brain edema formation, cerebral atrophy and apoptosis of nerve cells preferentially in the cortical penumbra area, and also significantly reduced mortality and improved neurological outcomes. Expression of various inflammatory mediator molecules in both hemispheres was markedly suppressed by ibudilast. We conclude that ibudilast exerts beneficial effects against acute brain ischemia in an animal model.

1. Introduction

Ibudilast (3-isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine), a non-selective inhibitor of cyclic nucleotide phosphodiesterases (PDEs) (Souness et al., 1994), is used to treat patients with cerebrovascular disorders (e.g., cerebral hypoperfusion, post-stroke dizziness) in Korea and Japan, based on its inhibitory activity on platelet aggregation and improvement of cerebral blood flow (Armstead et al., 1988; Fukuyama et al., 1993; Rile et al., 2001). However, it is practically used in the chronic stage of stroke while there has been no evidence supporting the use of ibudilast in patients with acute ischemic stroke.

Recent studies have suggested that ibudilast could exert neuroprotective effects in damaged brain cells. In cultured

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Abbreviations: PDE, phosphodiesterase; MCAO, middle cerebral artery occlusion; CCA, common carotid artery; ECA, external carotid artery; ICA, internal carotid artery; CBF, cerebral blood flow; TTC, 2,3,5-triphenyl tetrazolium chloride; RT-PCR, Reverse transcriptase-polymerase chain reaction

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Table 1 – Oligonucleotide primers used for RT-PCR analysis.

Gene (GenBank accession no.)	Oligonucleotide primer sequences	PCR product size (bp)
IL-1β (M98820)	5′-AGC ATC CAG CTT CAA ATC TCA-3′ 5′-CGA GGC ATT TTT GTT GTT CAT-3′	268
TNF-α (L00981)	5′-CCT CAG CCT CTT CTC ATT CCT-3′ 5′-CTC TGC TTG GTG GTT TGC TAC-3′	211
IL-6 (M26744)	5′-CAA GAG ACT TCC AGC CAG TTG-3′ 5′-GAA ACG GAA CTC CAG AAG ACC-3′	350
COX-2 (S67722),	5′-GAT TGA CAG CCC ACC AAC TTA-3′ 5′-CTC AGG ATG CTC CTG TTT GAG-3′	598
MMP-9 (NM031055)	5′-CAG ATG ATG GGA GAG AAG CAG-3′ 5′-TGT TAT GAT GGT GCC ACT TGA-3′	187
ICAM-1 (BC081837)	5′-GAC CCT GGA GAT GGA GAA GAC-3′ 5′-GAG TCT GCT GAG ACC CCT CTT-3′	398
iNOS (U03699),	5′-AAG AAC GTG TTC ACC ATG AGG-3′ 5′-CCA GTA GCT GCC ACT CTC ATC-3′	252
β-actin (AF122902)	5′-TGT CAC CAA CTG GGA CGA TA-3′ 5′-GGG GTG TTG AAG GTC TCA AA-3′	165

astrocytes, ibudilast elevates the level of cGMP, leading to attenuation of H_2O_2-triggered apoptosis via cGMP-dependent protein kinase (PKG) activation (Takuma et al., 2001). The compound protects glia or neurons against excitotoxicity by reducing the intracellular Ca^{2+} concentration and maintaining a high intracellular cAMP level (Souness et al., 1994; Takuma et al., 2001; Tominaga et al., 1996; Yoshioka et al., 1998). Moreover, ibudilast attenuates neuronal cell death and improves passive avoidance responses in rats subjected to transient cerebral global ischemia (Yoshioka et al., 2002).

Since ibudilast inhibits PDE4, an enzyme involved in the regulation of inflammatory responses (Huang et al., 2006; Souness et al., 1994; Spina, 2008), the neuroprotective potential of this compound is possibly associated with its anti-inflammatory activity (Hutchinson et al., 2009; Ledeboer et al., 2006; Mizuno et al., 2004). Ibudilast attenuates cell death in neuron culture exposed to lipopolysaccharide (LPS) or interferon-γ (IFN-γ) (Mizuno et al., 2004). Moreover, an increasing number of *in vivo* studies have documented its potential utility in the treatment of various neurological diseases associated with neuroinflammation (Barkhof et al., 2010; Kagitani-Shimono et al., 2005).

In view of its multifaceted effects of ibudilast including neuroprotective and anti-inflammatory properties, we hypothesized that it may be useful in the treatment of acute ischemic stroke. In this report, we explored the potential utility of ibudilast in reducing brain damage in a rat model of acute ischemic stroke.

2. Results

2.1. Physiological parameters

Physiological parameters were evaluated before occlusion and after reperfusion and drug treatment. No statistical differences were evident between vehicle- and ibudilast-treated groups with regard to all physiological parameters assayed ($P>0.05$), including blood pH, PO_2, PCO_2, glucose (Glu), hematocrit (Hct), hemoglobin (Hgb), mean arterial blood pressure, rectal core and temporalis muscle temperature (Table 2).

2.2. Dose-dependent reduction in infarct volume

At 24 h after MCAO, all rats developed cerebral infarction. Compared to the sham operation, MCAO led to a significant evolution of infarction, whereby no differences in infarct volume were observed between untreated groups (Fig. 1A) and those pre-treated (Fig. 1B) with vehicle (1% Arabic gum) (284.7±19.9 vs. 276.7±19.8 mm^3). Upon injection of ibudilast into the tail vein at a range of doses 30 min before MCAO, infarct volume was decreased in a dose-dependent manner (Figs. 1C and D). The largest reduction was observed at a dose of 10 mg/kg ibudilast, whereby the infarct volume of the vehicle-treated group was decreased by 52.8%.

2.3. Evaluation of the time window of the efficacy

Significant reduction in infarction occurred upon administration of ibudilast (10 mg/kg, i.v.) 30 min prior to MCAO and at 0, 30 and 60 min after reperfusion (46.8%, 42.1%, 40.6%, and 46.9% reduction, respectively), compared to the non-treated group (Fig. 2). The effect of ibudilast was still evident when administered 2 h later (34.0% reduction), but not at 3 h after reperfusion (14.3% reduction; $P>0.05$).

Table 2 – Physiological parameters.

	Before MCAO		After MCAO	
	Vehicle	Ibudilast	Vehicle	Ibudilast
Blood pH	7.35±0.01 (4)*	7.34±0.00 (5)	7.38±0.01 (4)	7.38±0.01 (5)
PCO_2 (mm Hg)	52.68±2.54 (4)	51.04±0.32 (5)	48.48±1.40 (4)	44.30±1.20 (5)
PO_2 (mm Hg)	52.95±1.27 (4)	49.62±2.12 (5)	57.95±3.28 (4)	55.00±1.82 (5)
Glucose (mg/dL)	117.50±1.04 (4)	116.00±6.89 (5)	180.0±13.58 (4)	171.6±9.50 (5)
Hematocrit (%PCV)	44.25±0.25 (4)	44.20±0.73 (5)	44.75±0.48 (4)	44.20±0.37 (5)
Hemoglobin (g/dL)	15.23±0.14 (4)	15.04±0.26 (5)	15.23±0.14 (4)	15.24±0.11 (5)
Rectal core temperature (°C)	37.07±0.10 (13)	36.89±0.10 (15)	37.13±0.13 (13)	37.19±0.11 (15)
Temporalis muscle temperature (°C)	37.10±0.14 (7)	37.17±0.20 (6)	37.06±0.16 (7)	37.05±0.22 (6)
Mean arterial blood pressure (mm Hg)	n.d.#	n.d.	90.8±2.08 (5)	88.5±1.84 (6)

* Mean±SEM (animal number).
# Not determined.