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Research Report

Neuroprotection by cilostazol, a phosphodiesterase type 3 inhibitor, against apoptotic white matter changes in rat after chronic cerebral hypoperfusion

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TNF-α, tumor necrosis factor-α
GFAP, glial fibrillary acidic protein

ABSTRACT

In the present study, we elucidated effect of cilostazol to prevent the occurrence of vacuolation and rarefaction of the white matter in association with apoptosis induced by bilateral occlusion of common carotid arteries in the male Wistar rats. Rats orally received vehicle (DMSO) or 60 mg kg^{-1} day^{-1} (orally) cilostazol for 3, 7, 14 or 30 days. In the vehicle group, increased vacuolation and rarefactions in the white matter were accompanied by extensive activation of both microglial and astroglial cells with suppression of oligodendrocytes in association with increased TNF-α production, caspase-3 immunoreactivity and TUNEL-positive cells in the white matter including optic tract. Post-treatment with cilostazol (60 mg kg^{-1} day^{-1}) strongly suppressed not only elevated activation of astroglia and microglia but also diminished oligodendrocytes following chronic cerebral hypoperfusion. In conclusion, cilostazol (60 mg kg^{-1} day^{-1}, orally) significantly reduced the apoptotic cell death in association with decreased TNF-α production and caspase-3-positive cells in the white matter of rat brains subjected to bilateral occlusion of common carotid arteries, consequently ameliorating vacuoles and rarefaction changes in the white matter.

1. Introduction

A reduction in cerebral blood flow is implicated not only in the vascular dementia, but also in other types of dementia, including Alzheimer's disease (Deutsch and Tweedy, 1987). The white matter lesions are frequently observed in aging, hypertension and cerebrovascular disease and are responsible for cognitive decline and gait disorders in the elderly population (Boone et al., 1992). Demyelination and axonal damage were identified by chronic cerebral ischemia experimentally induced by the permanent occlusion of common carotid artery of rat, and this experimental model has been

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proposed as a model for vascular dementia and cerebrovascular white matter lesions (Ihara et al., 2001; Tanaka et al., 1996; Wakita et al., 1994).

Wakita et al. (1999) reported the suppression of the activated microglia and attenuation of the white matter lesions by using anti-inflammatory drugs, suggestive of importance of inflammatory reaction in provoking the white matter lesion. Microglia and astrocytes are the major sources of TNF-α (Sawada et al., 1989). TNF-α, as a pro-inflammatory cytokine, induces various effects on the pathological conditions, including edema formation after ischemia and demyelination (Taupin et al., 1997). On the other hand, oligodendroglial cell death was reported to occur following bilateral carotid artery occlusion in gerbil (Kurumatani et al., 1998) and transient global ischemia in rat (Petito et al., 1998).

Cilostazol (6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-dihydro-2-(1H)-quinolinone) has been known to increase intracellular cyclic AMP level by blocking its hydrolysis by type III phosphodiesterase (Kimura et al., 1985). Cilostazol was further reported to scavenge the hydroxyl and peroxyl radicals (Kim et al., 2002), and to increase the outward K^+ currents by activating maxi-K channels (Hong et al., 2003). Recently, cilostazol (1–100 μmol/l) concentration-dependently and significantly suppressed not only NAD(P)H oxidase-dependent superoxide production but also TNF-α and interleukin-1β releases with reduction of DNA fragmentation (Shin et al., 2004). Most recently, cilostazol significantly repressed monocyte adhesion to the endothelial cells in association with suppression of cell surface expression of VCAM-1, ICAM-1, E-selectin, and MCP-1, which were significantly antagonized by iberiotoxin, a maxi-K channel blocker (Park et al., 2005).

Given the suppression by cilostazol of the production of superoxide and release of cytokines such as TNF-α in association with suppression of monocyte adhesion, it is likely predictable that long-term treatment with cilostazol may provide a potential strategy for prevention of apoptotic cell death with suppression of TNF-α formation and caspase activation in the white matter lesion induced by chronic cerebral hypoperfusion, consequently reducing the vacuole formation.

Thus, to investigate cilostazol effects to prevent the occurrence of vacuolation and rarefaction of the white matter, we examined whether cilostazol suppresses activation of microglial and astroglial cells with the recover of oligodendrocytes in association with suppression of TNF-α production, caspase-3 immunoreactivity and consequently reduction of TUNEL-positive cells in the white matter lesion induced by bilateral common carotid arteries occlusion in male Wistar rats.

2. Results

2.1. Physiological variables

There was no significant difference in the physiological variables including mean arterial blood pressure, blood pH, $PaCO_2$, PaO_2 and rectal temperature after bilateral common carotid artery occlusion between vehicle and cilostazol-treated groups as summarized in Table 1.

2.2. White matter lesions

Fig. 1 shows representative photomicrographs of the white matter lesion by employing Klüver–Barrera staining. After 14 days of ligation, the severe white matter lesions were observed in the optic tract of the rats subjected to chronic cerebral hypoperfusion (Fig. 1B). Less intense changes were observed in the medial part of the corpus callosum (Fig. 1H) adjoining the internal capsule (Fig. 1E) in comparison with the sham operated rat brain (Figs. 1A, 1D, 1G). In the 60 mg kg^{-1} day^{-1} cilostazol-treated animals, the grading score significantly decreased in the optic tract (Fig. 1C) and corpus callosum (Fig. 1I), while a marginal reduction was observed in the internal capsule (Fig. 1F).

The animals received oral administration of vehicle (Figs. 2A, B, C, D) and 60 mg kg^{-1} day^{-1} of cilostazol (E, F, G, H) for 3, 7, 14 and 30 days. In the vehicle-treated rats subjected to chronic cerebral hypoperfusion for 14 and 30 days (Figs. 2C, D), vacuole formation was significantly increased with marked rarefaction, while at 7 days vacuolation (Fig. 2B) was increasingly manifested but in moderate degree. In rats treated with 60 mg kg^{-1} day^{-1} cilostazol for 14 days, vacuolar changes were significantly reduced (Figs. 2G, 3D). A moderate reduction was observed by 30 mg kg^{-1} day^{-1} cilostazol (Figs. 3C). For further study, we observed immunohistochemical findings, measured TNF-α and caspase-3-positive cells, and identified TUNEL-positive cells in rats treated with 60 mg kg^{-1} day^{-1} cilostazol for 14 days.

2.3. Immunohistochemical findings

Chronic cerebral hypoperfusion caused a large increase in the number of glial fibrillary acidic protein (GFAP, a marker for astroglia) immunoreactivity to 384.4 ± 40.4 per 0.25 mm^2 (sham, 87.5 ± 29.8 per 0.25 mm^2, $P < 0.001$) in the optic tract (Fig. 4B). Following cilostazol treatment, the number of GFAP-immuno-positive astroglial cells significantly decreased to 210.4 ± 32.5 per 0.25 mm^2 ($P < 0.01$) in the optic tract of the cilostazol (60 mg kg^{-1} day^{-1} for 14 days)-treated rats (Fig. 4C).

Table 1 – Physiological variables at 14 days after bilateral common carotid artery occlusion

	MABP (mm Hg)	pH	pCO_2	pO_2	Rectal temperature (°C)
Vehicle	103 ± 10	7.45 ± 0.01	36.97 ± 0.7	86.00 ± 1.9	37.5 ± 0.4
Cilostazol	109 ± 13	7.40 ± 0.04	35.50 ± 0.2	84.57 ± 0.7	37.5 ± 0.2

MABP, mean arterial blood pressure. Each value represents mean ± SEM. ($n = 6$). During the observation periods, all variables were in the normal ranges and did not change significantly.