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BRAIN RESEARCH

Research Report

Effects of gemfibrozil on outcome after permanent middle cerebral artery occlusion in mice

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ABSTRACT

Fibrates are lipid lowering drugs and found as ligands for peroxisome proliferator-activated receptors (PPARs). A clinical study has shown that one type of fibrate gemfibrozil reduces stroke incidence in men. However, it remains unknown whether gemfibrozil improves outcome after stroke. We hypothesized that prophylactic administration of gemfibrozil improves outcome after ischemic stroke. In this study, we measured the impact of gemfibrozil in two permanent middle cerebral artery occlusion (MCAO) models in young adult male mice on normal diet. First, we tested gemfibrozil in a filamentous MCAO model. Pretreatment with gemfibrozil (30 mg/kg) for 7 days moderately but significantly reduced infarct size at 24 h after MCAO. A higher dose (120 mg/kg) did not attenuate infarct size. Rather, it tended to increase brain swelling. Second, we tested in a distal MCAO model. Gemfibrozil (30 mg/kg) for 7 days before and after stroke significantly attenuated cortical lesion size at 7 days after MCAO. Cortical blood flow measured by laser speckle imaging was improved by gemfibrozil in the ischemic hemisphere. In non-stroke animals gemfibrozil also altered gene expression levels of PPARs in both the aorta and brain in organ specific manners; however, endothelial nitric oxide synthase (eNOS) was not significantly affected. These findings suggested the possibility that the observed infarct reductions and cortical blood flow improvements in ischemic brains were not through eNOS-mediated mechanisms. Further investigations may be meritorious to examine whether prophylactic usage of gemfibrozil against stroke is beneficial.

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcriptional factors belonging to the so-called nuclear receptor family (Chinetti et al., 2000). Several classes of PPARs have been identified, namely PPARα, PPARβ, and PPARγ. PPARγ consists of two isoforms, PPARγ1 and PPARγ2. All of them are involved in regulation of lipid or glucose metabolism. PPAR agonists have been demonstrated to induce pleiotropic effects in different organs, such as vessels, heart, and kidney (Chinetti et al., 2000). For example, beyond the metabolic effects, the activation of PPARα and PPARγ has been shown to exert multiple cellular functions, such as anti-inflammatory and anti-oxidative effects (Staels et al., 1998; Escher and Wahli, 2000; Von Knethen and Brune, 2001). These pleiotropic

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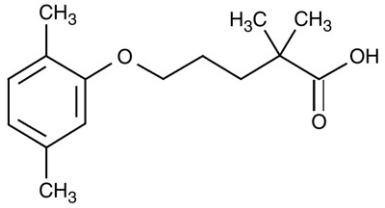

Fig. 1 – Chemical structure of gemfibrozil.

effects may be attributable to the mode of tissue protection by PPAR agonists against ischemia in several different organs (Yue et al., 2001; Sivarajah et al., 2002; Wayman et al., 2002; Cuzzocrea et al., 2004).

PPAR agonists have been shown to protect the nervous system under several disease conditions. For example, experimental studies have shown that fibrates attenuate brain tissue injury associated with inflammation (for review, Bordet et al., 2006; Heneka and Landreth, 2007). Lovett-Racke et al. (2004) demonstrated the effect of fenofibrate and gemfibrozil on clinical signs of experimental autoimmune encephalomyelitis in mice. Regarding stroke, two independent studies demonstrated that fenofibrate attenuated brain infarct formation in mice subjected to focal cerebral ischemia (Deplanque et al., 2003; Inoue et al., 2003). Inoue et al. (2003) also showed that another PPARα agonist Wy-14643 protected the brain against permanent focal cerebral ischemia in mice. Moreover, PPARγ agonists and PPARβ agonists have been shown to protect the brain against focal ischemia (Shimazu et al., 2005; Zhao et al., 2005; Iwashita et al., 2007). Thus, PPARs seem to be potential therapeutic molecular targets to predispose the brain to be more resistant against ischemic stroke.

Gemfibrozil (Fig. 1), one of PPAR agonist, has been prescribed in hyperlipidemia patients to lower the level of triglycerides. Gemfibrozil has a higher affinity to PPARα compared to PPARγ and PPARβ. This drug has been shown to decrease the incidence of stroke in men who previously had coronary heart disease (the Veterans Affairs HDL Intervention Trial (VA-HIT) (Bloomfield Rubins et al., 2001). However, its effects on stroke outcome have not been studied. Since other PPARα agonists attenuated infarct size after permanent and transient focal cerebral ischemia in mice (Deplanque et al., 2003; Inoue et al., 2003), we hypothesized that gemfibrozil protects brain against ischemic stroke. Therefore, we investigated the effects of gemfibrozil on brain infarct formation using two mouse models of ischemic stroke, permanent middle cerebral artery occlusion (MCAO). We tested 7 days pretreatment with gemfibrozil at 30 or 120 mg/kg bw. The two doses were selected based on the following three facts: (1) the VA-HIT study (Bloomfield Rubins et al., 2001) administered gemfibrozil at 1200 mg daily; (2) the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study (Keech et al., 2005) administered fenofibrate at 200 mg daily; and (3) in our previous study, fenofibrate (30 mg/kg bw daily) was effective to significantly attenuate infarct size in young adult mice after permanent MCAO (Inoue et al., 2003).

2. Results

2.1. *Effects on stroke outcome after filamentous MCAO*

Body temperature measured before and after filamentous MCAO did not show significant difference among the vehicle and gemfibrozil groups (Table 1). The difference of regional cerebral blood flow (rCBF) at the ischemic core area after MCAO was not statistically significant among the three groups (Table 1).

At 24 h after MCAO, the infarct tissue was visible as the area of pallor which was sharply demarcated from the adjacent tissue (Fig. 2A). Gemfibrozil had a dose dependent effect in affecting the infarct volume ($p<0.05$ by ANOVA). When the dosage of gemfibrozil was 30 mg/kg bw, the infarct volume was significantly reduced by 20% compared with vehicle (Fig. 2B). The dose at 120 mg/kg did not reduce the infarct volume compared with vehicle.

Brain swelling in the vehicle group at 24 h after MCAO was 6.3±4.9% of the contralateral hemispheric volume. Gemfibrozil showed a tendency to aggravate brain swelling but the tendency was not statistically significant (Fig. 2C). As the tendency of brain swelling suggested that gemfibrozil may worsen long-term outcome, we tested gemfibrozil in another model by distal MCAO combined with ipsilateral permanent carotid artery ligation, which allowed longer-term outcome measurements.

2.2. *Effects on stroke outcome after distal MCAO*

Based on the results from filamentous MCAO, we tested only 30 mg/kg in this distal MCAO model. In addition to the pre-stroke treatment, we continued treating until day 7 after MCAO. All animals survived for 7 days after distal MCAO in both gemfibrozil and vehicle groups. Body weight changes across MCAO did not significantly differ between the groups (Fig. 3). On day 7, the infarct tissue was found mainly in the parietal cortex with minor involvements in the temporal and occipital cortices (Figs. 4A, B). Many animals showed cortical atrophy. Gemfibrozil significantly reduced infarct size by 42% compared with vehicle ($p<0.001$ by Mann–Whitney) (Fig. 4C).

We measured cortical surface blood flow by laser speckle imaging through intact skull. In the vehicle treated animals, at 10 min after MCAO, CBF within the hemispheric cortical area between the linea temporalis and midline was reduced to 60% of the basal value (Figs. 5A, B). When measurement was limited to the MCA territory, the CBF was reduced to 40% of the basal (Fig. 5C). At 1 day after MCAO, these numbers were

Table 1 – Regional cerebral blood flow (rCBF) and body temperature (BT).

Gemfibrozil	(mg/kg)	0 ($n=9$)	30 ($n=8$)	120 ($n=5$)
% rCBF	Before	100	100	100
	After	12.1±2.7	13.6±3.3	15.8±3.0
BT (°C)	Before	36.9±0.2	37.0±0.2	37.0±0.2
	After	37.0±0.2	37.0±0.2	37.0±0.2