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Research Report

# Anti-inflammatory effects of OBA-09, a salicylic acid/pyruvate ester, in the postischemic brain

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ABSTRACT

Cerebral ischemia leads to brain injury via a complex series of pathophysiological events, and therefore, multi-drug treatments or multi-targeting drug treatments provide attractive options with respect to limiting brain damage. Previously, we reported that a novel multi-functional compound oxopropanoyloxy benzoic acid (OBA-09, a simple ester of pyruvate and salicylic acid) affords robust neuroprotective effects in the postischemic rat brain. OBA-09 exhibited anti-oxidative effects that appeared to be executed by OBA-09 and by the salicylic acid afforded by hydrolysis. Here, we report the anti-inflammatory effects of OBA-09. Microglial activation observed at 2 days post-middle cerebral artery occlusion (MCAO, 90 min) and at 1 day after a LPS injection (0.5 mg/kg, intravenously) in the brains of Sprague-Dawley rats were markedly suppressed by the administration of OBA-09 (10 mg/kg). Inductions of proinflammatory markers (TNF-α, IL-1β, iNOS, and COX-2) were also suppressed by OBA-09 in both the LPS and MCAO models. Moreover, the anti-inflammatory effect of OBA-09 was accompanied by the suppression of infarct formation in the postischemic brain, but appeared to be independent of neuroprotection in LPS-treated rats. The inductions of proinflammatory markers were also inhibited by OBA-09 in LPS-treated BV2 cells (a microglia cell line) and in LPS-treated-primary neutrophils, possibly due to the suppression of NF-κB activity. Interestingly, the anti-inflammatory effect of OBA-09 was greater than that of equivalent co-treatment with pyruvate and salicylic acid. Together these results indicate that OBA-09 is a potent multi-modal neuroprotectant in the postischemic brain, and that its anti-inflammatory effect contributes to its neuroprotective function.



Abbreviations: OBA-09, oxopropanoyloxy benzoic acid; COX-2, cyclooxygenase-2; iNOS, inducible no synthase; Iba-1, ionized calcium binding adaptor molecule 1; IL-1B, interleukin-1beta; Mac-2, macrophage galactose-specific lectin-2; MCAO, middle cerebral artery occlusion; MMP-9, matrix metalloproteinase-9; MPO-1, myeloperoxidase-1; NeuN, neuronal nuclei; NF-kB, nuclear factor kappa-light-chain-enhancer of activated b cells; TNF-α, tumor necrosis factor-alpha; ROS, reactive oxygen species

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## 1. Introduction

In the postischemic brain, neuronal injury progresses through a complex series of pathophysiological events, which include glutamate-induced excitotoxicity, oxidative stress, inflammation, and apoptosis (Dirnagl et al., 1999). In the ischemic core, acute and massive neuronal death occurs due to excitotoxicity and $Zn^{2+}$ toxicity (Lipton, 1999), whereas inflammation and apoptosis are responsible for delayed neuronal injury in the region surrounding the ischemic core (Graham and Chen, 2001). This delayed neuronal death may occur over a few hours to days after primary ischemic events and insidiously expands brain damage (Kirino, 2000).

Immediately after arterial occlusion, activated endothelial cells and platelets rapidly produce adhesion molecules and proinflammatory signals, which cause platelet aggregation and leukocytes adhesion and consequently lead to microvascular occlusion (Zhang et al., 1998; Amantea et al., 2009). In addition, microglia and macrophages localized in brain or derived from blood exacerbate cerebral ischemic damage in the early stage (Tomita and Fukuuchi, 1996). In particular, microglial cells, the resident immune cells of the CNS, have been reported to be activated by ischemic damage (Morioka et al., 1993) and to exacerbate inflammation by producing ROS and cytokines (Colton and Gilbert, 1987; Sawada et al., 1989). In addition to microglia, blood-derived neutrophils also play a crucial role in inflammatory response in the postischemic brain (Hallenbeck et al., 1986; Bednar et al., 1991).

In a previous report, we showed that ethyl pyruvate and aspirin (acetylsalicylic acid) co-treatment provides synergistic neuroprotection in the postischemic brain (Kim et al., 2010). Ethyl pyruvate has been reported to suppress infarct formation potently in the postischemic brain (Yu et al., 2005; Kim et al., 2005). Furthermore, ethyl pyruvate is converted to pyruvate, a well-known $H_2O_2$ scavenger (Desagher et al., 1997) and ameliorator of zinc toxicity (Sheline et al., 2000). On the other hand, aspirin has been used for primary and secondary stroke prevention due to its anti-platelet (Smith and Willis, 1971; Antithrombotic Trialists' Collaboration, 2002), anti-inflammatory (Roth et al., 1975; DeWitt et al., 1990), and anti-excitotoxic effects (De Cristóbal et al., 2001; Castillo et al., 2003). It has also been reported that pyruvate and salicylic acid, the main metabolites of ethyl pyruvate and aspirin, respectively, exert beneficial effects in stroke, that is, pyruvate acts as an anti-oxidant and anti-inflammatory molecule (Dobsak et al., 1999; Wang et al., 2009) and salicylic acid inhibits the NF-κB pathway (Kopp and Ghosh, 1994) and the transcription of COX-2 (Xu et al., 1999).

Based on the above-mentioned observations, Kim et al. (2011) developed the novel multi-functional compound oxopropanoyloxy benzoic acid (OBA-09). OBA-09 is a simple ester of pyruvate and salicylic acid and was originally chosen because it would allow the release of these components slowly in vivo via ester hydrolysis (Kim et al., 2011). Furthermore, in a rat middle cerebral artery occlusion (MCAO) model, OBA-09 was found to reduce infarct volumes significantly in parallel with ameliorations of neurological and behavioral deficits. OBA-09 was found to have anti-oxidative effects, which were attributed in part to its scavenging of hydroxyl radicals, and this effect appeared to be due to the ester (OBA-09) and salicylic acid (Kim et al., 2011). In addition, OBA-09 was found to mitigate $Zn^{2+}$-toxicity and NMDA-induced excitotoxicity (Kim et al., 2011).

In the present study, we investigated the anti-inflammatory effects of OBA-09 in postischemic and LPS-treated rat brains. We found that OBA-09 exerted anti-inflammatory effects, and that these were in part responsible for the robust neuroprotective effect of OBA-09. It was also found that the anti-inflammatory effects of OBA-09 were significantly greater than those obtained by co-treating equivalent quantities of salicylic acid and pyruvate.

## 2. Results

### 2.1. OBA-09 suppressed infarct formation in the 90 min-MCAO animal model

In a previous report, we showed that OBA-09 has a robust neuroprotective effect in the postischemic brain (MCAO, 60 min) (Kim et al., 2011). Here, we confirmed the neuroprotective effect of OBA-09 in a 90 min-MCAO model. The administration of 10, 20, or 30 mg/kg of OBA-09 (i.v.) at 30 min post-MCAO reduced infarct volumes to 63.2±6.0%, 46.6±7.4%, and 34.5±2.8% ($n=5$, $p<0.01$) of that in the MCAO-control group (Fig. 1A). In addition, infarct volume was reduced to 59.7±9.8%, at 48 h after MCAO when 30 mg/kg of OBA-09 was administered as late as 6 h post-MCAO (Fig. 1B). Mean modified neurological severity scores (mNSS) were 4.0±1.0 ($n=12$, $p<0.01$) and 8.7±0.7 ($n=12$, $p<0.01$) at 48 h post-MCAO, when 30 mg/kg of OBA-09 was administered at 30 min or 6 h post-MCAO, respectively, which was significantly lower than 12.3±0.6 ($n=12$, $p<0.01$) in the MCAO-control group (Fig. 1C). Physiological variables, including rectal temperature, pH, $PaO_2$, and $PaCO_2$, did not differ significantly between OBA-09-treated and untreated animals (Table 1). However, blood glucose levels were marginally higher in OBA-09-treated animals (Table 1), which might be due to the transient glucose increase induced by pyruvate (gluconeogenesis) released by the hydrolysis of OBA-09 (Kim et al., 2011).

### 2.2. OBA-09 suppressed the inflammatory process in the postischemic brain

To investigate the anti-inflammatory effects of OBA-09 in the postischemic brain, OBA-09 (10 or 20 mg/kg, i.v.) was administered at 30 min post-MCAO, and microglia and neutrophil activations were examined by immunostaining with antibodies against Iba-1, a marker of cells of myeloid origin (Imai et al., 1996), Mac2, a marker of activated resident microglia/macrophages (Lalancette-Hébert et al, 2007), and MPO-1, a marker of neutrophil (Rausch et al., 1978) at 48 h post-MCAO. In sham-operated animals, Iba-$1^+$ cells were detected throughout brains, including contralateral hemispheres, and exhibited a ramified morphology (Fig. 2B). However, in penumbra of MCAO-control animals (the region indicated