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BRAIN RESEARCH

Research Report

# Influence of age on the response to fibroblast growth factor-2 treatment in a rat model of stroke

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*Abbreviations:*
FGF-2, basic fibroblast growth factor
CCAs, common carotid arteries
MCAO, middle cerebral artery occlusion
aCSF, artificial cerebrospinal fluid
BrdU, bromodeoxyuridine
TTC, triphenyltetrazolium chloride
DCX, doublecortin
SVZ, subventricular zone

ABSTRACT

Basic fibroblast growth factor (FGF-2) has been reported to protect against ischemic injury in the brains of young adult rodents. However, little is known about whether FGF-2 retains this capability in the aged ischemic brain. Since stroke in human is much more common in older people than among younger adults, to address this question is clinically important. In this study, aged (24-month-old) rats were treated with intracerebroventricular infusion of FGF-2 or vehicle for 3 days, beginning 48 h before (pre-ischemia), 24 h after (early post-ischemia), or 96 h after (late post-ischemia) 60 min of middle cerebral artery occlusion, and were killed 10 days after ischemia. Aged rats given FGF-2 pre-ischemia showed better symmetry of movement and forepaw outstretching, and reduced infarct volumes, compared to rats treated with vehicle, but no significant improvement was found in aged rats given FGF-2 after focal ischemia. In contrast, young adult (3-month-old) rats treated with FGF-2 for 3 days beginning 24 h post-ischemia showed significant neurobehavioral improvement and better histological outcome. In addition, we also found that newborn neurons in the rostral subventricular zone (SVZ) were increased in aged rats treated with FGF-2 prior to ischemia. However, unlike in young adult ischemic rats, only a few of newly generated cells migrated into the damaged region in aged brain after focal ischemia. These findings point to differences in the response of aged versus young adult rats to FGF-2 in cerebral ischemia, and suggest that such differences need to be considered in the development of neuroprotective agents for stroke.



## 1. Introduction

Despite a large number of animal studies with promising neuroprotective agents, no clinically successful strategy for neuroprotection has emerged (Pitkanen, 2003). Whether this discrepancy is due to species differences or differences between the pathophysiology of human stroke and the animal models used is uncertain. Although stroke in humans usually afflicts the elderly (Arnold, 1981; Ramirez-Lassepas, 1998), most experimental studies on stroke have used young adult animals due to their greater availability, lower cost and fewer health problems. This is the case notwithstanding that

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abnormalities in glycolytic flux, lactate production, oxidation and energy production are more pronounced with advancing age, suggesting a reduced ability of the brain to adapt to stress (Hoyer, 1987). For example, ischemic lesion in the hippocampal CA1 region after global ischemia and in cerebral infarct size after focal ischemia increases in severity with increasing age (Arnold, 1981; Hoyer, 1987; Yao et al., 1991). After ischemia, mild memory impairment is observed in aged rats, while changes in some exploratory behaviors are observed in young adult rats (Andersen et al., 1999). These findings suggest that the outcome after cerebral ischemia might be affected significantly by advancing age.

Basic fibroblast growth factor (bFGF or FGF-2) is a polypeptide with potent trophic and protective effects on the brain. FGF-2 has been reported to exert neuroprotection against a wide variety of insults, including ischemic neuronal injury (Wada et al., 2003). A number of studies showed that administration of FGF-2 significantly reduced infarct volume and improved limb placing tests and rotarod fall latency in a model of focal cerebral ischemia in the rat compared to animals treated with vehicle alone (Ay et al., 1999; Bethel et al., 1997; Li and Stephenson, 2002). The mechanisms of enhanced functional recovery may include stimulation of neural sprouting and neural stem/progenitor cells in brain (Berry et al., 2005; Wada et al., 2003). However, little is known about the efficacy of FGF-2 in aged rodents subjected to focal ischemia (Ooboshi et al., 2000).

In this study, we investigated the effect FGF-2, given at different times in relation to the onset of focal cerebral ischemia, in aged compared to young adult rats. We found differences between these two groups in the effective time window for FGF-2 administration and in the neurogenesis response to FGF-2. Because of these differences, models of focal cerebral ischemia that employ aged animals may be more relevant to human stroke, and could have greater predictive value in the search for new modes of treatment (Fig. 1).

## 2. Results

Physiological data including arterial pressure, arterial blood gas and blood glucose were measured in rats treated with or without FGF-2 before and after focal ischemia, and no significant difference was found between groups before MCA occlusion and early reperfusion.

First, we conducted neurobehavioral testing, including tests for symmetry of movement and forepaw outstretching, and found normal results (score=3) in all young adult and aged rats before MCAO, but profound behavioral deficits (score=0) in all after 60 min of MCAO. Thus, none of the animals tested was excluded because of an inadequate degree of cerebral ischemia. Symmetry of limb movement and of forepaw outstretching was monitored daily for 10 days. Aged rats treated with FGF-2 48 h before ischemia (Plan A) showed significant improvement in symmetry of movement and forepaw outstretching, compared to vehicle-treated aged rats ($P<0.05$; two-way ANOVA with the Bonferroni correction). Significant improvement of symmetry of movement ($P<0.05$), but not forepaw outstretching ($P>0.05$), was observed when aged rats were given FGF-2 beginning 24 h (Plan B) after ischemia. No significant improvement was observed when aged rats were given FGF-2 beginning 96 h (Plan C) after ischemia ($P>0.05$; two-way ANOVA with the Bonferroni correction). In contrast, young adult rats did show improvement when treated according to Plan B ($P<0.05$).

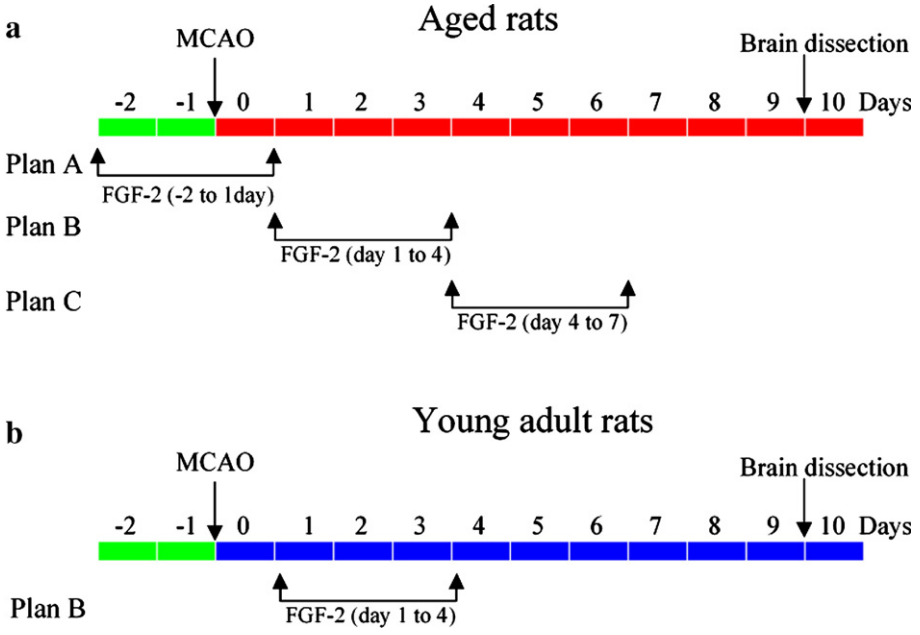


**Fig. 1 – Scheme for administration of FGF-2 before and after MCAO in rats. MCAO was induced in aged rats on day 0. FGF-2 or aCSF was administered by the intraventricular route for 3 days, beginning 2 days before (Plan A), or 1 day (Plan B) or 4 days after (Plan C) MCAO (a). Young adult rats were treated beginning 1 day after MCAO (Plan B) (b). Rats were killed 10 days after MCAO and brains analyzed by cresyl violet staining, BrdU immunohistochemistry, and cell counting.**