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Research Report

The neuroprotective effects of NMDAR antagonist, ifenprodil and ASIC1a inhibitor, flurbiprofen on post-ischemic cerebral injury☆

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ABSTRACT

Owing to the complex and multifactorial pathology of cerebral stroke, multiple drug therapy had long been advocated by STAIR committee for stroke successful treatment. In this context, we analyzed the effect of Ifenprodil, an NR2b selective NMDAR antagonist and its combination at lower doses with flurbiprofen, a selective ASIC1a inhibitor on rat model of focal cerebral ischemia. We found that the combination produced significant neuroprotective effect as produced by ifenprodil at higher doses, which was evidenced by reduction in infarct volume, neurological deficit and MDA levels. Further, histopathological studies revealed that, the combination not only attenuated the cell damage in striatal regions of ischemic brain, but also significantly inhibited apoptotic cell death, which was more pronounced than monotherapy with ifenprodil or flurbiprofen. Thus, it appears that the combination therapy will be more efficacious in offering neuroprotection on one hand and also lower the risks associated by mono-therapy with ifenprodil at higher doses.

1. Introduction

Cerebral stroke is a major cause for mortality and morbidity worldwide. Despite tremendous research efforts for development of neuroprotectants, the only drug therapy available for stroke is rt-PA, which has a narrow therapeutic window with hemorrhagic complications. Among various reasons cited, a major reason for the failure of neuroprotectants in clinical trials was attributed to research focused on single drug intervention therapies for cerebral ischemia, despite the complex and multifactorial pathology of stroke (Mehta et al, 2007; Doyle et al, 2008).

Brain injury following stroke results from the complex interplay of multiple pathways including excitotoxicity, acidotoxicity, oxidative stress, inflammation and apoptosis. Each of the above pathophysiological processes, though are overlapping but occur at distinct time frames following ischemia (Doyle et al, 2008). Excitotoxicity, the earliest one, results from the over activity of excitatory neurotransmitter glutamate on NMDA receptors, which causes Ca^{2+} ions overload in ischemic brain thus triggering multiple cell death signaling pathways. However, clinical trials in humans to prevent brain injury by the use of NMDA receptor antagonists have been disappointing owing to lack of efficacy and adverse effects (Ikonomidou and Turski, 2002; Birmingham 2002). Lately, NR2b selective antagonists (ifenprodil and traxoprodil) were reported to be significant neuroprotective with lesser side effects as compared to conventional NMDA receptor blockers

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(Gotti et al, 1988). But still, ifenprodil clinical trial for stroke in humans was abandoned due to high risk/benefit ratio arising from adverse cardiovascular consequences at higher doses (Small and Buchan, 1997).

Recently, Xiong and other researchers had reported glutamate independent Ca^{2+} ions overload in ischemic brain by acidosis activated acid sensing ion channels 1a (ASIC1a) (Waldmann et al., 1997; Xiong et al., 2004; Yermolaieva et al., 2004). Further, the ASIC1a inhibition by psalmotoxin1 (PcTX1) proved significantly neuroprotective in mouse model of focal ischemia with time window of 5 h, far beyond achieved by glutamate antagonists (Xiong et al., 2004; Simon, 2006). We have also demonstrated that ASIC1a inhibition with flurbirprofen, a NSAID, afforded significant neuroprotection, with therapeutic time window of upto 4 h in focal cerebral ischemia (Mishra et al., 2010). Interestingly, NR2b containing NMDA receptors were reported to be functionally coupled with ASIC currents, which results in an increased Ca^{2+} influx through ASIC1a following ischemia and aggravates cell death (Gao et al., 2005). It was also found that memantine, a NMDA receptor antagonist, in presence of PcTX1 produced additive neuroprotection with extended therapeutic window (Pignataro et al., 2007).

In the above context, we assumed that combination of NR2b selective NMDA receptors antagonist, at early time point, with ASIC1a channels inhibitor, at later time point of ischemic insult, could provide maximal neuroprotective effects even at lower doses by targeting two distinct pathways. Thus, the present study was focused on analyzing the neuroprotective effect of ifenprodil, a selective NR2b NMDA receptor antagonist, alone and in combination with flurbiprofen, an ASIC1a inhibitor, in rat model of focal cerebral ischemia. The neuroprotection was evaluated on various parameters based on reduction in neurological deficit scores, cerebral infarct volume and level of oxidative stress marker, i.e., MDA. Further, effect of the treatment was also analyzed on necrotic and apoptotic cell death by H&E staining and TUNEL assay, respectively (Fig. 1).

2. Results

2.1. *Effect of ifenprodil pre-ischemic treatment on neurological deficit and infarct volume*

At the outset, significant optimal neuroprotective dose for ifenprodil was determined based on its efficacy in reducing neurological deficit and cerebral infarct volume of rats subjected to 1/24 h of ischemia/reperfusion (I/R) injury. Neurological deficit was analyzed on the basis of neurological scores obtained after 24 h of reperfusion in all experimental groups. A dose-related significant improvement in neurological deficit score was found in ifenprodil pretreated rats compared to vehicle treated group (Fig. 2a). Further, the mean infarct volume in vehicle treated control rats was 219.1±15.6 mm^3, whereas rats pretreated with ifenprodil with 5, 10, 20 mg/kg, i.p. was found to be 180.1±17.2, 130.2±18.7, 95.1±15.3 mm^3, respectively, indicating significant reduction in infarct volume (Fig. 2b and c).

2.2. *Effect of ifenprodil and flurbiprofen combination treatment on neurological deficit and infarct volume*

Since ifenprodil 10 mg/kg i.p. was found to significantly improve the neurological deficit and cerebral infarct volume in ischemic rats, it was chosen as an effective dose for combination therapy with flurbiprofen. As shown in Fig. 3a combination of ifenprodil (10 mg/kg) and flurbiprofen (10 mg/kg) led to significant ($P<0.01$) improvement in neurological deficit. Further, increasing the dose of ifenprodil to 20 mg/kg with flurbiprofen at 10 mg/kg did not seem to enhance the observed effects (Fig. 3a). The cerebral infarct volume in ifenprodil, flurbiprofen and combination of ifenprodil (10 mg/kg) and flurbiprofen (10 mg/kg) treated rats were 130.2±19.2, 116.7±17.8, 80.74±17.5 mm^3, respectively. Thus combination effectively reduced the neurological deficit and infarct volume, which was more significant than ifenprodil or flurbiprofen alone treatment (Fig. 3b and c). The ifenprodil treatment was also evaluated in post-ischemic conditions and it was found that ifenprodil post 30 min MCAo treatment produced effects similar to ifenprodil 30 min pre-ischemic treatment, and the same was also reflected in combination study with flurbiprofen (Fig. 4a, b and c).

2.3. *Effect of ifenprodil alone and in combination with flurbiprofen on brain MDA levels*

The MDA levels were measured post 24 h of ischemic injury in cortex and striatal rat brain regions. A significant ($P<0.01$) increase in brain MDA levels was observed in both brain regions of ischemic rats as compared to sham rats. The MDA levels in vehicle treated rats were found to be 10.6±0.61 and 12.7±0.63 nmol/mg protein in cortex and striatum, respectively. In flurbiprofen (10 mg/kg, i.p.) treated rats MDA levels were found to be 6.56±0.51 and 7.11±0.51 nmol/mg protein, in

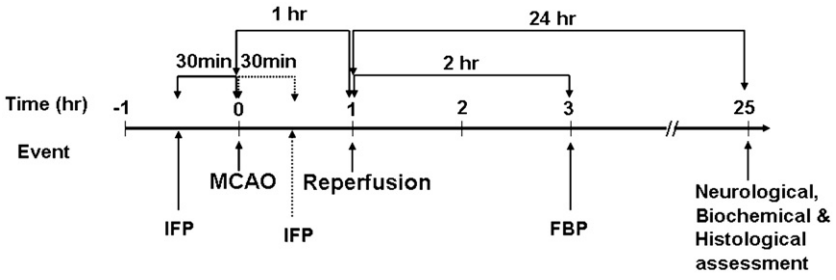

Fig. 1 – Schematic presentation of the study design for assessment of neuroprotective efficacy of ifenprodil (IFP) and its combination with flurbiprofen (FBP) in rat MCAo model; ifenprodil pre MCAo efficacy study (→), ifenprodil post MCAo efficacy study (⇢).