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BRAIN RESEARCH

Research Report

Neuroprotective properties of the non-peptidyl radical scavenger IAC in rats following transient focal cerebral ischemia

Antti Nurmi[a], *Taina-Kaisa Miettinen*[a], *Jukka Puoliväli*[a], *Raimo Pussinen*[a], *Antonio Soleti*[b], *Karim Bagate*[c], *Francesca Riccardino*[b], *Robert I. Grundy*[a], *Juha Yrjänheikki*[a], *Donatella Canistro*[d], *Moreno Paolini*[d,*]

[a]*Cerebricon Ltd, Finland*
[b]*Medestea Research, Italy*
[c]*Forenap Pharma, France*
[d]*Alma Mater Studiorum, University of Bologna, Italy*

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ABSTRACT

Experimental evidence suggests that reactive free radicals are generated during brain ischemia. We investigated the effect of a novel brain penetrant, low molecular weight, non-peptidyl carbon, oxygen- and nitrogen-centered radical scavenger, IAC, on infarct volume and sensory-motor performance in a rat transient middle cerebral artery occlusion model (tMCAO). Rats received 90 min tMCAO and treated with *i.p.* or *i.v.* injections of vehicle or IAC following tMCAO. Sensory-motor performance was evaluated by neuroscore tests (NS). Cerebral infarct volume was evaluated at 72 h after tMCAO. Rats treated with IAC *i.p.* (1 or 6 h after the onset of tMCAO) or *i.v.* (1 h after the onset of tMCAO) showed significant improvement in NS during the 3 or 21 day follow-up period when compared to vehicle treated rats. Cerebral infarct volumes were significantly decreased compared to vehicle in rats receiving IAC *i.p.* 1 h or 6 h after occlusion, ~30.5% decrease compared to vehicle, or *i.v.* 1 h after the onset of tMCAO, 48.6% decrease compared to vehicle. These results demonstrate that IAC has neuroprotective properties with a wide therapeutic window following tMCAO in rats. IAC could therefore be a candidate for the treatment of stroke.

1. Introduction

Stroke is a sudden loss of brain function stemming from interference with blood supply to the central nervous system. It is the third most common cause of death in North America and Europe and the leading medical cause of acquired adult disability (Lees et al., 2000). Rapid intervention after the onset of stroke can limit neurological damage and improve patient recovery of functioning. At present the only pharmacological treatment for acute ischemic stroke is the use of thrombolytic agents such as alteplase. However, the brief time window (3 h) together with the required neuroimaging and the training

* *Corresponding author*. Department of Pharmacology, Alma Mater Studiorum, University of Bologna, Via Irnerio 48, 40126, Bologna, Italy. Fax: +39 0512096331.
E-mail address: labpaolini.farmacologia@unibo.it (M. Paolini).

Abbreviations: tMCAO, Transient Middle Cerebral Artery Occlusion; NS, Neuroscore; IAC, bis(1-hydroxy-2,2,6,6-tetramethyl-4-piperidinyl)-decandioate; CCA, Common carotid artery; ECA, External carotid artery; ICA, Internal carotid artery; *i.p.*, Intraperitoneally; *i.v.*, Intravenously

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necessary for the use of this drug, have seriously limited its application to less than 5% of patients. There is therefore a clear need to develop more widely applicable therapies to combat the effects of acute ischemic stroke.

Ischemic damage to brain cells caused by clinical stroke is reported to involve a complex cascade of events that involves a number of phenomena including excitotoxicity, inflammation cytokines released by activated microglia and invading neutrophils, and oxidative damage caused by free radicals (Green and Shuaib, 2006). It is this oxidative damage to cells caused by excess production of free radicals on reperfusion that has been the focus of much attention regarding the development of non-peptidyl low molecular weight molecules with anti-oxidant properties. Of them, the free radical scavenging agents (tirilazad, ebselen and edavarone) or radical trapping compounds (NXY-059) have gained much attention over the past 5 years. Most notably the anti-oxidant NXY-059 has been shown to be effective in numerous pre-clinical models of stroke including transient and permanent occlusion of the middle cerebral artery (MCA) in rat (Sydserff et al., 2002), embolic stroke in rabbits (Lapchak et al., 2002) and permanent occlusion of the MCA in primates (Marshall et al., 2001). Having followed the recommendations of the Stroke Therapy Academic Industry Roundtable (STAIR) recommendations for pre-clinical development of new drugs for stroke, NXY-059 entered into clinical studies which were initially successful (Lees et al., 2006). However, although this early success was not mirrored in a large phase III trial and clinical development of NXY-059 has been discontinued (Savitz 2007), the rational for the development of anti-oxidant therapies for the treatment of acute ischemic stroke is still strong.

We have developed an innovative non-peptidyl low-molecular weight radical scavenger bis(1-hydroxy-2,2,6,6-tetramethyl-4-piperidinyl)-decandioate (IAC) (Filosa et al., 2005). A distinct advantage of this anti-oxidant is afforded by its unique activity in reacting rapidly with most (if not all) carbon-, nitrogen- and oxygen-centered radicals of biological interest — including peroxyl, superoxide, and peroxynitrite radicals (Valgimigli et al., 2001). Furthermore, additional insight has come from the physical–chemical properties of IAC that affect its partition properties in cell membranes and intra–extra-cellular compartments. The protonated form administered to a biological system is completely water-soluble and will distribute in the extra cellular compartments, but it is also in equilibrium with the free form, which is highly lipophilic (the calculated logP is 4.01) and will readily cross the cell membrane to allow distribution in any compartment where the production of free radicals can take place (Valgimigli et al., 2001).

In the current study we demonstrate that IAC confers protection against transient middle cerebral artery occlusion (tMCAO) in the rat when administered up to 6 h following occlusion as demonstrated by a reduction in lesion volume and improvement in neurological functions.

2. Results

2.1. Physiological variables

Rectal temperature was monitored and maintained during surgical procedure. No significant differences were found in rectal temperature between vehicle and any IAC treated groups before or immediately after tMCAO. Rats subjected to 90 min tMCAO exhibited substantial loss of body weight during the follow-up period in both experiments. However, in experiment I, rats treated with IAC 10 mg/kg b.w. at 1 h or 90 mg/kg b.w. at 6 h *i.p.* had significantly higher body weights at 24, 48 and 72 h after tMCAO if compared to vehicle group ($p<0.05$; data not shown). In experiment II, body weight loss was transient recovering to pre-MCAO level at day 21 in vehicle treated rats. However, rats treated with IAC 1 mg/kg b.w. at 1 h *i.v.* had significantly higher body weights at days 3, 4, 14, 17 and 21 after tMCAO if compared to vehicle group ($p<0.05$; data not shown). Mortality, with the exclusion of hemorrhagic cases (0 to 1 per group), was similar between vehicle and IACVITA treated groups in both experiments (0–3 cases per group).

2.2. Sensory-motor performance

In both experiments (experiments I and II) all rats had normal neurological status before tMCAO. When tested at 1 (experiment 1) or 2 (experiment 2) hours after 90 min tMCAO, all rats exhibited clear deficits in sensory-motor functions detectable with both scoring systems.

In experiment I, deficits in sensory-motor functions persisted during 72 h follow-up period in vehicle treated rats. In contrast, rats treated with IAC 10 mg/kg b.w. *i.p.* at 1 h (but not 3 or 6 h) after the onset of MCA occlusion had a significantly higher 28-point neuroscore values at 72 h after tMCAO if compared to vehicle treated rats (Fig. 1). No significant changes were seen in seven point neuroscore test in rats treated with IAC 10 mg/kg b.w. *i.p.* if compared to vehicle treated rats. In the same experiment, rats treated with IAC 90 mg/kg b.w. *i.p.* showed no significant increase in 7 or 28-point neuroscore when compared to vehicle treated rats (Fig. 2).

In experiment II, sensory-motor deficits were stable for the first 72 h, after which there was some degree of spontaneous recovery in neuroscores in vehicle treated rats. However, rats treated with IAC 1 mg/kg b.w. *i.v.* at 1 h (but not 3 or 6 h) after the onset of MCA occlusion, showed significantly higher 28-point neuroscores at day 21 after tMCAO if compared to vehicle treated rats ($p<0.05$). In addition, seven point neuroscore test showed significantly higher scores for the rats treated with IAC 1 mg/kg b.w. *i.v.* 1 h after the occlusion at days 14 and 21 if compared to vehicle treated rats ($p<0.05$, Fig. 3).

2.3. Evaluation of brain damage

Brain damage after 90 min middle cerebral artery occlusion was evaluated with histology (experiment I) or with T2-weighed magnetic resonance imaging (T2-MRI) (experiment II). In experiment I, 72 h after tMCAO vehicle treated rats had unilateral infarcts comprising cortical and subcortical regions of the forebrain (total infarct 321.2±13.3 mm^3; cortical infarct 273.0±12.5 mm^3; and subcortical infarct 48.2±1.6 mm^3) as evidenced by TTC-staining and image analysis of coronal brain sections. If compared to the vehicle group, rats treated with IAC 10 mg/kg b.w. *i.p.* starting at 1 h (but not at 3 or 6 h) after the onset of occlusion of MCA had significantly smaller total (220±31.5 mm^3 (−31.4%); $p<0.05$, Fig. 4) and cortical (180±31.5 mm^3 (−33.9%); $p<0.05$, Fig. 4), but not subcortical infarcts