available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Neuroprotective effect of STAZN, a novel azulenyl nitrone antioxidant, in focal cerebral ischemia in rats: Dose–response and therapeutic window

James J. Ley[a], *Ludmila Belayev*[a], *Isabel Saul*[a], *David A. Becker*[b], *Myron D. Ginsberg*[a,*]

[a]*Cerebral Vascular Disease Research Center, Department of Neurology (D4-5) University of Miami Miller School of Medicine PO Box 016960 Miami, Florida 33101, USA*

[b]*Department of Chemistry, Florida International University, Miami, Florida, USA*

ARTICLE INFO

Article history:
Accepted 14 May 2007
Available online 26 May 2007

Keywords:
Stroke
Cerebral ischemia
Reperfusion injury
Free radical
Antioxidant
Neuroprotection

ABSTRACT

Stilbazulenyl nitrone (STAZN) is a potent antioxidant that, in a rat model of transient focal cerebral ischemia, confers significant enduring functional and morphological neuroprotection. This study investigated the influence of dose and time of administration on the neuroprotective effects of STAZN in the intraluminal suture model of middle cerebral artery occlusion (MCAo). *Dose response*: At 2 and 4 h after the onset of MCAo, animals received intravenously either STAZN (low dose = 0.07 mg/kg, $n=8$; medium dose = 0.7 mg/kg, $n=9$; high dose = 3.5 mg/kg, $n=9$), an equivalent volume of vehicle (30% Solutol HS15 and 70% isotonic saline, 0.37 ml/kg, $n=5$) or saline (0.37 ml/kg, $n=5$). Only the medium dose improved scores ($p<0.05$) on a standardized neurobehavioral test at 1, 2 and 3 days after MCAo. Only the medium dose reduced the total infarction (51%, $p=0.014$) compared to controls. These results indicate that STAZN exhibits maximal neuroprotection at the 0.7 mg/kg dose. *Therapeutic window:* STAZN (0.6 mg/kg) dissolved in dimethylsulfoxide was given intraperitoneally at 2 and 4 h ($n=11$), 3 and 5 h ($n=10$), 4 and 6 h ($n=10$) or 5 and 7 h ($n=7$) after the onset of MCAo. Additional doses were given at 24 and 48 h. Vehicle (dimethylsulfoxide, 2.0 ml/kg, $n=6$) was administered at 3, 5, 24 and 48 h. STAZN treatment initiated at 2 or 3 h after the onset of MCAo improved neurological scores ($p<0.001$) and reduced total infarction (42.2%, $p<0.05$) compared to controls.

1. Introduction

An abundance of evidence has established that free radicals and the oxidative stress that they engender contribute to ischemia-induced injury (Fiskum et al., 2004; Crack and Taylor, 2005). This fact has provided the rationale for the testing of various antioxidant therapeutics in animal models of cerebral ischemia (Margaill et al., 2005; Weinberger, 2006). We have

* *Corresponding author*. Fax: +1 305 243 9727.
E-mail address: mginsberg@med.miami.edu (M.D. Ginsberg).

Abbreviations: NXY-059, disodium 4-[(tert-butylimino)methyl] benzene-1,3-disulfonate *N*-oxide; STAZN, stilbazulenyl nitrone; MCA, middle cerebral artery; DMSO, dimethyl sulfoxide; MCAo, middle cerebral artery occlusion; FAM, 40% formaldehyde, glacial acetic acid and absolute methanol, 1:1:8 by volume; PBN, α-phenyl-*N*-tert-butyl nitrone; AZN, azulenyl nitrone

0006-8993/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2007.05.028

previously demonstrated the marked, enduring neuroprotective effects of a novel antioxidant free-radical scavenger, stilbazulenyl nitrone (STAZN), in transient focal cerebral ischemia (Ginsberg et al., 2003; Ley et al., 2005). The successful translation of antioxidant therapy from animal models to the clinic, however, is not always straightforward (Committee, 2000; Green and Ashwood, 2005). For example, although free radicals and oxidative stress are implicated in most human diseases, a recent meta-analysis of antioxidant supplements, such as vitamin E, vitamin A and β-carotene, found that antioxidant therapy did not confer benefit but rather increased all-cause mortality in a variety of pathologies including neurological, cardiovascular, ocular, renal, endocrinological, gastrointestinal and dermatological diseases (Bjelakovic et al., 2007). Thus, in order to translate the potential of antioxidants to reduce free-radical-mediated damage into clinically significant neuroprotection, a more detailed understanding of the subtleties of antioxidant therapy may be required (Sena et al., 2007). Here we report on the effects of dose and time of administration of STAZN on its neuroprotective efficacy in a rat model of transient focal cerebral ischemia produced by intraluminal occlusion of the middle cerebral artery (MCAo) for 2 h, followed by recirculation (Belayev et al., 1996). Neurobehavioral score was assessed sequentially, and quantitative histopathology was performed at 3 days.

2. Results

2.1. Dose–response series

In this series, rats received 2-h MCAo and were treated with STAZN or vehicle i.v. at 2 h and 4 h after onset of ischemia. STAZN dosing-groups were either 0.07, 0.7 or 3.5 mg/kg.

2.1.1. Physiological variables

These are shown in Table 1. Physiological variables were generally similar in the four treatment groups at all times studied. Exceptions, however, were cranial and rectal temperature measurements at 2 h after onset of MCAo, which were higher in the pooled controls than in STAZN-treated rats (Table 1, $p<0.05$). This was not the case prior to MCAo or at subsequent times during the 3-day survival period (Table 1).

2.1.2. Neurological score

Prior to MCAo, the total neuroscore was zero in every rat. When re-tested at ~105 min of MCAo, each rat of the entire series showed a neuroscore of 11, indicating a severe neurological deficit (Fig. 1). Neuroscores in control rats treated with saline or with Solutol vehicle did not differ; hence, these groups were pooled for analysis. Neuroscores during MCAo and following treatment are shown in Fig. 1. As early as 3.75 h

Table 1 – Physiological variables—dose–response series

	Pooled controls ($n=10$)	STAZN (0.07 mg/kg) ($n=8$)	STAZN (0.7 mg/kg) ($n=8$)	STAZN (3.5 mg/kg) ($n=9$)
Before MCAo (15 min)				
Cranial temperature (°C)	36.9±0.2	36.9±0.2	36.9±0.2	36.7±0.3
Rectal temperature (°C)	36.7±0.6	36.8±0.5	36.7±0.3	36.7±0.4
Arterial pH	7.44±0.03	7.45±0.03	7.49±0.13	7.45±0.02
PaO_2, mm Hg	129±24	102±12	119±33	113±11
$PaCO_2$, mm Hg	38.1±2.0	37.9±1.5	37.2±2.3	38.6±3.0
MABP, mm Hg	115±10	110±10	108±10	113±9
Plasma glucose, mg/dL	141±14	137±14	146±26	137±21
Body weight	316±18	306±16	309±14	307±12
During MCAo (15 min)				
Cranial temperature (°C)	36.8±0.3	36.9±0.3	36.9±0.2	36.9±0.3
Rectal temperature (°C)	37.0±0.5	37.0±0.3	36.9±0.3	36.9±0.2
Arterial pH	7.43±0.03	7.42±0.05	7.43±0.02	7.47±0.03
PaO_2, mm Hg	128±19	108±9	121±18	125±14
$PaCO_2$, mm Hg	40.0±2.3	39.9±2.2	37.8±1.6	37.9±1.6
MABP, mm Hg	126±13	128±11	121±14	132±11
Plasma glucose, mg/dL	155±15	149±16	176±14	157±22
After MCAo (2 h)				
Cranial temperature (°C)	38.2±0.8	37.2±0.4*	37.1±0.6*	37.2±0.8*
Rectal temperature (°C)	37.9±0.5	37.1±0.6*	37.5±0.8*	37.3±0.8*
MABP, mm Hg	103±15	104±12	102±12	98±6
During 3-day survival				
Rectal temperature (°C)—1 day	37.6±0.6	37.6±0.5	37.5±0.8	37.6±0.4
Rectal temperature (°C)—2 days	37.5±0.5	37.1±0.7	37.3±1.0	37.2±0.5
Rectal temperature (°C)—3 days	37.2±0.9	37.1±0.9	36.9±2.0	37.2±0.6

Values are mean±SD.
* Different from vehicle group ($p<0.05$, one-way ANOVA followed by Holm–Sidak test).