Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Neurodegeneration after transient brain ischemia in aged mice: Beneficial effects of bilobalide

*Tina M. Schwarzkopf, Konrad A. Koch, Jochen Klein**

Department of Pharmacology, College of Pharmacy, Goethe University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany

ARTICLE INFO

Article history:
Accepted 3 July 2013
Available online 11 July 2013

Keywords:
Ageing
Microdialysis
Middle cerebral artery occlusion
Mitochondrial respiration
Mitochondrial swelling
Stroke

ABSTRACT

Bilobalide, an active constituent of *Ginkgo biloba*, has neuroprotective properties in experimental stroke models, but nearly all published studies were carried out in young animals. As ischemic strokes in humans are much more frequent in old age, we investigated bilobalide's effects in aged mice (age 18–22 month) using a model of transient ischemia induced by occlusion of the middle cerebral artery (MCAO) for 60 min. When bilobalide was administered locally into the striatum via microdialysis, a significant reduction of infarct size by almost 70% was observed. Concomitantly, the extensive, twelve-fold increase of extracellular glutamate which was observed in untreated animals was strongly reduced during the infusion of bilobalide. Glucose levels, in contrast, were not affected by bilobalide. In further experiments, bilobalide was given as an intraperitoneal injection (10/mg/kg) 1 h before MCAO onset. ATP levels (measured in brain homogenates) were significantly reduced by transient MCAO but pretreatment with bilobalide prevented this loss. In *ex vivo* experiments with isolated mitochondria from aged mice, we found that the activity of the mitochondrial respiratory chain was only slightly impaired after 60 min of ischemia, and bilobalide showed no benefit in this experiment. However, aged mitochondria proved to be very sensitive to calcium-induced swelling which was significantly increased after ischemia. In this assay, pretreatment with bilobalide lowered the extent of swelling nearly to control levels. In behavioural tests, pretreatment of aged mice with bilobalide significantly improved the outcome in the Rotarod and the Corner test. In conclusion, aged mice show some differences in their response to transient ischemia when compared with young mice. Bilobalide has prominent neuroprotective properties in mice of all ages.

1. Introduction

Extracts of *Ginkgo biloba* are widely used for the treatment of neurodegenerative diseases such as Alzheimer's disease (Christen and Maixent, 2002; Birks and Grimley, 2009). Several studies have also shown beneficial effects of Ginkgo extracts for brain ischemia (Ahlemeyer and Krieglstein, 2003). Bilobalide, a sesquiterpene lactone that constitutes 2.9–3.2% of the Ginkgo extract EGb761, has shown promising neuroprotective properties in experimental stroke models (DeFeudis, 2002;

Abbreviations: ETS, electron transfer system; MCAO, middle cerebral artery occlusion; mPT, mitochondrial permeability transition pore; OXPHOS, oxidative phosphorylation

**Corresponding author*. Fax: +49 69 798 29277.
E-mail address: klein@em.uni-frankfurt.de (J. Klein).

0006-8993/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2013.07.003

Mdzinarishvili et al., 2007). It is well tolerated in humans and may be useful for prevention of ischemic damage in patients with high risk for stroke (Lang et al., 2011). However, while surveying the literature, we noticed that most published studies about bilobalide were carried out in young animals. As the risk of stroke increases with age (O'Donnell et al., 2010), the present study focuses entirely on bilobalide's effects in aged mice.

The mechanism of action of bilobalide remains poorly defined. According to the "mitochondrial free radical theory of aging" (Harman, 1956), mitochondrial damage may be responsible for the formation of oxygen radicals and age-associated neurodegeneration (Lenaz et al., 2002). Accordingly, mitochondrial respiratory chain dysfunction was suggested to be causally related to the well-known susceptibility of aged individuals to stroke-related brain damage (Moran et al., 2012). Limited data indicate that bilobalide may interfere with mitochondrial energy metabolism. For instance, we have shown that breakdown of membrane phospholipids is prevented by bilobalide, possibly by restoration of the cellular energy status (Klein et al., 1997; Weichel et al., 1999). Preservation of complex I function was reported in ischemic endothelial cells and in neurons (Janssens et al., 2000; Chandrasekaran et al., 2002), and stabilization of mitochondrial function was found in cells treated with Ginkgo extracts (Abdel-Kader et al., 2007). Very recently, we have observed that bilobalide reduces glutamate release from ischemic brain, an effect that may be explained by preservation of cellular energy (Lang et al., 2011). In the present study, we investigated mitochondrial function in aged mice treated with bilobalide. To our knowledge, this is the first study of bilobalide and its neuroprotective effects in aged animals.

2. Results

2.1. *Local infusion of bilobalide: effect on stroke area*

Neuroprotective effects of bilobalide in aged mice were determined after infusion of drug (10 μM) into the striatum (core region of ischemic damage). MCAO for 60 min caused widespread cell death as indicated by TTC staining of brain slices 24 h past stroke (Fig. 1). Ischemic slices (Fig. 1A, middle panel) showed a white necrotic area in the left hemisphere. Bilobalide-treated mice had reduced necrotic areas, and slices showed a pink-reddish staining (Fig. 1A, lower panel), resembling sham-operated mice (Fig. 1A, upper panel). Infarct areas (Fig. 1B) were strongly reduced (by 70%; $p<0.01$) in the presence of bilobalide.

2.2. *Extracellular levels of glucose and glutamate*

Fig. 2 displays extracellular concentrations of glucose and glutamate, measured by microdialysis in mouse striatum before, during and after 60 min of ischemia. At onset of MCAO, glucose levels dropped immediately to less than 10% of basal levels (from 0.21±0.05 mM to detection limit, 0.02 mM). Glutamate concentrations rose extensively to more than 1200% of baseline levels (from 1.6±0.8 μM to 19.9±9.9 μM). Glucose and glutamate levels recovered rapidly

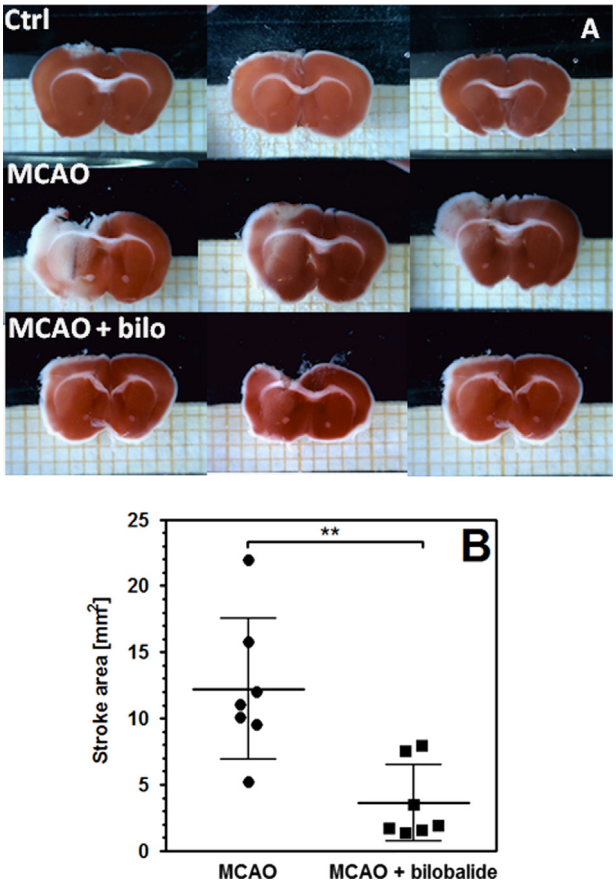

Fig. 1 – (A) Infarct area 24 h after transient middle cerebral artery occlusion (tMCAO, 60 min). Brain slices were stained with 2,3,5-triphenyl-tetrazolium chloride (TTC). Healthy tissue is colored red, ischemic tissue remains white. Upper panel: control animals. Middle panel: mice with transient MCAO. Lower panel: mice with transient MCAO that were treated locally with bilobalide (10 μM in aCSF) starting 1 h before ischemia. (B) Stroke area in mm² was calculated from slices as shown in (A) using Image J software. Data is expressed as means ±S.D. of N=7 experiments. **, $p<0.01$ (t-test, GraphPad Prism).

when transient MCAO was terminated (Fig. 2). Blood flow during reperfusion as measured by laser Doppler flowmetry revealed a recovery to about 50% of pre-ischemic values during reperfusion (data not illustrated). Importantly, in mice treated locally with 10 μM bilobalide through the dialysis probe, glucose levels dropped as in untreated mice, indicating that ischemia was complete; however, the neurotoxic rise of glutamate was significantly attenuated and reached only 400% of baseline level ($p<0.01$ vs. controls).

2.3. *Systemic administration of bilobalide: ATP levels*

ATP levels were measured to determine the energy state of the brain after 60 min of ischemia (Fig. 3). No significant differences were observed when old mice (121±8 μM cytosolic ATP) were compared with young mice (142±14 μM cytosolic ATP, N=6 each) (not illustrated). In old mice, 60 min ischemia caused a significant reduction in ATP content to 58%