European Journal of Pharmacology 666 (2011) 105–110

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and Analgesia

Therapeutic time window for treatment of focal cerebral ischemia reperfusion injury with XQ-1h in rats

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ARTICLE INFO

Article history:
Received 15 December 2010
Received in revised form 11 May 2011
Accepted 17 May 2011
Available online 1 June 2011

Keywords:
XQ-1h
Therapeutic time window
Focal cerebral ischemia reperfusion injury
Middle cerebral artery occlusion (MCAO)

ABSTRACT

Pervious experimental studies have shown that XQ-1h has beneficial neuroprotective effect in the cerebral ischemia reperfusion injury. However, the therapeutic time window for treatment of focal cerebral ischemia reperfusion injury with XQ-1h is not clear. Under chloral hydrate anesthesia, transient focal cerebral ischemia was induced in rats by 2 h of middle cerebral artery occlusion (MCAO), followed by 24 h of reperfusion. Saline as vehicle or XQ-1h at the doses of 31.2, 15.6 and 7.8 mg/kg *i.v.* was administered at 0.5, 1, 2, 3 h after induction of ischemia. Subsequently, 24 h after MCAO brain edema, infarct volume, neurological deficits and cerebral blood flow were evaluated. Administrations of XQ-1h at the doses of 31.2 mg/kg at 0.5, 1, and 2 h after reperfusion of MCAO significantly reduced infarct rate (%) by 75.6% (5.2 ± 1.7), 66.2% (7.2 ± 1.9), and 47.9% (11.1 ± 1.2), respectively. XQ-1h (31.2 mg/kg) treatment, 0.5, 1, and 2 h after reperfusion produced significant improvement in neurological score compared to vehicle-treated group ($P<0.01$). Administrations of XQ-1h at the doses of 31.2 mg/kg and 15.6 mg/kg at 0.5, 1, and 2 h after reperfusion of MCAO significantly increased cerebral blood flow (mv) by 16.9 ± 1.9, 11.7 ± 1.3, 9.5 ± 1.0, respectively ($P<0.01$). In conclusion the therapeutic time window of XQ-1h for cerebral ischemia reperfusion injury is within 2 h. Interestingly, we also discovered that the therapeutic time window of XQ-1h is deeply related with the activity of scavenging oxidative stress products. Further studies need to be conducted more drug combination therapy programs in order to assess the potential clinical application of XQ-1h.

1. Introduction

Stroke is one of the most frequent causes of death and disability worldwide, and has significant clinical and socioeconomic impact. The direct pathological consequence of the stroke is the cerebral ischemic-reperfusion injury a complex interplay of multiple pathways, which includes: depletion of adenosine-triphosphate (ATP), excitotoxic glutamate efflux, ionic imbalance, loss of metabolic function with increased acidosis, oxidative stress, and activation of inflammatory processes (Kristian et al., 2008). Ultimately, it involves the destruction and/or dysfunction of brain cells, for which a very few available drugs have proved to be partially effective.

Over the last decade, substantial scientific evidence has accumulated to suggest that ginkgolide-B, a major constituent of *Ginkgo biloba* extract, is a Platelet Activating Factor (PAF) highly selective and competitive receptor antagonist, which is exert beneficial effects in animal models of acute neurodegeneration (Ahlemeyer and Krieglstein, 2003; Bate et al., 2004). PAF is an important modifiable factor for cerebral ischemic-reperfusion injury, which regulates the Nitric Oxide Synthase (NOS) and deal with the inflammatory response of the leukocytes (Park et al., 1999).

Previous studies from our laboratory have shown that Ginkgolide B at dose of 16 and 8 mg/kg produced significant reduction in infarct volume, edema volume and neurological deficits when treatment was initiated within 4 h after the initiation of focal cerebral ischemia by MCAO, i.e. 2 h after reperfusion. (Weirong and Yan, 2010).

XQ-1h is a novel ginkgolide B derivative; its structure is shown in Fig. 1. From Fig. 1 we can see that the structural difference between them is that XQ-1h has a dimethylamino-ethoxy group combining with methane sulfonic acid. The possible mechanism of the protective effect of XQ-1h on the blood–brain barrier is that XQ-1h antagonizes the PAF receptor and thus inhibits PAF-induced calcium overload and up-regulation of iNOS (Yan and Weirong, 2009).

The previous study from our laboratory has shown that XQ-1h at doses of 15.6 and 7.8 mg/kg produced neuroprotective effects in pretreatment of cerebral ischemic-reperfusion injury in vivo (data not shown), but there has not yet been any detailed investigation of the therapeutic time window associated with the use of XQ-1h in the focal stroke model. Therefore it is very important to evaluate the therapeutic time window and its possible mechanisms by which XQ-1h exhibit potential for the treatment for stroke.

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doi:10.1016/j.ejphar.2011.05.048

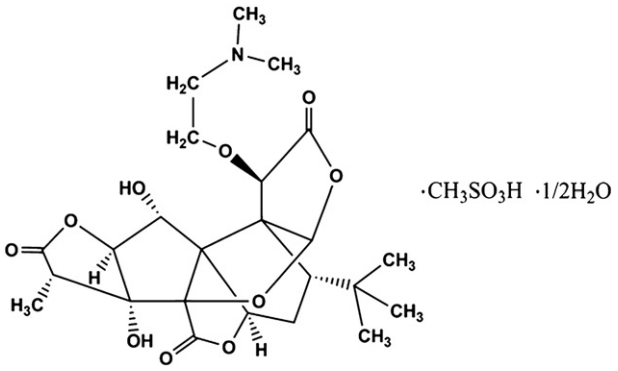

Fig. 1. Chemical structure of XQ-1h.

In this study, we delineated the window of opportunity for treatment of focal cerebral ischemic damage using XQ-1h after middle cerebral artery occlusion (MCAO). Infarct volume, water content, cerebral blood flow (CBF), and neurologic deficit scores were used to evaluate the efficacy of XQ-1h administered at different times after MCAO. We also study the production of oxidative stress in homogenates from the perifocal brains.

2. Materials and methods

2.1. Chemicals and reagents

XQ-1h was kindly provided by Jiangsu Kefeiping Pharmaceutical Company Limited. Ozagrel was bought from Haerbing Sanlian Pharmaceutical Company Limited. Superoxide dismutase (SOD) kit, malondialdehyde (MDA) kit and Na^+/K^+ ATPase kit were obtained from Nanjing Jiancheng Bioengineering Institute. All other reagents used were of analytical grade and commercially available.

2.2. Animals

Male Wistar rats weighing 250–300 g were obtained from Zhejiang Laboratory Animals Center, Zhejiang Academy of Medical Science (Hangzhou, China). Rats were maintained in a clean room (Animal Center for Pharmaceutical Research, China Pharmaceutical University, Nanjing, China) at a temperature between 20 and 23 °C, with a 12 h light–dark cycle and a relative humidity of 50%. Rats were housed in metabolic cages under the supply of filtered pathogen-free air with access to food and water ad libitum. The experimental protocols used in this study were approved by our ethics committees for animal experiment.

2.3. Grouping

Three hundred and sixty healthy Wistar male rats were randomly divided into 24 groups (n = 15) : 0.5 h sham-operation group, 0.5 h vehicle group, 0.5 h XQ-1h 31.2 mg/kg, 0.5 h XQ-1h 15.6 mg/kg, 0.5 h XQ-1h 7.8 mg/kg and 0.5 h Ozagrel 12 mg/kg group after reperfusion; 1 h sham-operation group, 1 h vehicle group, 1 h XQ-1h 31.2 mg/kg, 1 h XQ-1h 15.6 mg/kg, 1 h XQ-1h 7.8 mg/kg and 1 h Ozagrel 12 mg/kg group after reperfusion; 2 h sham-operation group, 2 h vehicle group, 2 h XQ-1h 31.2 mg/kg, 2 h XQ-1h 15.6 mg/kg, 2 h XQ-1h 7.8 mg/kg and 2 h Ozagrel 12 mg/kg group after reperfusion; 3 h sham-operation group, 3 h vehicle group, 3 h XQ-1h 31.2 mg/kg, 3 h XQ-1h 15.6 mg/kg, 3 h XQ-1h 7.8 mg/kg and 3 h Ozagrel 12 mg/kg group after reperfusion.

2.3.1. Model assessment (Ulrich, 2009)

Because the success rate of MCAO model is about 60%, the experimental design is calculated at 15 rats in each group to ensure that each group has 8 valid data.

The following exclusion criteria were applied during the experiment. Exclusion criteria:

- Mortality animals;
- No stroke: after MCAO surgery the awaken rats' neurological deficits has been tested. The rat which neurological deficits score (Longa et al., 1989) is 0 points will be excluded;
- Problems during induction of MCAO (excessive bleeding, prolonged operation time ≥15 min, thread placement).

The rats in the different groups were sacrificed 24 h after MCAO operation and the brains were removed respectively.

2.4. Middle cerebral artery occlusion (MCAO)

The right middle cerebral artery (MCA) was occluded using the intraluminal suture technique described by (Longa et al., 1989), with minor modification. Male rats weighing 260–300 g were anesthetized with 300 mg/kg chloral hydrate *i.p.* The right carotid region was exposed through a midline cervical incision. In order to block the origin of the MCA, a monofilament nylon suture (diameter about 0.26 mm) was prepared by rounding its tip by heating and coating with poly-L-lysine (Sigma). The nylon suture was introduced through the right external carotid artery into the internal carotid artery and advanced approximately 18–20 mm intracranially from the common carotid artery bifurcation. Body temperature was maintained within the normal physiological range with a heating lamp during the operation.

2.5. Neurological deficits

2 h after MCAO, the animals were anesthetized with aether and the MCA was reopened by withdrawing the inserted suture. 0.5, 1, 2, and 3 h after reperfusion of MCAO, XQ-1h (31.2, 15.6, 7.8 mg/kg), ozagrel 12 mg/kg and vehicle were administrated *i.v.* respectively via vena caudalis (Fig. 2). Neurological deficits in the vehicle-treated group and XQ-1h-treated group were measured according to the method of Longa et al. (Longa et al., 1989) at 24 h after reperfusion.

- Score 0: no apparent neurological deficits
- Score 1: contra lateral forelimb flexion
- Score 2: decreased resistance to lateral push
- Score 3: spontaneous movement in all directions and contra lateral circling when pulled by tail
- Score 4: did not walk spontaneously and had depressed levels of consciousness.

2.6. Cerebral blood flow (CBF)

CBF was continuously monitored using a laser-Doppler flow meter (AD Instruments, Australia) on the base of the skull at the level of the fronto parietal cortex. Rats were placed in a stereotaxic frame (Kopf)

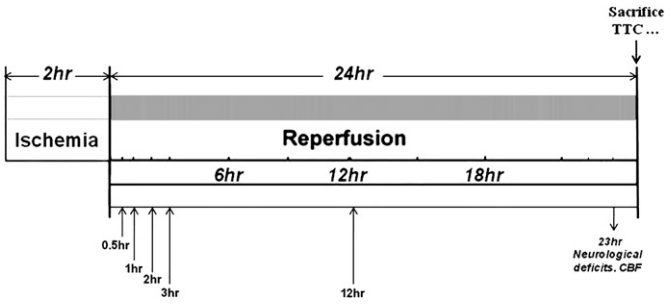

Fig. 2. Design for therapeutic time window study of XQ-1h in ischemia and reperfusion injured rats.