Behavioural Brain Research 283 (2015) 61–68

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Cilostazol but not sildenafil prevents memory impairment after chronic cerebral hypoperfusion in middle-aged rats

Jacqueline Godinho, Janaina Nicolau de Oliveira, Emilene Dias Fiuza Ferreira, Gislene Gonçalves D. Zaghi, Cristiano Correia Bacarin, Rúbia Maria Weffort de Oliveira, Humberto Milani*

Department of Pharmacology and Therapeutics, Health Science Center, State University of Maringá, Maringá, Paraná 87020-900, Brazil

HIGHLIGHTS

- Chronic cerebral hypoperfusion (CCH) causes retrograde amnesia in middle-aged rats.
- Cilostazol reversed that retrograde amnesia in the absence of neuronal rescue.
- The antiamnesic effect of cilostazol persisted for 4 weeks after the end of treatment.
- Sildenafil failed to prevent CCH-induced retrograde amnesia, despite neuronal rescue.
- Cilostazol but not sildenafil may be useful to treat memory deficit after CCH.

ARTICLE INFO

Article history:
Received 11 December 2014
Received in revised form 15 January 2015
Accepted 16 January 2015
Available online 24 January 2015

Keywords:
Chronic cerebral hypoperfusion
Middle-aged rat
Memory loss
Cilostazol
Sildenafil
Memory recovery

ABSTRACT

We previously reported that the phosphodiesterase-5 (PDE5) inhibitor sildenafil prevented neurodegeneration but not learning deficits in middle-aged rats that were subjected to the permanent, three-stage, four-vessel occlusion/internal carotid artery (4-VO/ICA) model of chronic cerebral hypoperfusion (CCH). In the present study, we examined whether the PDE3 inhibitor cilostazol alleviates the loss of long-term memory (*i.e.*, retrograde amnesia) caused by CCH. The effect of sildenafil was then compared to cilostazol. Naive rats (12–15 months old) were trained in a non-food-rewarded eight-arm radial maze and subjected to CCH. One week later, retrograde memory was assessed for 5 weeks. Cilostazol (50 mg/kg, p.o.) was administered for 42 days or 15 days, beginning approximately 45 min after the first occlusion stage. Sildenafil (3 mg/kg, p.o.) was similarly administered for 15 days only. Histological examination was performed after behavioral testing. Chronic cerebral hypoperfusion caused persistent retrograde amnesia, which was reversed by cilostazol after both short-term and long-term treatment. This antiamnesic effect of cilostazol was sustained throughout the experiment, even after discontinuing treatment (15-day treatment group). This effect occurred in the absence of neuronal rescue. Sildenafil failed to prevent CCH-induced retrograde amnesia, but it reduced hippocampal cell death. Extending previous findings from this laboratory, we conclude that sildenafil does not afford memory recovery after CCH, despite its neuroprotective effect. In contrast, cilostazol abolished CCH-induced retrograde amnesia, an effect that may not depend on histological neuroprotection. The present data suggest that cilostazol but not sildenafil represents a potential strategy for the treatment of cognitive sequelae associated with CCH.

1. Introduction

Chronic cerebral hypoperfusion (CCH) has been implicated in the genesis of aging-related dementia of vascular origin and/or the Alzheimer's dementia type. As age advances, CCH might emerge as a prodromal feature of dementia, and it is a common denominator in several aging-related comorbidities, including hypertension, coronary artery disease, atherosclerosis of the internal carotid arteries, hypercholesterolemia, and diabetes mellitus, among others [1–3]. Preventing or treating these comorbidities may reduce the prevalence of neurodegenerative diseases and cognitive decline associated with CCH [3,4]. However, the pharmacological control of vascular risk factors by antihypertensive, anticholesterolemic, and antidiabetic drugs has not been

* Corresponding author at: Departamento de Farmácia e Farmacologia, Centro de Ciências da Saúde, Universidade Estadual de Maringá, Av. Colombo, 5790, Maringá, Paraná CEP 87020-900, Brazil. Tel.: +55 44 30114814; fax: +55 44 30114999.
E-mail address: hmilani@uem.br (H. Milani).

http://dx.doi.org/10.1016/j.bbr.2015.01.026
0166-4328/

convincingly shown to be associated with a reduction of cognitive decline or dementia in the elderly [5]. Despite the importance of reducing the impact of vascular risk factors in the genesis and evolution of aging-related dementia, one unresolved issue is whether the progression of brain damage and cognitive impairment triggered by CCH can be mitigated pharmacologically.

We previously reported that the phosphodiesterase-5 (PDE5) inhibitor sildenafil reduced both mortality rate and hippocampal neurodegeneration in young rats that were subjected to the permanent, two-stage, four-vessel occlusion/internal carotid artery (4-VO/ICA) model of CCH [6]. In that study, however, we found that young rats (3–4 months old) were not cognitively impaired after permanent 4-VO/ICA, thus hindering the determination of possible beneficial effects of sildenafil on functional recovery. To reduce the mortality rate that is typically observed with the two-stage 4-VO/ICA model, we are currently using the three-stage 4-VO/ICA model, which consists of occluding the vertebral arteries (VAs) simultaneously, followed by stepwise ligation of each internal carotid artery (ICA) according to the sequence VA → ICA → ICA (*i.e.*, three-stage 4-VO/ICA), with an interstage interval (ISI) of 4 days. Under these conditions, the mortality rate was zero in young rats, but their resilience to learning/memory deficits still persisted, despite the occurrence of hippocampal damage [7]. In contrast, middle-aged rats (12–15 months old) that were subjected to permanent, three-stage 4-VO/ICA developed not only brain damage but also learning impairment in the radial maze task, and the mortality rate reached 25%, suggesting that aging is an important factor for the development of cognitive impairment after permanent, stepwise 4-VO/ICA [7].

We then used middle-aged rats to further examine whether sildenafil can attenuate learning deficits after permanent 4-VO/ICA [8]. In that study, sildenafil treatment covered the period of three-stage 4-VO/ICA implantation, and learning performance was assessed more than 3 months after 4-VO/ICA. Extending our previous data [7], sildenafil afforded neurohistological protection not only in the hippocampus but also in the cerebral cortex. It failed to attenuate, however, the learning deficit caused by CCH [8]. To explain the failure of sildenafil to prevent learning deficits after 4-VO/ICA, we speculated that distinct PDE types may play differential roles in the mediation of functional recovery by PDE inhibitors following CCH, and the effects of inhibitors of different PDE types should be investigated [8]. For example, the PDE3 inhibitor cilostazol was found to alleviate learning/memory deficits caused by permanent, bilateral ligation of the common carotid arteries (2-VO model of CCH) [9]. We also suggested that other treatment regimens or behavioral protocols should be used to further evaluate any eventual benefit of sildenafil against CCH-induced cognitive impairments in middle-aged rats [8]. We found that permanent, three-stage 4-VO/ICA caused robust and persistent retrograde amnesia in middle-aged rats in the aversive radial maze (AvRM) task [10,11].

In the present study, we used a retrograde memory protocol to evaluate whether the PDE3 inhibitor cilostazol and PDE5 inhibitor sildenafil can mitigate persistent retrograde amnesia caused by 4-VO/ICA in middle-aged rats. A sequence of experiments was designed to answer the following questions: (i) Does long-term treatment with cilostazol given throughout the experiment prevent retrograde amnesia caused by CCH? (ii) Is the antiamnesic effect of cilostazol also evident after reducing the treatment duration? (iii) Can the antiamnesic effect of cilostazol be sustained after the discontinuation of treatment? (iv) How do the effects of sildenafil compare with the effects of cilostazol following the same short-term treatment duration?

2. Materials and methods

2.1. Subjects

One hundred forty-four male, middle-aged Wistar rats (inbred strain, 12–15 months old) were acquired from the local vivarium. Ninety-four rats completed the experiments. Of these, 31 were assigned to sham operation (sham group), and 63 were subjected to CCH and assigned to the following treatments: vehicle (veh; $n = 27$), cilostazol for 42 days (Cilos43; $n = 14$), cilostazol for 15 days (cilos15; $n = 13$), and sildenafil for 15 days (sild; $n = 9$). The rats were housed under a controlled temperature ($22 \pm 1\,^{\circ}C$) on a 12 h alternating light/dark cycle (lights on at 7:00 AM). The animals had free access to tap water and a standard commercial chow diet (Nutrilab-CR1; Nuvital Nutrients, Curitiba, PR, Brazil). The experimental procedures were approved by our Ethics Committee on Animal Experimentation (protocol no. 137/2012).

2.2. Surgery

The animals were anesthetized by isoflurane inhalation (Isoflorine, Cristália, SP, Brazil) that was delivered through a mask adapted to the nose. The vaporizer was regulated to a minimal burble flow of 0.5 L/min. Under these conditions, the volume of isoflurane delivered to the animal was further regulated and monitored to maintain the minimal isoflurane concentration required for efficient anesthesia (evaluated by pinching the animal's tail). Permanent 4-VO/ICA or sham surgery was performed gradually in three stages according to the sequence VA → ICA → ICA, with an interstage interval (ISI) of 4 days. For bilateral occlusion of the VAs, the tip of a unipolar electrode was inserted into the alar foramen of the first cervical vertebra and gently rotated until the presence of hemorrhage ensured vessel rupture. The hemorrhage was then immediately staunched by a 3–4 mA electrical current. This procedure ensures complete and irreversible VA occlusion. An incision into the ventral neck exposed the internal carotid arteries, which were carefully dissected from adjacent tissues and permanently ligated using cotton thread. After each occlusion stage, the incision was sutured, and the animal was returned to its home cage until the next surgery. Rectal temperature was monitored with a digital thermometer (Minipa, APPA MT-520, São Paulo, Brazil) using a rectal probe inserted to a depth of approximately 6 cm. Core temperature was controlled only during surgery and maintained at approximately 37.5 °C by a heating blanket. The animals that were assigned to the sham surgery group were subjected to the same surgical procedures as their counterparts but did not receive vessel occlusions.

2.3. Timeline of drug administration relative to three-stage 4-VO/ICA surgery and behavioral testing

Fig. 1 schematically shows the entire experimental protocol. Naive rats were trained for 15 days to learn the radial maze task (see procedural details below) and then assigned to different groups. On the following day, the three-stage 4-VO/ICA surgeries were performed (Days 0, 4, and 8). All of the drug treatments began approximately 45 min after the first occlusion stage (VA occlusion; *i.e.*, when the animals fully recovered from anesthesia). Subsequent doses were administered daily between 2:00 PM and 3:00 PM. In the first experiment, cilostazol (50 mg/kg) or vehicle was administered for 42 days consecutively, covering the entire periods of 4-VO/ICA and the retention memory trials (RMTs). In the second experiment, the duration of cilostazol treatment was reduced to 15 days. According to this protocol, cilostazol administration did not coincide with behavioral testing. Based on the results obtained with cilostazol, a third experiment evaluated the effect of sildenafil