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Laboratory Study

Ramipril protects from free radical induced white matter damage in chronic hypoperfusion in the rat

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Abstract

We investigated whether the angiotensin converting enzyme inhibitor, ramipril, could attenuate white matter lesions caused by chronic hypoperfusion in the rat, and whether suppression of oxidative stress is involved in the resulting neuroprotection. The ramipril treatment group showed significant protection from development of white matter lesions in the optic tract, the anterior commissure, the corpus callosum, the internal capsule and the caudoputamen. The level of malondialdehyde (MDA) and the oxidized glutathione (GSSG)/total glutathione (GSH_t) ratio was also significantly decreased in the ramipril group compared to the vehicle-treated group. These results suggest that ramipril can protect against white matter lesions that result from chronic ischemia due to its effects on free radical scavenging. Further efficacy should be studied in the treatment of cerebrovascular insufficiency states and vascular dementia.

Keywords: Free radical; Scavenger; Ramipril; Chronic cerebral hypoperfusion; Rat

1. Introduction

Cerebrovascular white matter lesions are frequently observed in human cerebrovascular diseases, and are believed to be responsible for cognitive impairment.[1] These lesions are most likely caused by chronic cerebral ischemia,[1] and indeed, can be experimentally induced in the rat brain by permanent occlusion of both common carotid arteries.[2,3] In this model, animals exhibit blood-brain barrier dysfunction, delayed white matter lesions and learning impairment, and it can therefore be used as a model for vascular dementia.[4–7]

Recent experimental and epidemiological data suggest that the angiotensin converting enzyme inhibitor (ACE-I) ramipril can lower the risk of atherosclerotic disease events and death independent of the effect on blood pressure.[8–11] Although there have been many investigations on the protective effects of this ACE-I, the mechanism of action remains poorly understood.

In this study, we investigated whether the ACE-I drug ramipril could attenuate white matter lesions produced by chronic hypoperfusion in the rat, and whether suppression of oxidative stress is involved in the mechanism of neuroprotection.

2. Methods

The present investigation was approved by the Catholic Ethics Committee of the Catholic University of Korea. Sprague–Dawley rats weighing 260–320 g (Samtako, Seoul, Korea) were subjected to global ischemia by permanent occlusion of the common carotid arteries. The rats were housed at a constant temperature of 22 °C and relative humidity of 50% with a 12-h light-dark cycle; they were allowed free access to food and water.

A total of 72 rats were divided into three groups containing 24 rats each. The animals were anesthetized with a

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mixture of 80 mg/kg ketamine hydrochloride and 8 mg/kg xylazine hydrochloride. Both common carotid arteries were dissected and permanently ligated with silk thread as previously described.[3,6,7,12] A sham operation group underwent the same operation without dissection and ligation of both common carotid arteries. The body temperature was maintained during the operation at about 37 °C. In all experiments, ramipril (20 mg/kg per day, donated by Aventis Pharmaceutics, Seoul, Korea) or vehicle was administered intraperitoneally just after the operative procedure and daily thereafter. At 14 days after ligation, the animals were sacrificed for evaluation of white matter damage and for evaluation of possible markers for oxidative stress.

The animals were deeply anesthetized with 18% urethane, perfused transcardially with 0.01 M phosphate-buffered saline (PBS), and then perfused with a fixative containing 4% paraformaldhyde in 0.1 M phosphate buffer (PB, pH 7.4) containing 0.2% glutaraldehyde. The brain was removed rapidly and fixed in 4% paraformaldhyde. Each brain was cut into 30-μm thick sections using a cryostat (Microtome, Stauffenberg, Germany). The sections were stained with Klüver-Barara stains, and white matter lesions were graded in five regions: the optic chiasm, anterior commissure, internal capsule, corpus callosum and caudoputamen. The severity was graded by two independent investigators as normal (grade 0), disarrangement of nerve fibers (grade 1), formation of marked vacuoles (grade 2) and disappearance of myelinated nerve fibers (grade 3) (Fig. 1).[6,7,12] Twelve animals from each group were examined.

The malondialdehyde levels (MDA) and oxidized glutathione (GSSG)/total glutathione (GSH_t) ratio, used as markers for lipid peroxidation, were estimated at 14 days after ligation of the common carotid arteries. The measurements of MDA and $GSSH/GSH_t$ were conducted using a BIOXYTECH LPO-586 assay kit (OxisResearch, Portland, OR, USA) and a glutathione assay kit (Cayman, Ann Arbor, MI, USA). The measurements were performed as described previously.[13] Briefly, for the measurement of MDA, brain homogenate was diluted 10-fold with 20 mM ice-cold Tris buffer (pH 7.4); 0.5 M butylated hydoxytoluene (Sigma-Aldrich, St Louis, MO, USA) per 1 mL homogenate was added to prevent oxidation. The homogenate was centrifuged at 3000 g and 4 °C for 10 min; next 200 μL of the prepared sample was mixed with 650 μL of *N*-methyl-2-phenylindole (OxisResearch) and 150 μL of 37% HCl, both continuously added to the mixture. The reaction proceeded at 45 °C for 60 min, and was then centrifuged at 15 000 g for 10 min. From the supernatant obtained, the concentration of MDA was estimated by reading the absorbance at 586 nm with a spectrometer (Smartspec 3000, Biorad, Hayward, CA, USA). For the estimation of the $GSSG/GSH_t$ ratio, 100 μL of the diluted homogenate was mixed with 100 μL of 10% metaphosphoric acid (Sigma-Aldrich); the mixed sample was centrifuged at 2000 g for 2 min. Then 100 μL of this supernatant was mixed with 1 μL of 1 M 2-vinylpyridine (Sigma-Aldrich). A GSH sample was prepared by the same method as the preparation of the GSSG sample, but with 5 μL of TEAM reagent (4M triethanolamine, Sigma-Aldrich), instead of

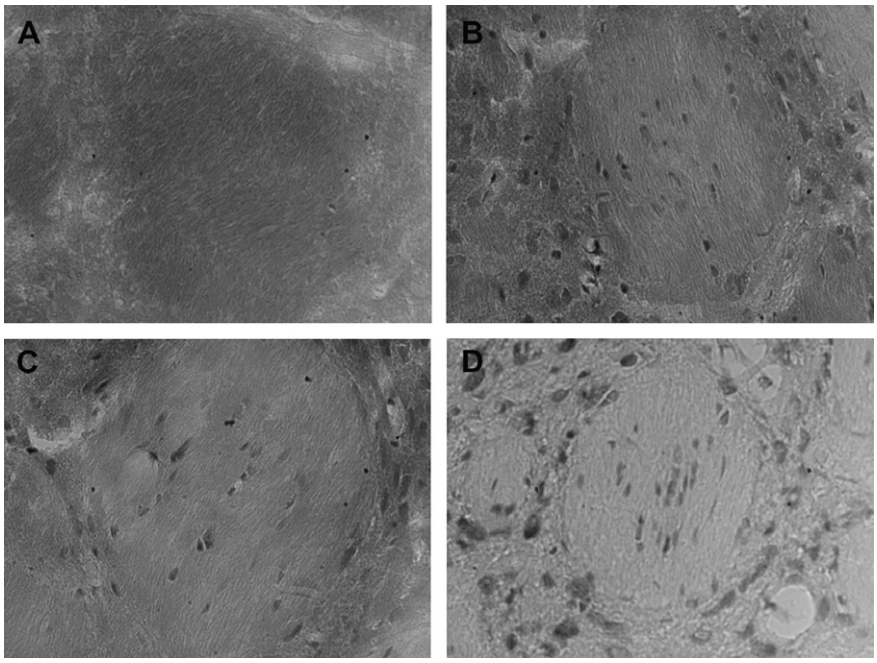

Fig. 1. Representative photomicrographs of Klüver-Barrera staining and the white matter lesion scores. Note the normal findings in A (grade 0), the disarrangement of the nerve fibers in B (grade 1), the formation of marked vacuoles in C (grade 2), and the disappearance of myelinated fibers in D (grade 3).