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BRAIN RESEARCH

Research Report

Marine compound Xyloketal B protects PC12 cells against OGD-induced cell damage

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ABSTRACT

Xyloketal B is a novel marine compound with unique chemical structure isolated from mangrove fungus Xylaria sp. (no. 2508). Recently, we have demonstrated that Xyloketal B is an antioxidant and can protect against oxidized low density lipoprotein (LDL)-induced cell injury. In the present study, we investigated whether Xyloketal B can protect against ischemia-induced cell injury in an *in vitro* oxygen glucose deprivation (OGD) model of ischemic stroke in PC12 cells. We found that Xyloketal B could directly scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and protect PC12 cells against OGD insult. Furthermore, Xyloketal B alleviated OGD-induced mitochondria superoxide, mitochondria fragmentation and GTPase dynamin-related protein 1 (Drp1) overexpression as well as reduction of mitochondrial membrane potential. All together, the present study demonstrates that Xyloketal B protects PC12 cells against OGD-induced cell injury and that the anti-oxidative property and protective action on mitochondria may account for its neuroprotective actions.

1. Introduction

Stroke is one of the leading causes of disability and death in developing countries. Approximately 40% of stroke deaths in developing countries were in China and the majority of survivors are disabled (Reddy and Yusuf, 1998). However, the therapeutic option for stroke is very limited. Although recombinant tissue plasminogen activator has been approved by US Food and Drug Administration as an effective stroke therapy, it is only beneficial to 5–10% of acute ischemic stroke

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Abbreviations: PC12 cells, rat pheochromocytoma; OGD, oxygen-glucose deprivation; ROS, reactive oxygen species; DPPH, 1,1-diphenyl-2-picrylhydrazyl; Drp1, GTPase dynamin-related protein 1; MTT, 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide; LDL, low density lipoprotein

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patients (Brown et al., 2005). Therefore, it is an urgent task to develop effective and safe therapies for acute ischemic stroke.

Ischemic stroke accounts for 80% to 90% of all stroke cases and oxidative stress plays an important role in the pathophysiology of ischemic stroke (Kontos, 2001; Andersen, 2004). Reactive oxygen species (ROS) formed during ischemia and reperfusion contribute to neuronal injury whereas suppression of ROS alleviates ischemia-induced neuronal damage (McCord, 1985; Greenlund et al., 1995). Mitochondria are an important therapeutic target for ischemic stroke. During ischemia, mitochondria generate huge amounts of ROS. On the other hand, excessive ROS also damage mitochondria (Schild and Reiser, 2005; Yamamoto et al., 2007). Consequently, the injured mitochondria in turn produce more ROS, causing a vicious cycle.

Antioxidants have been shown to neutralize hazardous free radicals and prevent cell death in animal models of stroke even when given after stroke. When combined with other therapies, antioxidants can have additional benefits in stroke models. Thus, antioxidants are thought to be a promising treatment for strokes. Despite the fact that lots of antioxidative agents have failed to show significant clinical benefits in the treatment of stroke (Kamat et al., 2008), antioxidant Edaravone has been approved in Japan for clinical use in stroke patients. The efficacy of Edaravone in acute cerebral infarction has been proved in a randomized, placebo-controlled, double-blind clinical trail conducted at multiple centers (Edaravone Acute Infarction Study Group, 2003).

Xyloketal B is a novel marine compound with unique chemical structure isolated from mangrove fungus Xylaria sp. (no. 2508). Recently, we have demonstrated that Xyloketal B protects against oxidized LDL-induced cell injury mainly through its antioxidative activity (Chen et al., 2009). Xyloketal B is quite safe and high concentrations of Xyloketal B do not cause toxic or proliferative effects on cells. Structurally, Xyloketal B has a hydroxyl-phenol radical structure, suggesting that Xyloketal B may have direct free radical scavenging activity. Collectively, Xyloketal B is an attractive candidate for stroke drug development.

In the present study, we first investigated whether Xyloketal can directly scavenge DPPH free radical in a cell free system and then studied the neuroprotective potential of Xyloketal B in an *in vitro* model of ischemic stroke in PC12 cells. Additionally, we also explored the mitochondrial mechanisms underlying protective potential of Xyloketal B.

2. Results

2.1. *Free radical scavenging ability of Xyloketal B*

DPPH assay was used to investigate the free radical scavenging ability of Xyloketal B without the involvement of cells or animals. DPPH is a stable free radical and can accept an electron or hydrogen radical to become a stable diamagnetic molecule. This feature made it a sensitive agent for detecting antioxidant activities (Prasad et al., 2009). DPPH assay showed that Xyloketal B at a concentration range of 12.5 to 800 μM significantly reduced DPPH free radical (Fig. 1B).

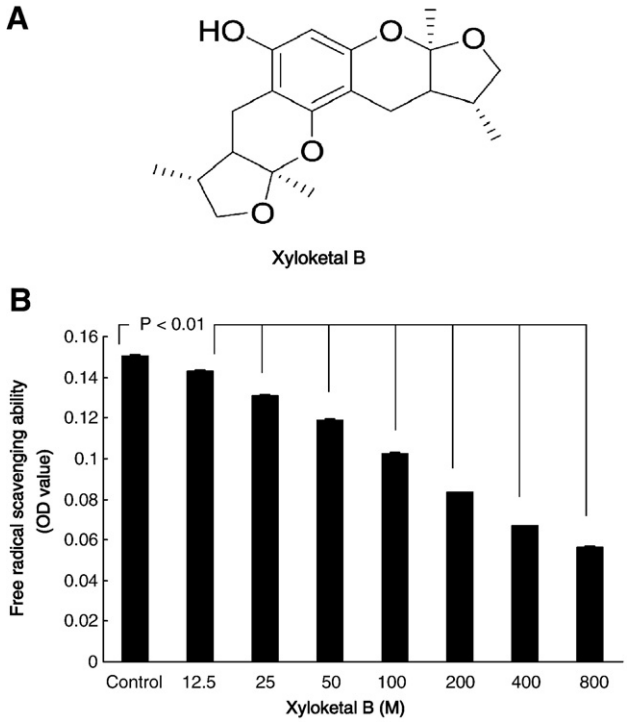

Fig. 1 – Effect of Xyloketal B on DPPH free radical. (A) The structure of Xyloketal B. (B) Xyloketal B significantly inhibited DPPH in a concentration-dependent manner. ($P<0.01$ vs. control, $n=6$ wells for each group, Kruskal–Wallis test). The results were obtained from three independent experiments.

2.2. *Effect of Xyloketal B on OGD-induced injury in PC12 cells*

3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide (MTT) assay and nuclear morphological analysis were used to evaluate the cell viability. To test weather Xyloketal B alone is toxic to PC12 cells, different doses (12.5 to 200 μM) of Xyloketal B were added to normal PC12 cell cultures, respectively. There was no significant difference in cell-viability between Xyloketal B-treated groups and vehicle-treated group (data not shown). We then examined whether Xyloketal B can protect PC12 cells against OGD insult. OGD 4 h plus 24 h reperfusion led to approximately 40% reduction of MTT value. Xyloketal B at a concentration range of 50 to 200 μM significantly prevented OGD-induced decrease of MTT values in a concentration-dependent manner (Fig. 2B). Nuclear morphological analysis yielded similar results. The morphological changes were almost invisible in the normal control cells. In contrast, OGD 4 h plus 24 h reperfusion led to substantial morphological changes such as crenation and condensation in PC12 cells. The extents of cell damage were 2.87±0.62%, 25.63±1.92%, and 6.30±0.86% in control group, OGD group and Xyloketal B+ OGD group, respectively. Pretreatment with Xyloketal B significantly attenuated the number of cells with abnormal nuclear morphology induced by OGD (Fig. 2A). According to the concentration-effect trend of Xyloketal B, Xyloketal B at 100 μM was applied to all subsequent experiments.