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Effects of marine compound xyloketal B on neuroprotection and potential drug development

Sammen Huang[1,2], Wenliang Chen[1,2,3], Jiyan Pang[4,5], Zhong Pei[6], Guan-Lei Wang[5,7*], Hong-Shuo Sun[1,2,3,8*]

[1]*Department of Surgery, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A8*

[2]*Department of Physiology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A8*

[3]*Department of Pharmacology and Toxicology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A8*

[4]*Department of Applied Chemistry, School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou 510275, China*

[5]*Key Laboratory of Functional Molecules from Oceanic Microorganisms (Sun Yat-Sen University), Department of Education of Guangdong Province, 510080, China*

[6]*Department of Neurology, The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510080, China*

[7]*Department of Pharmacology, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou 510080, China*

[8]*Institute of Medical Science, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A8*

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ABSTRACT

Coastal medicine is a promising field for research and drug development, because several natural products isolated from marine organisms have been found to be therapeutically useful in a clinical setting. In comparison to terrestrial products, the merit of marine compounds arises from the diversity in their chemical structure and unique bioactivity. A recently discovered marine compound, xyloketal B, displays neuroprotective and antioxidative effects in *in-vitro* and *in-vivo* experimental models. It elicits these beneficial effects through mitochondrial protection, free radical scavenging, reducing reactive oxygen species production and suppressing apoptotic signaling. In addition, unpublished data from our lab revealed that transient receptor potential melastatin 7 channel activity, implicated in a myriad of neurodegenerative diseases, was inhibited following xyloketal B administration. In this review, the therapeutic effect of xyloketal B will be evaluated based on existing evidence and its potential for drug development will be also discussed.

1. Introduction

Neurodegenerative disease is an umbrella term that describes the gradual decline of cognitive ability marked by the progressive dysfunction or death of neurons. These disorders are sporadic but are generally associated with genetic anomalies that remain dormant in early stages of life and have an onset of disease symptoms during middle to old age. Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, amyotrophic lateral sclerosis and stroke-related dementia are all classified as neurodegenerative disorders that pose a huge health and economic problem. In 2014, the United States provided an estimated 17.7 billion hours of care to patients with dementia which included AD and stroke cases and this amounted to a cost of over $ 220 billion[1]. As the incidence for neurodegenerative diseases is expected to increase in our aging population, we should make continuous efforts to develop new and effective treatments.

Over the past decades, the development of marine compounds has garnered a lot of attention. In fact, the annual discovery of new marine compounds has increased 5-fold since the 1980s[2]. The popularity of marine drugs originates from the unique characteristics of marine organisms that make up over 87% of Earth's life. However, obtaining a large supply of a single marine compound remains a challenging task in marine drug development due to the low yield of product synthesis. Nonetheless, several marine drugs have been proven to be clinically successful in therapeutically

*Corresponding author: Guan-Lei Wang, Key Laboratory of Functional Molecules from Oceanic Microorganisms (Sun Yat-Sen University), Department of Education of Guangdong Province, 510080, China.
E-mail: wangglei@mail.sysu.edu.cn
Hong-Shuo Sun, Department of Surgery, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A8.
E-mail: hss.sun@utoronto.ca
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treating cancer, viral infection, hypertriglyceridemia and neuropathic pain[3]. Marine compounds that act through targeting ion channels are well-established, making it a useful pharmacological tool to study neurodegenerative diseases including AD, PD and ischemic stroke. This is important to coastal medicine research because ion channels make up the second largest class of drug receptors in the market[4].

Xyloketals are a class of marine drugs that were isolated from mangrove fungus strain No. 2508 reported to have unique neuroprotection and antioxidant effects. There are 8 naturally found xyloketals named xyloketals A-H but only xyloketals A, B, C, D and G analogues have been efficiently synthesized[5]. Of these compounds, xyloketal B has shown: 1) to protect endothelial cells against oxidized low-density lipoprotein (oxLDL)-induced cell injury[6], 2) to provide antioxidant activity though heme oxygenase-1 (HO-1) in endothelial cells[7], 3) to reduce oxygen and glucose deprivation (OGD)-induced neuronal cell death *in vitro* in primary cortical cell culture[8], and for the first time 4) to reduce neonatal hypoxic-ischemic (HI) brain injury *in vivo* in mice[8]. Recent studies demonstrated that xyloketal B act by targeting free radicals[6,7,9,10], apoptotic proteins and mitochondrion proteins[6-10]. In addition, unpublished data from our lab revealed that xyloketal B was also an inhibitor of transient receptor potential melastatin 7 (TRPM7) which is a nonselective cation channel strongly implicated in neurodegenerative diseases. In this review, we will discuss the potential therapeutic effects of xyloketal B regarding its neuroprotective and antioxidative actions with evidence from *in-vitro* and *in-vivo* studies. Also, we will also discuss its potential in drug development.

2. Antioxidative properties of xyloketals

Oxidative stress is known to play a key role in a myriad of neurodegenerative diseases including AD, PD and ischemic stroke. The imbalance of reactive species with endogenous antioxidants is believed to intensify disease progression; this sparked the development of antioxidant-based therapies. There has been a growing interest in the antioxidative properties of xyloketals and drug development studies on xyloketal derivatives have been promising. Recently, Liu and colleagues demonstrated that novel xyloketal derivatives protected against H_2O_2-induced injury in an *in-vitro* model of oxidative damage[11]. In addition, the same group has previously synthesized xyloketal derivatives that significantly attenuated reactive oxygen species (ROS) production in zebrafish, increased the survival rates of *Caenorhabditis elegans* and protected against neurodegeneration in C57BL/6 mice in an *in-vivo* model of PD[12]. These neuroprotective effects are likely mediated by the antioxidative properties of xyloketals. Xyloketal B demonstrated in multiple *in-vitro* and *in-vivo* models that it possessed antioxidative effects that act through similar pathways. However, the full mechanistic profile of xyloketal B remains to be elucidated; a bioinformatic study revealed that xyloketal B could potentially regulate over 324 functional proteins and 61 related signaling pathways[13]. Most notably, xyloketal B can regulate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, protein kinase B (Akt), extracellular-signal-regulated kinase (ERK) 1/2 and caspase 1, 3, 7 and 8 proteins known to be involved in redox homeostasis, cell proliferation and survival, and apoptotic signaling pathways, respectively.

3. Xyloketal B protects against oxLDL-induced cell injury and angiotensin-II-induced apoptosis

OxLDL plays a central role in vascular endothelial cell apoptosis that leads to plaque development and thrombosis which are hallmarks of atherosclerosis. Atherosclerosis is one of the leading causes of ischemic stroke and its pathogenesis largely involves the production of excessive reactive oxygen/nitrogen species via activation of NADPH oxidase, reduction of nitric oxide (NO) and decreased expression of the anti-apoptotic protein, B-cell lymphoma 2 (Bcl-2)[6].

Xyloketal B was found to be cytoprotective and it dose-dependently reduced oxLDL-induced damage in human umbilical vein endothelial cells (HUVECs)[6]. Specifically, it attenuated oxLDL-stimulated NADPH oxidase production of ROS[6]. Interestingly, it also decreased the expression of two NADPH oxidase subunits, gp91phox and p47phox[6]. This suggests the xyloketal B may elicit its effects through interaction with these subunits. In addition, xyloketal B promoted the release of NO and inhibited apoptosis by restoring the expression of Bcl-2[6]. NO is an important signaling molecule in the nervous system and maintaining it at physiological levels is important for normal neurological functions such as synaptic transmission. This evidence further supports that xyloketal B has an effect on multiple downstream targets and is likely to act through multiple mechanisms. Li and colleagues discovered that one of those mechanisms involved Akt and ERK 1/2 phosphorylation which induces HO-1, an antioxidant enzyme, in HUVECs and zebrafish[7]. They found that inhibition of HO-l removed the protective effect of xyloketal B against angiotensin-II-induced apoptosis leading to believe that HO-1 induction is a critical mediator of the antioxidative actions of xyloketal B. Secondary to HO-1 induction, activating the Akt and ERK 1/2 pathway affects a cascade of signaling proteins involved in cell proliferation, survival and metabolism suggesting a modulatory role for xyloketal B in these cellular processes[7].

4. Xyloketal B protects against OGD-induced injury

Oxygen and glucose are essential for neuronal survival and a deficiency of either component can lead to cell death and irreversible brain damage. OGD is a commonly used *in-vitro* model of cerebral stroke that is described by the removal of oxygen and glucose from cell culture medium followed by incubation of cells in an anaerobic chamber at various time intervals. Zhao and colleagues found that xyloketal B protected pheochromocytoma 12 cells against OGD-induced injury[9]. Using the 1,1-diphenyl-2-picrylhydrazyl assay, they demonstrated that xyloketal B concentration-dependently scavenged free radicals in a cell free system[9]. This suggests that other than attenuating ROS production from NADPH oxidase, xyloketal B can exert its antioxidative actions through scavenging free radicals. However, it remains unclear whether xyloketal B can scavenge superoxide radicals from the mitochondria that are critically important in OGD-induced injury.

The mitochondrion plays an important role in metabolism and bioenergy production. Several lines of evidence suggest that events that affect mitochondrial morphology and cause mitochondrial dysfunction are early signs of neurodegeneration[14]. It is well understood that OGD-induced injury causes changes in mitochondrial morphology leading to increased release of ROS and mitochondrial fragmentation. Treatment with xyloketal B attenuated mitochondrial fragmentation and resulted in the reduction of GTPase dynamin-related protein 1, a marker of apoptosis and mitochondrion dysfunction[9]. Mounting evidences suggest that the neuroprotective actions of xyloketal B are a result of primarily targeting the mitochondria and its substrates. More studies need to be done to confirm these findings and further investigate the effects of this drug on the mitochondrion.

5. Neuroprotective effects of xyloketal B in MPTP/MPP$^+$ model

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin commonly used to selectively destroy dopaminergic neurons in the substantia nigra and induce PD-like symptoms. While MPTP is not toxic, its active metabolite, 1-methyl-4-phenylpyridinium (MPP$^+$) interferes with oxidative phosphorylation in complex I of the electron transport chain leading to energy depletion, mitochondrial dysfunction, apoptosis and neurodegeneration. In particular, hallmarks of MPP$^+$-induced damage include increased ROS production[15], reduction of antioxidant levels and increased release of apoptosis initiation factor, cytochrome C, from the mitochondria[16,17]. Several studies have reported that reduction of oxidative stress via inhibition or removal of ROS production results in neuroprotection from MPP$^+$-induced toxicity[15,18].

Lu and colleagues demonstrated that xyloketal B provided neuroprotection against MPP$^+$-induced toxicity in pheochromocytoma 12 cells and *Caenorhabditis elegans* in a dose-dependent manner[10]. Similar to the aforementioned approach, xyloketal B significantly reduced MPP$^+$-induced intracellular ROS production at a dose between 50 and 250 μmol/L[10]. Further investigation revealed that the MPP$^+$-mediated reduction in glutathione (GSH) levels[10], the most abundant antioxidant in cells, was restored by xyloketal B administration. However, it is unclear whether xyloketal B directly promoted the synthesis of GSH or prevented the MPP$^+$-mediated loss of GSH. In addition, xyloketal B appears to have a direct protective effect on the mitochondria by attenuating the reduction in mitochondrial membrane potential following administration of MPP$^+$[10]. This suggests that the mitochondrial membrane integrity was maintained and the amount of cytochrome C released into the cytoplasm was reduced.

6. Neuroprotective effect of xyloketal B in neonatal hypoxic ischemic model

Neonatal HI brain injury could lead to neonatal hypoxic-ischemic encephalopathy that is characterized by neurological impairment in children due to a lack of oxygen and blood flow to the brain during the pregnancy, labor or post natal period. Numerous experimental studies have utilized neonatal HI injury in rodent models to mimic the disease[19,20]. The model consists of a unilateral ligation of the common carotid artery followed by a period of hypoxia that leads to decreased blood flow and neuronal death in the ipsilateral hemisphere of the brain. During the injury period, a number of physiological processes are affected in the damaged hemisphere. Most notably, there is calcium overload, increased ROS production, inflammation and pro-apoptotic signaling in the neurons, as well as neurobehavioral deficits in the rodents[21].

Xiao and colleagues demonstrated for the first time the neuroprotective effect of xyloketal B in the mouse neonatal HI brain injury model *in vivo* and in OGD-induced neuronal cell death *in vitro* in mouse primary cortical cell culture[8]. They performed 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) staining, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, neurobehavioral assessments and histological analysis to evaluate brain damage following HI injury. TTC is a redox indicator used to detect cellular respiration that would stain either red or white depending on if the area of the brain was metabolically active or inactive, respectively. The infarct volume, represented by the white stain, was reduced in mice pre-treated with xyloketal B 1 day after HI. It remains unclear if administration of xyloketal B after HI injury would result in the same neuroprotective effect. TUNEL staining is an assay used to detect DNA fragmentation in cells which is an indicator of apoptosis. Xyloketal B was discovered to decrease the number of TUNEL-positive cells in the penumbra area of ipsilateral hemisphere 3 days after HI, possibly by interfering with the apoptotic signaling pathway[8]. Behavior tests were performed 1, 3 and 7 days after HI and included the geotaxis reflex, cliff aversion reaction and grip test used to assess sensorimotor function. The tests demonstrated that xyloketal B improved functional behavioral recovery following HI injury[8]. Whole brains retrieved 7 days after HI showed a smaller injury site in the xyloketal B-treated groups in comparison to the vehicle-treated group[8]. Further histological analysis with Nissl staining using the same brains revealed that mice which displayed poor sensorimotor function in the behavior tests had severe brain damage and this effect was reversed in mice pre-treated with xyloketal B[8]. In their *in-vitro* model, Xiao and colleagues measured OGD-induced neuronal cell death using a membrane impermeant molecule, propidium iodide, which fluoresced when bound to nucleic acids. Fluorescence density analysis revealed that xyloketal B protected against OGD-induced neuronal cell death[8].

At the molecular level, Fura-2 calcium imaging and western blot analysis showed that xyloketal B reduced calcium influx in the neurons, reduced caspase 3 levels and increased Bcl-2 to Bax protein ratio[8]. Calcium homeostasis is essential to precisely control neuronal excitability and a symphony of cellular processes that include modification of synaptic proteins, signal transduction and neuronal gene expression. Failure to regulate calcium levels in the cell can result in activation of caspase 3-mediated apoptotic signaling[22]. Caspase 3 is a mediator of the mitochondria apoptotic signaling pathway and it has been previously reported that caspase 3 loss-of-function mice had delayed events of Bax translocation and cytochrome C release[23]. This suggests that xyloketal B may inhibit the early events of apoptosis by restoring calcium homeostasis. In addition, it also increased Bcl-2 anti-apoptotic protein levels indicating multiple pathways that can elicit its neuroprotective effect[8]. It remains unclear through which mechanism xyloketal B reduces calcium influx but previous studies have shown that xyloketal B has a modest inhibitory effect on L-type calcium channels in rat hippocampal cells[24]. We believe that it is also likely to be associated with TRPM7 channel because unpublished data from our lab found that xyloketal B was a TRPM7 inhibitor and could directly block calcium entry into the cell.

7. Blocking TRPM7 and its therapeutic implications

TRPM7, the seventh member in the melastatin subfamily of TRP channels, is an outward rectifying non-selective cation channel that predominantly permeates calcium and magnesium divalent ions. This channel is ubiquitously expressed in all cell types, and like other TRPM channels, has six transmembrane segments with a pore region between S5-S6. Remarkably, TRPM7 has an enzymatic serine/threonine kinase domain that is believed to be functionally linked to channel activity. In particular, the kinase domain can regulate actomyosin contractility and cell adhesion through a kinase-dependent mechanism which in turn can affect channel opening[25]. TRPM7 is critically important in many physiological functions including magnesium homeostasis[26], cell survival[27] and proliferation[28]. Despite of this, evidence has pointed towards the involvement of TRPM7 in the pathology of neurodegenerative and cardiovascular diseases thus identifying a role for TRPM7 in health and disease[29].

TRPM7 is a novel ion channel that has been implicated in ischemia-mediated neurotoxicity through a non-glutamate mechanism[30]. Interestingly, TRPM7 channel activity is potentiated by oxidative stress which is a hallmark of ischemic stroke. It was found that suppression of TRPM7 with small hairpin RNA in hippocampal CA1

neurons resulted in resilience to neuronal death following global ischemia[31]. In addition, the TRPM7 inhibitor carvacrol reduced brain damage in neonatal HI brain injury[32]. Although not extensively tested, TRPM7 is also implicated in other neurodegenerative diseases such as AD, PD and Huntington's disease due to their link to calcium deregulation in disease pathophysiology[33]. Henceforth, xyloketal B has a therapeutic potential for drug development to treat neurodegenerative diseases due to its ability to inhibit TRPM7 current. However, degree of pharmacological inhibition and biophysical changes is not known and further research should focus on the drug-channel interaction for xyloketal B and TRPM7.

Mounting evidence from both *in-vitro* and *in-vivo* studies has demonstrated the neuroprotective and antioxidative effects of the marine drug xyloketal B. However, our current understanding of the pathways that involve xyloketal B is still at the beginning stage and the full action of xyloketal B remains to be elucidated. Despite of this, the presented preliminary data are convincing and it shows that marine drug xyloketal B is a promising neuroprotective compound for drug development to treat neurodegenerative diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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