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BRAIN RESEARCH

Research Report

Neuroprotective effects of a nanocrystal formulation of $sPLA_2$ inhibitor PX-18 in cerebral ischemia/reperfusion in gerbils

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ABSTRACT

The group IIA secretory phospholipase A2 ($sPLA_2$-IIA) has been studied extensively because of its involvement in inflammatory processes. Up-regulation of this enzyme has been shown in a number of neurodegenerative diseases including cerebral ischemia and Alzheimer's disease. PX-18 is a selective $sPLA_2$ inhibitor effective in reducing tissue damage resulting from myocardial infarction. However, its use as a neuroprotective agent has been hampered due to its low solubility. In this study, we test the possible neuroprotective effects of PX-18 formulated as a suspension of nanocrystals. Transient global cerebral ischemia was induced in gerbils by occlusion of both common carotid arteries for 5 min. Four days after ischemia/reperfusion (I/R), extensive delayed neuronal death, DNA damage, and increases in reactive astrocytes and microglial cells were observed in the hippocampal CA1 region. PX-18 nanocrystals (30 and 60 mg/kg body wt) and vehicle controls were injected i.p. immediately after I/R. PX-18 nanocrystal injection significantly reduced delayed neuronal death, DNA damage, as well as glial cell activation. These findings demonstrated the effective neuroprotection of PX-18 in the form of nanocrystal against I/R-induced neuronal damage. The results also suggest that nanocrystals hold promise as an effective strategy for the delivery of compounds with poor solubility that would otherwise be precluded from preclinical development.

1. Introduction

Phospholipases A_2 (PLA_2) are essential enzymes for maintenance and regulation of cell membrane phospholipids. These enzymes are generally grouped into three broad types, namely, the group IV Ca^{2+}-dependent cytosolic PLA_2, the group VI Ca^{2+}-independent PLA_2, and the small molecular weight secretory $sPLA_2$ (Burke and Dennis, 2009; Masuda et al., 2005;

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Abbreviations: CCA, common carotid arteries; I/R, ischemia/reperfusion; DND, delayed neuronal death; GFAP, glial fibrillary acidic protein; rCBF, regional cerebral blood flow; ROS, reactive oxygen species; DAPI, 4′,6-diamidine-2′-phenylindole; sPLA2-IIA, group IIA secretory phospholipase A2; AA, arachidonic acid; PGD2, prostaglandin D2; PX-18, 2-[N,N-bis(2-oleoyloxyethyl)amine]-1-ethanesulfonic acid

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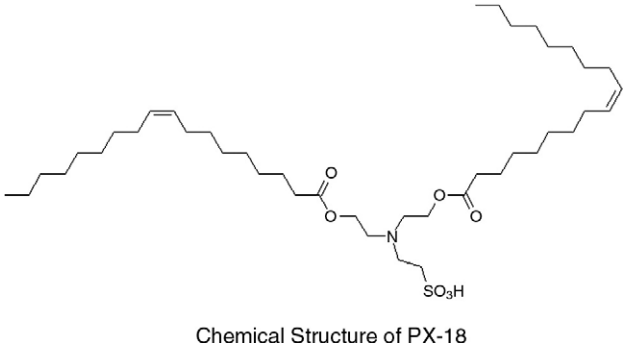

Chemical Structure of PX-18

Fig. 1 – Chemical structure of PX-18.

Murakami and Kudo, 2002; Sun et al., 2004). Among more than 12 isoforms of $sPLA_2$ widely distributed in mammalian cells, much attention has focused on the group IIA $sPLA_2$ ($sPLA_2$-IIA) because of its role in the pathogenesis of many inflammatory diseases (Murakami and Kudo, 2004). In the peripheral system, this enzyme is regarded as a mediator connecting innate and adaptive immunity, and is upregulated in a number of inflammatory diseases including coronary artery diseases, atherosclerosis, sepsis, arthritis, and infection (Camargo et al., 2008; Ibeas et al., 2009; Kimura-Matsumoto et al., 2008; Krijnen et al., 2006; Leitinger et al., 1999; Mallat et al., 2007; Tietge et al., 2005). Although less is known about the role of $sPLA_2$ in the central nervous system, up-regulation of $sPLA_2$-IIA expression and increase in $sPLA_2$-IIA activity has been reported in rat brain after cerebral ischemia (Adibhatla and Hatcher, 2007b; Lauritzen et al., 1994; Smart et al., 2004). A recent study further demonstrated up-regulation of this enzyme in reactive astrocytes in Alzheimer's disease brains as compared with age-matched controls (Moses et al., 2006). Besides fatty acid release, $sPLA_2$-IIA has been shown to alter membrane functions including stimulation of voltage-sensitive Ca^{2+} channels in neurons, potentiation of glutamate excitotoxicity, and induction of neuronal apoptosis (DeCoster et al., 2002; Kolko et al., 2002; Yagami et al., 2002, 2003). These studies resulted in increasing interest in developing specific inhibitors targeting this type of $sPLA_2$ (Yagami et al., 2005).

PX-18 (2-*N*,*N*-Bis(oleoyloxyethyl)amino-1-ethanesulfonic acid) (Fig. 1) is a lipid compound shown to offer cytoprotective properties (Franson and Rosenthal, 1989). These earlier studies also demonstrated ability for PX-18 to stabilize membrane, protect mitochondria through inhibition of PLA_2. This type of PLA_2 inhibitor could also inhibit thrombin-stimulated PGE2 and prostacyclin release in coronary artery endothelial cells (Meyer et al., 2005a), and sPLA2 in ischemic myocardium (Nijmeijer et al., 2003, 2008). Its ability to inhibit $sPLA_2$ and prostaglandin release in human endothelial cells appears to offer promising therapeutic potential as an anti-inflammatory agent (Rastogi et al., 2007). Nevertheless, the wide therapeutic use of this compound has been limited by its low aqueous solubility (Meyer et al., 2005b).

In this study, we investigated the possibility to overcome the solubility problem by formulating the compound as nanocrystals. This type of drug nanocrystals are nanoparticles with particle size <1 μm (typically 200–500 nm) and are comprised of 100% drug without any matrix material (Keck and Müller, 2006). Nanonization of drugs can increase their saturation solubility, which can be explained by the Kelvin and Ostwald–Freundlich equations, as well as their dissolution rate, which is described in the Noyes–Whitney and Prandtl equations (Böhm et al., 1998; Müller and Peters, 1997; Müller and Akkar, 2004). An increase in the bioavailability of drugs formulated as nanosuspensions has been reported (Möschwitzer and Müller, 2006; Müller and Keck, 2004; Müller et al., 2006). Therefore, the ability to formulate drugs or compounds with low solubility as nanocrystals displays a new paradigm in pharmacotherapy and drug delivery. In this study, we test whether PX-18 drug nanocrystals may offer protective effects against neuronal damage after cerebral ischemia, a model well established in our laboratory (Wang et al., 2002).

2. Results

2.1. *PX-18 nanocrystals*

An average particle size of 41 nm was achieved by applying 20 homogenization cycles at 1500 bar at 5 °C. Particle sizes as small as this have been previously reported as nanosuspensions and produced only using a combination technology where the material is lyophilized and subsequently subjected to high-pressure homogenization (Möschwitzer and Lemke, 2005). The 1% PX-18 nanosuspension was physically stable over a period of 180 days when stored at 4–6 °C (Fig. 2). No changes in particle size and polidispersity index (PI) occurred during the observation period. This protocol ensures a constant quality of the nanosuspension during the *in vivo* study (Fig. 2).

2.2. *PX-18 is neuroprotective against cerebral I/R-induced DND*

Four days after a 5-min CCA occlusion, extensive DND were observed in the hippocampal CA1 subfield (Fig. 3B vs. A). PX-18 administration resulted in a marked reduction of DND (Fig. 3C vs. B). Analysis of the numbers of viable neurons indicated

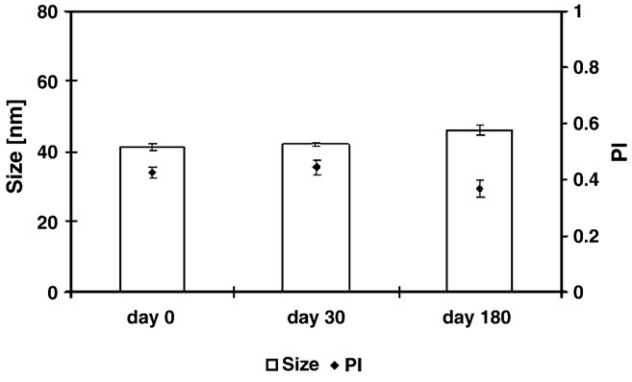

Fig. 2 – Average particle diameter and polydispersity index (PI) of the 1% PX-18 nanosuspension immediately after production (day 0), and after 30 days and 180 days of storage at 4–8 °C.