SUPPRESSION OF OTOTOXICITY BY BAMBOO SALT-PHARMACEUTICAL ACUPUNCTURE

Noh-Yil Myung[1], Su-Jin Kim[2], Hong-Kun Rim[2], Hyun-Ja Jeong[3], Seung-Heon Hong[4], Hyung-Min Kim[2], Jae-Young Um[1,2,*]

[1]Acupuncture and Meridian Science Research Center, Kyung Hee University, Seoul, 130-701 Republic of Korea; [2]Department of Pharmacology, College of Oriental Medicine, Institute of Oriental Medicine, Kyung Hee University, Hoegi-Dong, Dongdaemun-Gu, Seoul 130-701, Republic of Korea; [3]Biochip Research Center, Hoseo University, 165, Sechul-ri, Baebang-myun, Asan, chungnam 336-795, Republic of Korea; [4]College of Pharmacy, VestibuloCochlear Research Center of Wonkwang University, Iksan, Jeonbuk, 570–749, Republic of Korea

Correspondence to Jae-young Um; Department of Pharmacology, College of Oriental Medicine, Kyung Hee University, 1 Hoegi-Dong, Dongdaemun-Gu, Seoul, 130-701, Republic of Korea Tel: 82-2-961-9448, Fax: 82-2-967-7707. E-mail: jyum@khu.ac.kr

ABSTRACT: Bamboo salt is a specially processed salt according to the traditional recipe using solar salt and bamboo etc. It has been commonly used for medicinal, skin-care and general health purposes in Korea for more than 1,000 years. Purple bamboo salt (PBS) is the best bamboo salt manufactured through a unique method. We report PBS-pharmaceutical acupuncture prevents the cisplatin-induced ototoxicity. Recent studies have showed that IL-6 expression was distinctively induced in inner ear under various damaging conditions. When PBS was acupunctured at acupoint of triple energiger meridian 21 (TE21) after administration of cisplatin, PBS suppressed the IL-6 production and caspase-3 activation induced by cisplatin in mice cochlear. In addition, PBS inhibits IL-6 production, bcl-2 expression, and extracellular signal-related kinase activation in an auditory cell line, HEI-OC1. Our findings indicate that PBS-pharmaceutical acupuncture may contribute to hearing loss correction.

Key words: Purple bamboo salt; cisplatin; caspase-3; extracellular signal-related kinase, hearing loss correction

INTRODUCTION

Bamboo salt (BS) is a specially processed salt according to the traditional recipe using normal salt, bamboo, pine tree fire wood, pine resin, and yellow earth. It contains abundant minerals such as natural sodium, potassium, calcium, chloride, and magnesium. BS has been gaining more popularity in improving our health. Korean has long history of oriental medical science and developed many kinds of excellent traditional medical treatments. Traditionally in Korea, BS was used as an important medicine material. It has many different medical efficacy varies from its stub, roots, and leaves. Now BS is well known as one of the most famous traditional medical treatments. Also many pharmaceutical scientists all around the world are researching BS's special therapeutics like anti-cancer effect and anti-virus effect. Purple bamboo salt (PBS) is a specially processed salt according to the traditional recipe using normal salt and bamboo etc in relatively high temperature. Compared with crude salt, the contents of iron, silicon, potassium, and phosphate in the PBS were higher whereas the sulfate content was lower (Kim et al., 1998). It is known to have various therapeutic effects on diseases such as inflammations, viral disease, diabetes, circulation organ

disorder and cancer etc (Kim et al., 1993; Min et al., 1995; Yang et al., 1999; Huh et al., 2001). However, the protective effect of PBS on hearing loss is not completely understood.

Cisplatin is a highly effective and widely used anticancer agent (Trimmer and Essigmann, 1999). The risk of ototoxic and nephrotoxic side effects commonly hinders the use of higher doses that could maximize its antineoplastic effects (Humes, 1999). Also, cisplatin has been shown to induce auditory sensory cell apoptosis in vitro (Liu et al., 1998; Cheng et al., 1999) and in vivo (Alam et al., 2000). Cisplatin ototoxicity occurs primarily in the cochleae, especially in the outer hair cells (OHCs) of the organ of corti (McAlpine and Johnstone 1990).

Recently, it was reported that the inner ear has the capacity to generate an active immune response (Satoh et al. 2003), resulting in hearing loss in some individuals caused by aggravated immune responses in the inner ear (McCabe 2004). Accumulating evidence indicates that cochlear and vestibular functions, including hearing and balance, are influenced by immune responses in the inner ear (Rahman et al. 2001). Although immune function in the inner ear is very important in the protection from infectious diseases such as labyrinthitis, immunerelated inflammatory responses also cause damage to the delicate tissues of inner ear compartments and can often lead to cochlear degeneration and permanent hearing loss (Ryan et al. 2002; Satoh et al. 2003). Pro-inflammatory cytokine is a deteriorating factors for cochlea inflammation. It induces secondary inflammatory responses, including leukocyte infiltration, and scar formation in other injured. These reports indicate the involvement of pro-inflammatory cytokines in cochlear damage (Satoh et al., 2002). Other study reported that cisplatin caused ototoxicity through the apoptotic death of OHCs and supporting cells in the organ of corti accompanying the secretion and expression of the pro-inflammatory cytokines (So et al. 2007).

Apoptosis plays not only an essential role in development and tissue homeostasis but is also involved in a wide range of pathological conditions (De Martinis et al. 2007; Gatzka and Walsh 2007). In mammalian cells, there are two major pathways: the extrinsic and intrinsic pathways. In the extrinsic pathway, binding of the death receptors causes the activation of caspase-8, which is an initiator caspase. In the intrinsic pathway, various forms of cellular stress cause mitochondrial alterations leading to mitochondrial membrane depolarization (MMP) and the release of cytochrome c (cyt c). In the cytosol, cyt c binds to and activates Apaf-1, which then activates pro-caspase-9. Active caspase-9 directly cleaves and activates the effector protease, caspase-3. Cisplatin toxicity also was associated with an increase in caspase activity, cyt c release, apoptosis-inducing factor (AIF) translocation, and reactive oxygen species (ROS) generation.

In mammalian cells, three major mitogen-activated protein kinases (MAPKs) have been defined; extracellular signal-regulated kinase (ERK) pathway, the stress-activated pathways of the c-jun N-terminal kinase (JNK), and the p38 MAPK. These pathways are central components of the intracellular signaling networks that control many aspects of mammalian cellular physiology including cell proliferation, differentiation, and apoptosis (Roux and Blenis 2004; Wada and Penninger 2004). In general, the ERK signaling cascade is activated by growth factors and is associated with cell survival and proliferation (McCubrey et al. 2007; Puddicombe and Davies 2000). On the other hand, p38 and JNK are primarily activated by cellular stress and are often associated with inflammation and apoptosis. However, it has been suggested that these signaling pathways play more complex roles in the regulation of distinct cellular effects. The cellular functions regulated by ERK, p38 or JNK appear to depend on the cell type and stimulus as well as the duration and strength of the kinase activities. Many studies have reported the correlation between hearing loss and MAPK in the cochlea. JNK has been shown to be activated in hair cells as a result of amino-glycoside and noise-induced damage. Moreover, pharmacological prevention of JNK activation protects against hearing loss from those ototoxicity insults (Pirvola et al., 2000; Wang et al., 2004; Matsui et al., 2004). It was reported that hearing loss induced by cisplatin is through the activation of ERK (Battaglia et al., 2003). Pharmacologic inhibition of ERK significantly attenuated the death of auditory cells caused by cisplatin (Kim et al., 2008).

Caspase-1 is a member of the cystein-aspartic acid protease family

(Thornberry et al., 1992). Caspase-1 was identified by its ability to activate the inactive precursors of interleukin (IL)-1 and IL-18, cytokines involved in inflammation. It contains an N-terminal caspase recruitment domain (CARD). CARD promotes proteolytic activation of recruited caspases in apoptosis and inflammation (Lamkanfi et al., 2004). Specific adaptor molecules, receptor interacting protein-2 (RIP2, CARD containing kinase) regulate the activation of caspase-1 through CARD-CARD interaction (Ogura et al., 20001; Yoo et al., 2002). Caspase-1 is activated in a variety of cell death paradigms, caspase-1 was activated by treatment cisplatin (Kondo et al., 1995).

Acupuncture is the insertion of needles in specific cutaneous locations of the body, known as acupoints, for the treatment or prevention of several diseases (Habacher et al., 2006; Luna et al., 2008). Pharmaceutical acupuncture is a traditional Oriental therapeutic technique that combines acupuncture with herbal treatment. This technique involves injecting an herbal extract into certain acupuncture points, according to Oriental medical theory (Yim et al., 2007).
The overall aim of this study was to gain further insights into the protective mechanism of PBS-pharmaceutical acupuncture against cisplatin-induced toxicity in auditory HEI-OC1 cells as well as in vivo. The specific aims are as follows: (I) to understand the mechanism of PBS against cisplatin-induced ototoxicity in HEI-OC1 cells (II) To examine the effect of PBS-pharmaceutical acupuncture on cochlear damage induced by cisplatin in mice.

RESULTS

Effect of PBS on cisplatin-induced cell death, ROS generation and IL-6 production in HEI-OC1 cells

To determine the effects of PBS on cisplatin-induced cell death, ROS generation, and IL-6 production, cells were exposed to cisplatin (20 μM) in combination with increasing concentration of PBS (0.02- 2 mg/ml). We first assessed the effect of PBS on viability of HEI-OC1 cells using MTT assay. As a result, when the cells were treated for 48 h with cisplatin, the cell viability significantly decreased compared with media control. However, PBS inhibited cell death induced by cisplatin in HEI-OC1 cells. In addition, we showed that treatment of PBS reduced the ROS generation, and IL-6 production induced by cisplatin in a dose-dependent manner.

Effect of PBS on cisplatin-induced cyt c release and caspase-3 activation in HEI-OC1 cells

The mitochondrial apoptotic cascade requires the release of cyt c into the cytosol (Li et al., 1997). To determine if PBS inhibits the release of cyt c induced by cisplatin, cells were exposed to cisplatin in the presence or absence of various concentrations PBS. Cyt c release from mitochondria into cytosol was evaluated by Western blot analysis. The result showed that cisplatin induced cyt c release into the cytosol, which was inhibited by treatment of PBS.

Caspase-3 activity is known to increase in cisplatin-induced apoptosis in HEI-OC1 cells (Jeong et al., 2007). To determine if PBS inhibits caspase-3 activation induced by cisplatin, caspase-3 activity assay was performed. The result showed that increased caspase-3 activity induced by cisplatin was significantly inhibited by treatment of PBS.

Effect of PBS on cisplatin-induced ERK activation in HEI-OC1 cells

Hearing loss induced by cisplatin was associated with an increase of ERK activation (Kim et al., 2008). To understand the protective mechanism of PBS, we investigate if PBS regulates the ERK activation. The result showed that increased ERK activation induced by cisplatin was significantly inhibited by treatment of PBS.

Effect of PBS on cisplatin-induced caspase-1 activation in HEI-OC1 cells

Caspase-1 is activated in a variety of cell death paradigms, caspase-1 was activated by treatment cisplatin (Kondo et al., 1995). We investigated the upstream and downstream mediators of the cell death pathway involving caspase-1. To determine if PBS inhibits caspase-1 activation induced by cisplatin, caspase-1 assay was performed. Extracts prepared from HEI-OC1 cells exposed to cisplatin contained strong caspase-1 activity compared with unstimulated cells. However, PBS significantly inhibited the increased caspase-1 activity.

Effect of PBS-pharmaceutical acupuncture on cisplatin-induced IL-6 expression and caspase-3 activation in mice.

We acupunctured PBS at acupoint of triple energiger meridian 21 (TE21) after administration of cisplatin for 14 days. After then, cochlear was isolated and isolated tissue

was homogenized. Group of cispatin treatment induced the IL-6 expression and caspase-3 activation in organ of corti from mice. However, group of PBS-pharmaceutical acupuncture reduced these phenomena.

Discussion

PBS is a specially processed salt according to the traditional recipe using normal salt and bamboo in Korea. In the practice of traditional medicine in the Oriental, PBS is known to be effective to certain illness. Our ancestors learn all this good knowledge through experience, even though they do not know how to conduct research like modern medicine. It is known to have therapeutic effects on disease such as viral disease, dental plaque, gastropathy, diabetes, circulation organ disorder, cancer, and anti-inflammatory disorder (Yang et al., 1999; Huh et al., 2001; Sharma et al., 2001; Shin et al., 2004). However, the protective effect of PBS on hearing loss is not completely understood.

Cisplatin is highly effective chemotherapeutic agent but with significant ototoxic side effects. Thus, combination chemotherapy must be optimized to increase tumor response and at the same time lower its toxicity. Apoptosis is an important mechanism of cochlear hair cell loss following exposure to an ototoxic level of cisplatin. In mammals, the mitochondria act as the central checkpoints for many forms of apoptosis. The mitochondrial pathway is believed to be the main target of the survival signaling system (Christophe and Nicolas 2006). The mitochondria commit the cell to undergo apoptosis by increasing the permeability of the outer mitochondrial membrane, and decreasing the MMP, the release of cyt c and the ROS generation. Cisplatin toxicity also was associated with these events. Therefore, this study focused on investigating the protective effect of PBS on these phenomenon. In present study, treatment of PBS reduced the cell death, ROS generation, and the release of cyt c induced by cispaltin in auditory HEI-OC1 cells. These results suggested that the protective effect of PBS is through regulation of ROS generation and mitochondria signaling pathways. However, the effect of PBS on oxidative stress triggers the apoptotic process was not reported. Therefore, further study will be needed to clarify the non-mitochondrial signaling pathways and delineate the role of mitochondria-mediated apoptosis in the broader spectrum of the apoptotic signaling mechanisms.

Caspases, which are a family of cysteine-dependent aspartate-directed proteases, play important roles in initiating and executing apoptosis. Some studies strongly suggest that caspases play a key role in cisplatin -induced hearing loss (Li et al., 1997). Therefore, it is believed that the apoptosis mechanism of cisplatin in auditory cells might occur, at least in part, through a caspase-dependent pathway. We showed that treatment of PBS inhibited the caspase-3 activation in HEI-OC1 cells. These results suggested that the protective effect of PBS is through suppression of caspase-3 activation in auditory HEI-OC1 cells.

The MAPKs pathways are the central components of the intracellular signaling networks that control many aspects of the mammalian cellular physiology including cell proliferation, differentiation, and apoptosis (Wada and Penninger 2004). It was reported that ototoxicity of cisplatin was associated with ERK activation in HEI-OC1 (Wang et al., 2004). This report suggests that the ERK pathway is a potential therapeutic target for preventing cisplatin-induced ototoxic damage. Therefore, we examined whether or not PBS affects ERK activation induced by cisplatin. As a result, we observed that PBS reduced the ERK activation. Although PBS attenuated ERK activation, the effect of ERK on the other pathways involving MAPK upstream / downstream and apoptosis marker was not determined. Therefore, further studies will be needed to clarify the role of PBS on the MAPK pathway in the auditory system

Pharmaceutical acupuncture is a traditional Oriental therapeutic technique that combines acupuncture with herbal treatment. The goal is to provide a prolonged mechanical stimulus in the acupoint. Other substances may also be used for this purpose like glucose and vitamins. For example, deer antler aqua-acupunture has been used in the treatment rheumatoid arthritis. Perillae fructus herbal acupuncture has been used in the treatment allergic bronchial asthma. Autologous blood (hemopuncture) and bee venom (apipuncture) are injected mainly for anti-inflammatory purposes (Lin et al., 2002; Lee et al., 2005; Baek et al., 2005; Zhao et al., 2006; Martin et al., 1987). In present study, we first report PBS-pharmaceutical

acupuncture prevents the cisplatin-induced ototoxicity. Recently, it was reported that cisplatin-induced cochlear injury is through the expression of pro-inflammatory cytokines. Also, flunarizine, known as a T-type Ca2+ channel antagonist, has a cytoprotective effect against cisplatin cytotoxicity through the inhibition of pro-inflammatory cytokine expression in HEI-OC1 cells (Kim et al., 2008). These reports suggested the involvement of pro-inflammatory cytokines in cisplatin-induced hearing loss. Hence, it is hypothesized that the protective effect of PBS is through the regulation of pro-inflammatory cytokines. In this study, we showed that PBS-pharmaceutical acupuncture inhibited the IL-6 expression in mice. These results implicated that protective effect of PBS-pharmaceutical acupuncture against cisplatin cytotoxicity is through the suppression of IL-6 expression induced by cisplatin in mice. Although PBS attenuated IL-6 expression, the effect of PBS on other cytokine and involved pathway was not determined. Therefore, further studies will be needed to clarify the role of PBS on the various pathways in the auditory system. We also showed that PBS-pharmaceutical acupuncture inhibited the caspase-3 activation in mice.

In conclusion, PBS inhibited the cell death, ROS generation, cyt c release, caspase-3 activation and increased the level of ERK activation induced by cisplatin in auditory cells. In addition, PBS-pharmaceutical acupuncture counteracts the ototoxicity by suppressing the expression of IL-6 and activation of caspase-3 in mice. Collectively, these findings show that blocking a critical step by PBS in apoptosis may be useful strategy to prevent harmful side effects of cisplatin ototoxicity in patients with having to undergo chemotherapy.

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