

# MINERAL-RICH BAMBOO SALT ALLEVIATES ALLERGIC INFLAMMATORY SYMPTOMS AND REDUCES CISPLATIN-MEDIATED VESTIBULO-TOXICITY IN MICE

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**Abstract:** Purple bamboo salt (PBS) is a specially processed salt according to the traditional recipe using normal solar salt and bamboo etc in high temperature. It has been used as a folk medicine for the purpose of prevention and treatment of various diseases in Korea. First of all, we investigated the effect of PBS on allergic reaction. PBS inhibited the ear swelling response and histamine release induced by compound 48/80. PBS inhibited the passive cutaneous anaphylaxis by oral administration. Second, we evaluated the anti-inflammatory effect of PBS on phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187-induced proinflammatory cytokines production from mast cells. PBS product, Powerdental also exhibited a significant antibacterial activity. PBS decreased the PMA plus A23187-induced interleukin (IL)-1, IL-6, and tumor necrosis factor- production and mRNA expression. Third, we investigated the effect of PBS product, Pilopool on performance of forced swimming test (FST). The immobility time was decreased in Pilopool-fed group in comparison with control group on FST. In blood serum, the contents of creatine kinase and lactic dehydrogenase were decreased and the contents of glucose and total protein were increased. Finally, we investigated the effect of PBS on cisplatin-induced apoptosis of auditory cells. We demonstrated that apoptosis of auditory cells induced by cisplatin was inhibited by treatment with PBS. Activation of caspase-3, caspase-8, and caspase-9 was observed within cisplatin-treated auditory cells. PBS also inhibited the activation of caspase-3, caspase-8, and caspase-9. Collectively, our results suggest that PBS importantly contributes to the prevention or treatment of allergic inflammatory diseases and predict a potential benefit of PBS as an immune regulator. In addition, our findings indicate 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.

**Keywords:** Purple bamboo salt; allergic inflammatory reactions; immune regulation; ototoxicity

Purple bamboo salt (PBS) is a specially processed salt according to the traditional recipe using normal salt, bamboo, pine tree fire wood, pine resin, and yellow earth in high temperature over 1,500°C. It contains abundant minerals such as natural sodium, potassium, calcium, chloride, and magnesium. 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, it is still unclear how to prevent various diseases in an experimental model. Therefore, we evaluated the effects of PBS on mechanism of various experimental models.

## Effect of PBS on allergic reaction

The mast cells are thought to play a major role in the development of many physiologic changes during anaphylactic and allergic responses (Wasserman and Marquardt, 1988). Degranulation of mast cells is caused by non-immunologic secretagogues like substance P, compound 48/80, extracellular ATP and so on, which result in rapid and marked histamine release (Hua et al., 1996; Shin et al., 1997; Sudo et al., 1996 and Pearce, 1989). Histamine-induced anaphylactic responses such as vasodilation, increased vascular permeability and contraction smooth muscles. Thus, mast cells may play some important role in diverse immunological and pathological processes (Wershil and Galli, 1994). The synthetic compound 48/80 is known to be one of the most potent secretagogues (Aridor et al., 1990). An appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mechanism of anaphylactic reaction (Allansmith et al., 1989). Studies on the compound 48/80-induced mast cell degranulation and ear swelling response have been continuously performed on these theoretical bases by our group (Kim et al., 1999; Na et al., 2002 and Yi et al., 2002).

The secretory response of mast cells can also be induced by aggregation of their cell surface-specific receptors for immunoglobulin E (IgE) by corresponding antigen (Kim et al., 1998a; Metzger et al., 1986 and Alber et al., 1991). The anti-IgE antibody has been established to induce passive cutaneous anaphylaxis (PCA) as a typical model for mast cell-dependent immediate-type allergic reaction (Saito and Nomura, 1989).

We have demonstrated that PBS potently compound 48/80-induced ear swelling response and anti-DNP IgE-induced PCA reaction. And PBS inhibited the histamine release from RPMC.

It is believed that stimulation of mast cells with compound 48/80 initiates the activation of a signal transduction pathway, which leads to histamine release. There have been some reports that compound 48/80 and other polybasic compounds are able to activate G proteins (Mousli et al., 1990a and Mousli et al., 1990b). Recently Chahdi et al. announced that compound 48/80 activates mast cell phospholipase D (PLD) via heterotrimeric GTP-binding proteins (2000). They identified that recombinant G ( $\beta 2\gamma 2$ ) subunit markedly

synergized PLD activation by compound 48/80 in permeabilized RBL-2H3 cells. Study on the compound 48/80-induced histamine release in murine mast cells is still of good use in experimental models (Alfonso et al., 2000). Our results indicate that mast cell-mediated immediate-type allergic reactions are inhibited by PBS. The report that compound 48/80 increased the permeability of the lipid bilayer membrane by causing a perturbation of the membrane (Tasaka et al., 1986) indicates that the membrane permeability increase may be an essential trigger for the release of mediators from mast cells. So, it is possible to suppose that PBS might act on the lipid bilayer membrane affecting the prevention of the perturbation being induced by compound 48/80.

Jaffery et al. (1994) reported that the number of mast cells in skin decreased by chronic treatment with compound 48/80 by differential alcian blue/safranin staining. There is a possibility that the mast cells cannot be detected by alcian blue because of the complete degranulation by compound 48/80. In the present study, we showed that PBS inhibited compound 48/80-induced ear swelling response.

The PBS-administered mouse is protected from IgE-mediated local allergic reaction. This is supported by the effect of PBS on PCA. The mechanism of the protection against anti-DNP IgE, while not clear at present, may be suggested only in some particular conditions. It is conceivable that PBS inhibits the initial phase of immediate-type allergic reactions, probably through interference with the degranulation system.

In conclusion, the results obtained in the present study provide evidence that PBS inhibited the immediate-type allergic reactions in vivo and in vitro in a murine model. These suggest a possible use of PBS in managing these symptoms, but further studies about the activity of PBS are needed.

## Effect of PBS on inflammatory reaction

Dental caries and periodontal disease are the most common chronic diseases in the dental fields (Shani et al., 2000 and Oh et al., 2002). Dental caries and periodontal disease are mainly caused by dental plaque, containing a lot of bacteria inhabit in the dental plaque. The bacteria in dental plaque metabolize carbohydrates to form organic



acids, which destroy the hard tissues of tooth and result in dental caries, and the periodontal disease is caused by the inflammatory response to the bacteria (Twetman and Lindqvist, 1985 and Schwartz et al., 1997). *Streptococcus mutans* is the most important bacteria in the formation of dental plaque and dental caries (Wiater et al., 1999).

Cytokines produced in response to plaque bacteria clearly play a key role in the periodontal diseases (Fletcher et al., 1997). Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is a pleiotropic cytokine capable of altering physiological and mediating pathophysiological responses of various disease conditions (Lederer and Czuprynski, 1995). TNF- $\alpha$  seems to be a target in therapy of inflammatory and immune diseases (Bondeson and Maini, 2001). Mast cells are also known to release pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\alpha$  and  $\beta$ , and IL-6 (Grabbe et al., 1994).

Previous reports have shown that the antibiotics such as penicillin and erythromycin are effective in inhibiting *Streptococcus mutans* for the prevention of dental caries and periodontal disease, but they are not used in dental clinics due to the development of antibiotic resistance for long-term use (Namba et al., 1982). The fluoride compounds also have been used in the control of the dental plaque (Guha-Chowdhury et al., 1995), but they show cytotoxicity if used at a concentration over 80 ppm (Jeng et al., 1998). In addition, several mouth rinses have been developed for prevention of dental caries and periodontal disease (Pan et al., 1999 and Marsh, 1993), but the fact that dental caries and periodontal disease are still major causes of tooth loss proves that these agents are not sufficiently effective. Therefore, development of more effective, practical, and safe preventive agents for dental caries and periodontal disease is needed. The bamboo salt, pine resin, and lophatheri herba have been used in traditional folk medicine for treatment of dental caries and periodontal disease and are still used as tooth pastes and mouth rinse agents in Korea. Powerdental was composed of bamboo salt (66.7%), pine resin (Pinaceae, 33.2%), lophatheri herba (*Lophatherum gracile* Brongn., Gramineae, 0.1%).

In the present study, the growth of *Streptococcus mutans* was reduced by the presence of the Powerdental (1 mg/ml), NaCl (1 mg/ml) significantly, and positive control

(1% NaF) also exhibited antibacterial activity. We also examined the inhibitory effect of Powerdental on the PMA plus A23187-stimulated secretion of TNF- $\alpha$  from HMC-1 cells. Powerdental inhibited the secretion of TNF- $\alpha$  in PMA plus A23187-stimulated HMC-1 cells.

Bamboo salt contains mineral materials such as Na, K, Ca, Mg, Fe, Mn, Zn, P, Si, Pb, and S (Kim et al., 1998). Pine resin, a component of Powerdental, is used for the therapy of burns, wounds, and purulent and inflammatory diseases (Simbirtsev et al., 2002). Lophatheri herba, another component of Powerdental, is used for the therapy of oral diseases (Shin, 2000). The main constituents of lophatheri herba were reported to be arundoin, cylindrin (Nishimoto et al., 1965 and Harris, 1984). Components of Powerdental have been used in folk medicine, which has been successfully used for the treatment of periodontitis and other oral diseases and inflammatory diseases. During the last decade, new therapeutic substances such as bamboo salt, pine resin, and xylitol have been added to dentifrice in order to improve its clinical effect against periodontal diseases and dental caries. However, its mechanism of action still remains unknown. In the present study, Powerdental showed a reduction in acid production by *Streptococcus mutans*. This finding appears to be attributable to the reduction in growth rate of *Streptococcus mutans* in the presence of Powerdental. Furthermore, it suggests that Powerdental may inhibit any enzyme activity associated with the growth/glycolyzing systems of *Streptococcus mutans*. Powerdental appears to exert its pharmacological actions through the synergistic effects of its components via drug interaction. However, we need further study on the effect of pine resin and lophatheri herba.

We have demonstrated that Powerdental inhibited secretion of pro-inflammatory cytokine TNF- $\alpha$  in PMA plus A23187-stimulated HMC-1 cells. This result indicates that Powerdental had an effective anti-inflammatory activity. In conclusion, we demonstrated that Powerdental inhibits the caries-inducing activity of *Streptococcus mutans* by reducing the rate of acid production and the secretion of TNF- $\alpha$  from HMC-1 cells. Thus, the application of Powerdental on a daily basis can be considered useful for the prevention of dental caries and inflammatory reactions.

## Effect of PBS on immune regulations

Forced swimming test (FST) is a behavioral test for rodents, which predicts the efficacy of antidepressant treatments. This test induces the development of immobility as a reflection of helplessness when subjected to an inescapable situation (tank of deep water). In this paradigm, mice are placed in the tank for an extended period. After an initial swimming period, the animal exhibits immobility behavior considered a depression-like response. FST is used to examine whether certain agents have an anti-fatigue effect and is also used as an immune-enhancement test. Pilopool is composed of high molecular weight water soluble chitosan, *Allium sativum* L. (garlic) extract, shiitake (mushroom) extract, *Dioscorea Batatas* D., and bamboo salt.

Anti-fatigue effect of Pilopool was investigated using forced swimming-treated mice. Pilopool was estimated to possess immune regulatory effects. Pilopool consists of five different materials. Chitosan, a non-acetylated or partially deacetylated chitin (a linear homopolymer of  $\beta$ -(1-4)-linked N-acetylglucosamine) has been proposed as biomaterial because of its apparent satisfactory biocompatibility. In mammals, chitosan has been reported to stimulate non-specific resistance against *Escherichia coli* infection and suppression of the growth of Meth A tumor in syngeneic Balb/c mice (Nishimura et al., 1984). The authors have previously found that high molecular weight water-soluble chitosan shows a stimulative effect of nitric oxide production in macrophages and an inhibitory effect of pro-inflammatory cytokine production (Jeong et al., 2000; Kim et al., 2002b). *Allium sativum* L. has played an important dietary, as well as medicinal, role for centuries. Even today the medicinal use of garlic is widespread and growing. A wide array of therapeutic effects of garlic such as hypolipidaemic, antiatherosclerotic, hypoglycaemic, anti-coagulant, antihypertensive, antimicrobial, anticancer, antidote (for heavy metal poisoning), hepatoprotective, and immunomodulation have been reported (Agarwal, 1996; Agusti, 1996). Shiitake has anti-bacterial activity (Hatvani, 2001), anti-tumor activity (Mizuno, 2000; Ng & Yap, 2002), and cholesterol-lowering effect (Fukushima et al., 2001). *Dioscorea Batatas* D. is known as

Chinese Yam or Sanyak. The pharmacology effects of Yam on immune improvement and anti-oxidation have been reported (Hou et al., 2002). PBS is a specially processed salt according to the traditional recipe using normal salt and bamboo in Korea. It is known to have various therapeutic effects on diseases such as inflammations, viral diseases, diabetes, circulation organ disorder, and cancer (Yang et al., 1999; Huh et al., 2001; Sharma et al., 2001; Shin et al., 2004).

In conclusion, the Pilopool-fed group decreased their immobility time during FST, changed the metabolites related to fatigue were changed. Therefore, the present results suggest that Pilopool may be useful for the development of immune-enhancement. However, this suggestion, based on these preliminary results, should be confirmed by further experimental studies.

## Effect of PBS on cisplatin-induced apoptosis of auditory cells

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). 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). Devarajan et al. recently have reported cisplatin-induced apoptosis in an immortalized cochlear cell line (2002). The cytotoxic effects of cisplatin may occur via several putative pathways. One well-studied mechanism is the formation of DNA adducts which blocks progression through the cell cycle. Another mechanism involves production of reactive oxygen species (ROS) (Jamieson and Lippard, 1999). Because hair cells do not regenerate in the mammalian cochlea, cell loss, e.g., because of noise, hypoxia, or cisplatin, is irreversible and cumulative (Cheng et al., 1999). Cisplatin primarily damages the outer hair cells (OHCs) of the organ of Corti, which are the specific effectors of mammalian cochlear amplification and frequency determination. Larger doses of cisplatin are associated with additional damage to auditory neurons, stria vascularis, and supporting cells near the OHCs (Cardinaal et al., 2000). Therefore, selective inhibition of these pathways may provide a strategy to minimize



cisplatin-induced ototoxicity. The present study investigated the effects of BS on cisplatin-induced apoptosis.

Caspase constitute family proteases that normally exist as inactive enzymes. These are cysteine-dependent, aspartate-specific proteases that function to mediate apoptotic destruction of the cell. A total of 14 caspase family members have been identified in mammalian (Strasser et al., 2000). Caspase-8 is an upstream family member that is tightly linked to the membrane-associated death domain-containing receptors. When respective ligands (including Fas ligand and TNF- ) bind these receptors, pro-caspase-8 is recruited at the intracellular levels. Activated caspase-8 then activates the downstream caspases, including caspase-3, caspase-6, and caspase-7 (Cheng et al., 2005). One main downstream caspase is caspase-3, which carries out the apoptotic program by cleaving proteins necessary for cell survival, including Bcl-2, inhibitors of deoxyribonucleases, and cytoskeletal proteins (Cheng et al., 1997; Lazebnik et al., 1994). Caspase-3 activation has been detected in hair cells damaged by cisplatin. From this, specific inhibitors of caspase-3 and caspase-8 protect against cisplatin-induced hair cell death and hearing loss. In our study, PBS inhibited cisplatin-induced caspase-3 and caspase-8 activity. Inhibition of caspases protects auditory cells against cisplatin. These data suggest that caspase activity may represent an appropriate therapeutic target.

In summary, we have demonstrated that treatment of PBS inhibited the cisplatin-induced apoptosis via inhibition of caspase activation. Further investigation is necessary to determine other possible anti-apoptotic mechanisms of PBS and to apply it clinically in ototoxicity environments.

## Conclusion

Increase of allergic reaction and disruption of immune balance presented the most common causes of various diseases. Deafness appears to have a various impact on daily life, depending on the severity of the hearing loss. Various diseases evoke an inflammatory response characterized by activation and release of inflammatory mediators. A wide selection of anti-inflammatory drugs are currently available that target one or more inflammatory mediators. However, successful treatment of

various diseases with anti-inflammatory agents has met with limited success. One consequence of such studies should be the development of new drug or health functional food to enhance recovery from diseases. PBS importantly contributes to the prevention or treatment of allergic inflammatory diseases. PBS product plays an important in immune regulation. PBS also contributes to prevent harmful side effects of cisplatin ototoxicity in patients with having to undergo chemotherapy. For that reason, we suppose that PBS can enhance the body's ability to prevent disease and to recuperate from disease damage by promoting beneficial immune responses and repressing the harmful ones. But further studies must go side by side with mechanical studies of PBS or their components.

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