Inhibitory Effects of Self-Fermented Pine Needle Extract on Catecholamine Release in the Rat Adrenal Medulla†

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Abstract – The aim of the present study was to investigate the effects of several fractions obtained from methylene chloride (CH₂Cl₂) extract of self-fermented pine needle (SFPNE) on the acetylcholine (ACh)-evoked CA release from the isolated perfused model of the rat adrenal medulla and to establish the mechanism of the most active fraction (Fr.)-induced inhibitory action on the CA release. We obtained 6 fractions from CH₂Cl₂ extract of self-fermented pine needle. For the ACh (5.32 mM)-evoked CA release, the following rank order of inhibitory potency was obtained: Fr.4-5 > Fr.8-11 >> Fr.3 > Fr.6 = Fr.1-2. Fr. 4 - 5 (60 µg/mL) perfused into an adrenal vein for 90 min produced relatively time-dependent inhibition of the CA secretory responses to ACh (5.32 mM), DMPP (100 µM), McN-A-343 (100 µM) and high K⁺ (56 mM). Fr. 4 - 5 itself did not affect basal CA secretion. Also, in the presence of Fr. 4 - 5 (60 µg/mL), the CA secretory responses to angiotensin II (AngII, 0.1 µM), veratridine (50 µM), Bay-K-8644 (10 µM), and cyclopiazonic acid (10 µM) were significantly reduced, respectively. In the simultaneous presence of Fr. 4 - 5 (60 µg/mL) and L-NAME (30 µM), the inhibitory responses of Fr. 4 - 5 on the CA secretion evoked by ACh, DMPP, high K⁺, AngII, Bay-K-8644 and veratridine were considerably recovered to the extent of the corresponding control secretion compared with that of Fr. 4 - 5-treatment alone. The level of NO released from adrenal medulla after the treatment of Fr. 4 - 5 (60 µg/mL) was greatly elevated compared with the basal level. Taken together, these results demonstrate that Fr. 4 - 5 inhibits the CA secretion from the isolated perfused rat adrenal medulla evoked by stimulation of cholinergic receptors as well as by direct membrane-depolarization. It seems that this inhibitory effect of Fr. 4 - 5 is mediated by blocking the influx of Ca²⁺ and Na⁺ into the adrenomedullary chromaffin cells as well as by inhibition of Ca²⁺ release from the cytoplasmic calcium store, which is evoked at least partly through the increased NO production due to the activation of NO synthase. Based on these results, it is also thought that Fr. 4 - 5 isolated from CH₂Cl₂ extract of pine needle may contain beneficial antihypertensive components to prevent or treat hypertension.

Keywords – Self-fermented pine needle extract, Catecholamine Release, Adrenal Medulla, NO release

Introduction

Recently, we found that both self-fermented pine needle for 2 years (SFPE2) and ethylacetate (EtOAc) fractions cause vascular relaxation in the aortic strips isolated from normotensive rats and SHRs as well as vasodepressor responses (Cheong & Lim, 2010). It seems that SFPE2 or the EtOAc fraction possesses active antihypertensive components, which are available to prevent or treat hypertension in future.

Generally, it has been known that red pine, the Pinus densiflora Sieb. et Zucc. (Pinaceae) grows naturally or is planted in mountain regions of Korea, Japan and China. Red pine needles have traditionally been used as a nourishing tonic drug in Korean folk medicines and are frequently used to brew a tea in Korea. Pine needle extract has been shown to cause several actions, such as an antioxidant activity in rats fed highly oxidized fat (Lee, 2003), cytotoxic effects on several cancer cell lines (Chung et al., 2002), inhibition of the pacemaker currents of interstitial cells of Cajal (ICC) by activating ATP-sensitive K⁺ channels via the production of PGs (Cheong et al., 2005), and nitrite scavenging activities (Park et al., 2002). Hsu and his co-workers (2005) have found that...
pine (*Pinus morrisonicola* Hay.) needle scavenges superoxide and inhibits the growth of leukemia cell U937. For the antioxidant activity of several fractions from *Pinus densiflora*, the following rank order of potency was obtained: ethyl acetate > n-butanol > water > dichloromethane fraction (Jung *et al*., 2003). Fitzpatrick and his co-workers (1998) have reported that the pine bark extract was able to stimulate in vitro the production of nitric oxide, thus counteracting the vasoconstriction by adrenaline or noradrenaline in isolated aortic rings from rats. Pine pollen powder, called ‘natural micro-nutrient storeroom’, is rich in many kinds of body-demanding amino acid, minerals, vitamin, enzyme, and flavonoids (Wang *et al*., 2005). Pollen lipids of a pine species have been shown to cause a remarkable inhibition of platelet activating factor activity (Siafaka-Kapadai *et al*., 1986). Lee *et al*., (2009) suggested that pine pollen is a potential antioxidant and beneficial for inflammatory conditions through down-regulation of IL-1β -induced JNK and matrix metalloproteinases (MMPs).

The present study was designed to investigate the effects of several fractions obtained from methylene chloride (CH₂Cl₂) extract of self-fermented pine needle (SFPNE) on the acetylcholine (ACh)-evoked CA release from the isolated perfused model of the rat adrenal medulla and to establish the mechanism of the most active fraction (Fr.)-induced inhibitory action on the CA release.

### Materials and Methods

**Preparation of pine needle fractions** – The leaves of Korean red pine trees (*Pinus densiflora* Sieb. et Zuc.) cultivated in Gokseong, Jeollanam-Do Province, Korea and harvested in 1999–2009 were collected to prepare for extraction. Pine needles were washed 3 - 4 times with tap water; dipped with charcoal water, dried, and ground for 1 minute to homogenize with con was allowed to put for 3 hours at 4°C and the supernatant was recovered. This supernatant sample was stored at 4°C for assay. Fresh pine needle extract (PE) was stored for years that favored emergence of microorganisms, which finally enabled spontaneous fermentation in extracts. The effects of the extract were examined for PE as well as after 2 years of self-fermentation designing as self-fermentation pine needle extract 3 years old (self-fermented pine needle). The extract was freezing-dried to obtain solid sample. Finally the combined extract was freeze-dried (105.7 g). This dried sample is referred to as the pine needle extract. As shown in Fig. 1, methylene chloride extract was fractionated to six fractions: Fr. 1 - 2 (240.9 mg), Fr. 3 (4.6 mg), Fr. 4 - 5 (66.1 mg), Fr. 6 (4.6 mg), Fr.7 (16.4 mg) and Fr. 8 - 11 (7.7 mg). The working solution of these fractions was prepared by dissolving in DMSO and then in 0.9% NaCl solution on the day of each experiment and filtered before administration.

**Experimental procedure** – Mature male Sprague-

![Fig. 1. Schematic diagram of fractionation form self-fermented pine needle extract (SFPNE). Yield of each fraction is expressed with milligrams.](image-url)