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Proof of the Mysterious Efficacy of Ginseng: Basic and Clinical Trials: Suppression of Adrenal Medullary Function In Vitro by Ginseng

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Abstract. The root of *Panax ginseng* C.A. MEYER has been reported to have an anti-stress action. Therefore, the effects of ginseng components on functions of adrenal medulla, which is one of the most important organs responsive to stress, were investigated in vitro. First, the components of ginseng were mainly divided into two fractions, that is, the saponin-rich and non-saponin fractions. The saponin-rich fraction greatly reduced the secretion of catecholamines from bovine adrenal chromaffin cells stimulated by acetylcholine (ACh), whereas the non-saponin fraction did not affect it at all. The protopanaxatriol-type saponins inhibited the ACh-evoked secretion much more strongly than the protopanaxadiol-type. On the other hand, only one oleanane-type saponin, ginsenoside-Ro, had no such effect. Recent reports have demonstrated that the saponins in ginseng are metabolized and absorbed in digestive tracts following oral administration of ginseng. All of the saponin metabolites greatly reduced the ACh-evoked secretion. M4 was the most effective inhibitor among the metabolites. M4 blocked ACh-induced Na^+ influx and ion inward current into the chromaffin cells and into the *Xenopus* oocytes expressing human $\alpha 3\beta 4$ nicotinic ACh receptors, respectively, suggesting that the saponin metabolites modulate nicotinic ACh receptors followed by the reduction of catecholamine secretion. It is highly possible that these effects of ginsenosides and their metabolites are associated with the anti-stress action of ginseng.

Keywords: ginseng, adrenal gland, chromaffin cell, catecholamine secretion, ginsenoside

Introduction

Ginseng, a root of *Panax ginseng* C.A. MEYER, is one of the most popular medicinal plants throughout the world because of its beneficial effects. The herb has been believed to have an aphrodisiac and a longevity action. Furthermore, it has been stated that the ginseng has many effects (e.g., replenishment of vital energy, tranquilization, and mood elevation) by the oldest Chinese medical book, *Sheng-nong Ben-cao Jing*. Thus, the ginseng seems to influence the function of various physiological systems and acts to maintain homeostasis against diseases. Stress influences many systems, that is, the endocrine, immune, and nervous systems, especially the central and autonomic nervous systems, consequently disturbing homeostasis, resulting in development of diseases.

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One of the most important stress-responding peripheral systems is the adrenal glands. These glands secret catecholamines and cortisol from the medulla and the cortex, respectively, under conditions of stress. Their stress hormones activate the organs coping with stress. Therefore, we investigated the influence of ginseng and its components on the function of adrenal medulla in vitro. This paper mainly reviews the results.

Effects of non-saponin and saponin-rich fractions on catecholamine secretion

First, ginseng components were roughly isolated as two major fractions, the non-saponin and saponin-rich fractions. The saponin-rich fraction reduced the secretion of catecholamines from the bovine adrenal chromaffin cells stimulated by acetylcholine (ACh), but the non-saponin fraction had no such effect (1). However, the saponin fraction did not affect the secretion from

the cells induced by high K^+, an activator of voltage-sensitive Ca^{2+} channels due to direct membrane depolarization. This suggests the possibility that the saponins in ginseng components are effective in inhibiting the catecholamine secretion induced by ACh.

Effects of ginseng saponins (ginsenosides) on catecholamine secretion

Over 30 kinds of ginseng saponins, which are called "ginsenosides", have been isolated and identified. They are classified into two groups, dammarane-type and oleanane-type saponins, on the basis of the chemical structures of their aglycones. The dammaranes are further divided into two types, protopanaxadiol and protopanaxatriol saponins (Fig. 1). The effects of 14 kinds of representative ginsenosides on the ACh-evoked secretion of catecholamines from the cells were explored (Fig. 2). The protopanaxatriols (ginsenoside-Re, -Rf, -Rg_1, etc.) strongly inhibited the ACh-evoked secretion, whereas the oleanane (ginsenoside-Ro) and the protopanaxadiols had little or no inhibitory effect except for ginsenoside-Rg_3 and -Rh_2 (2, 3). The IC_{50} values (μM) were as follows: Rb_1 (>100), Rb_2 (>100), Rb_3 (>100),

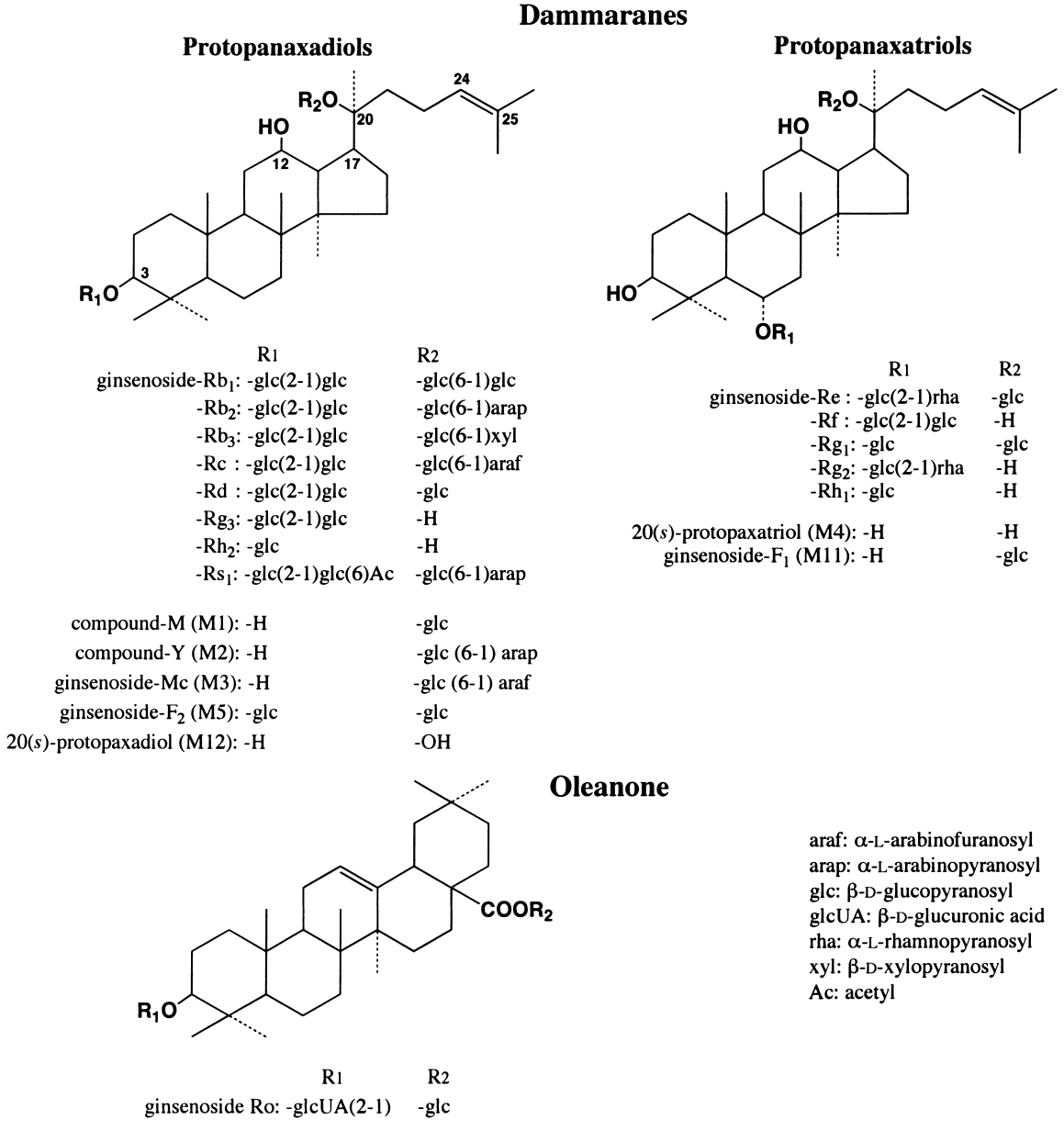

Fig. 1. Chemical structures of ginseng saponins (ginsenosides) and their metabolites.

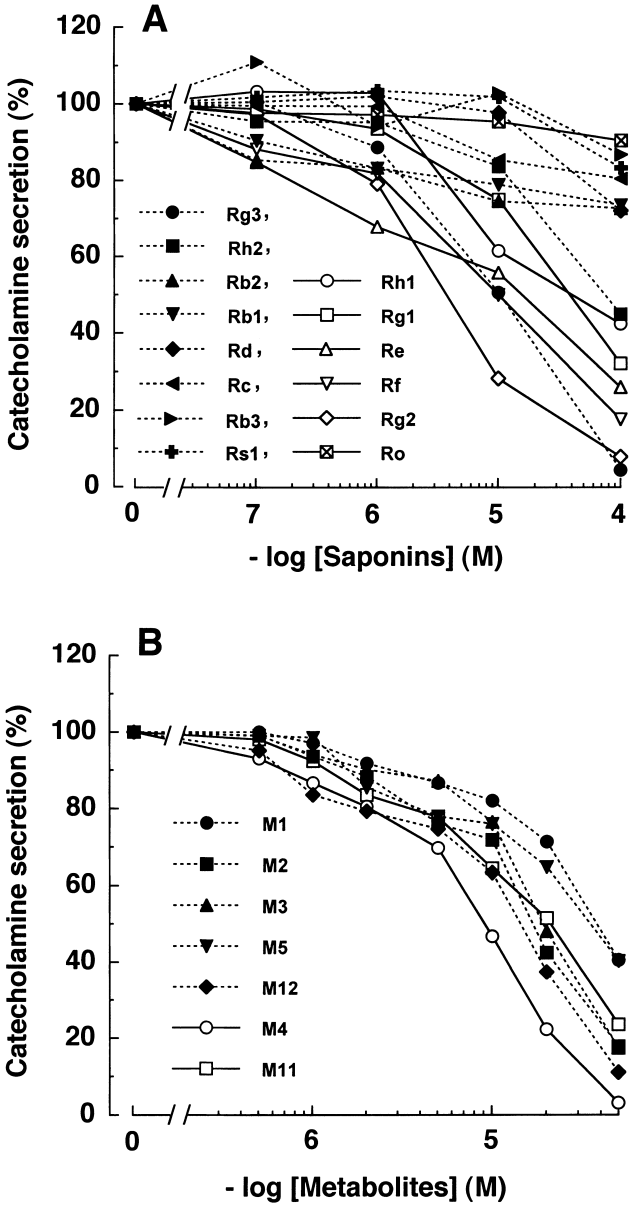

Fig. 2. Effects of ginsenosides and their metabolites on ACh-evoked secretion of catecholamines in bovine adrenal chromaffin cells. The cells were preincubated with or without ginsenosides or their metabolites for 10 – 15 min at 37°C and then incubated with or without the saponins (A) or their metabolites (B) used in the preincubation step in the presence of ACh for 7 min. The ACh-evoked secretion was assigned a value of 100%.

Rc (>100), Rd (>100), Re (16), Rf (10), Rg_1 (40), Rg_2 (4), Rg_3 (10), Rh_1 (30), Rh_2 (80), Rs_1 (>100). There was a relationship between the inhibitory effects and the structures of ginsenosides (3). However, other saponins (saikosaponins, glycyrrhizin, and cardiac glycosides) isolated from medicinal plants had only weak or no inhibitory effects on the secretion (3), suggesting that its effect is relatively specific for ginsenosides.

Of the protopanaxatriols, ginsenoside-Rg_2 was the most potent inhibitor. The saponin reduced Na^+ influx induced by ACh, whereas it did not affect the secretion of catecholamines induced by high K^+. These results strongly indicate that the inhibitory effect of the saponin-rich fraction is due to the saponins, particularly the protopanaxatriols, and that the secretory inhibition is due to the blockade of ACh-induced Na^+ influx into the cells through nicotinic ACh receptor-operated cation channels. This idea is also supported by the study that ginsenosides reduce the ACh-induced ion current into *Xenopus* oocytes expressing nicotinic ACh receptors (4).

Inhibition of catecholamine secretion by ginseng saponin metabolites

The recent reports have demonstrated that the saponins in ginseng are metabolized in the digestive tract following oral administration of ginseng (5, 6). The oligosaccharides connecting to the hydroxyl groups of aglycones are in turn hydrolyzed by gastric acid and enzymes of intestinal bacteria. The major metabolites derived from the protopanaxadiols are M1, M2, M3, M5, and M12, while those derived from the protopanaxatriols are M4 and M11. M1 (20-*O*-β-D-glucopyranosyl-20(*s*)-protopanaxadiol), and M4 (20(*s*)-protopanaxatriol) are end products from the protopanaxadiols and the protopanaxatriols, respectively (Fig. 1). All their saponin metabolites greatly inhibited the ACh-evoked secretion of catecholamines from chromaffin cells (Fig. 2) (7). Therefore, the protopanaxadiols seem to be prodrugs that reveal their inhibitory activities via the metabolism in the digestive tract. The IC_{50} values were as follows: M1 (38), M2 (18), M3 (19), M4 (9), M5 (36), M11 (22), and M12 (15). M4 was most effective.

M4 also inhibited the high K^+-induced secretion, whereas the inhibitory effect was much less than that on the ACh-evoked secretion. M4 reduced the ACh-induced Na^+ influx into the cells in a concentration-dependent manner similar to that of the inhibition of the ACh-evoked catecholamine secretion. From these results, it is highly probable that the ginseng saponins metabolized in the digestive tract as well as the saponins themselves antagonize the nicotinic ACh receptors, blocking Na^+ influx through the receptors and consequently reduce the secretion of catecholamines.

Effect of M4 on function of recombinant nicotinic ACh receptors

Recent studies have shown a gene family encoding a number of neuronal nicotinic ACh receptor subunits, which are nine types of α subunits ($\alpha 2 - \alpha 10$) and

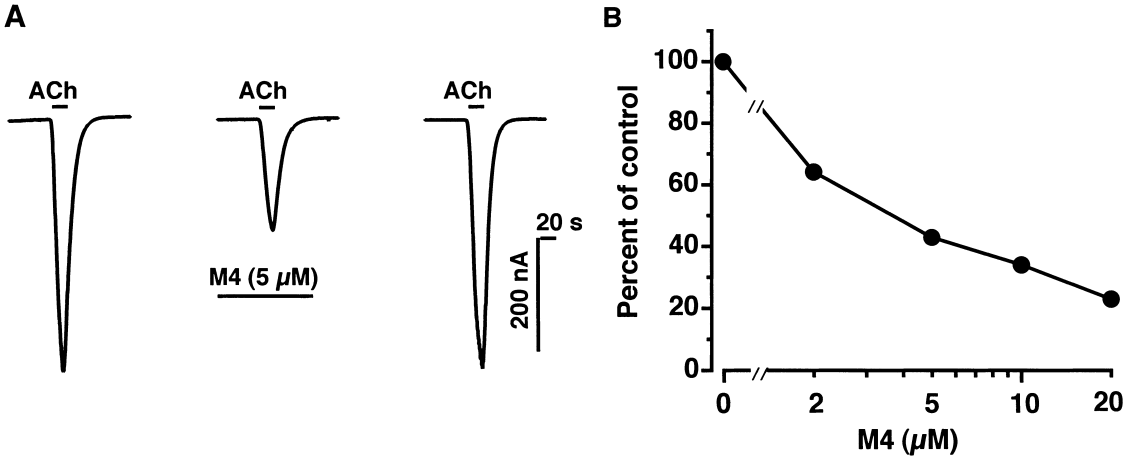

Fig. 3. Effects of M4 on ACh-induced inward currents in *Xenopus* oocytes expressing human $\alpha 3\beta 4$ nicotinic receptor. M4 was applied to the oocytes for 1 min before stimulation, and then the oocytes were perfused with ACh (100 μM) in the presence of M4 for 2 min. A: A representative sample of tracings obtained from a single oocyte clamped at –40 mV. B: Concentration-response curves for the inhibitory effect of M4 on the ACh-induced response. (Reproduced from Ref. 7 with permission from Elsevier)

three types of β subunits ($\beta 2 - \beta 4$) (8). The functional subunit combination of nicotinic ACh receptors associated with the secretion of catecholamines from bovine adrenal chromaffin cells is considered to be $\alpha 3$ and $\beta 4$ (9, 10). M4 attenuated the ACh-induced inward ion current into *Xenopus* oocytes expressing the human $\alpha 3\beta 4$ nicotinic receptors (Fig. 3) (7). Thus, saponin metabolites are presumed to act on the nicotinic ACh receptors.

Properties of M4 inhibition of catecholamine secretion

M4 did not alter the specific binding of [^{3}H]nicotine to chromaffin cells and the M4 inhibition of secretion was not overcome by increasing the ACh concentrations, indicating that the M4 inhibition is noncompetitive. Furthermore, based on the observation that M4 inhibition is dependent on the preincubation time of the cells with the saponin metabolite and is not completely reversible (7), it is possible that M4 enters the cells or the cell membranes and regulates the nicotinic ACh receptors from the cytoplasmic side of the plasma membrane or produces a change in the environment of membrane adjacent to the nicotinic receptors (Fig. 4). Léna and Changeux have proposed the binding sites of lipophilic, noncompetitive blockers of nicotinic ACh receptors lie at the interface between the receptor protein and membrane lipids (11).

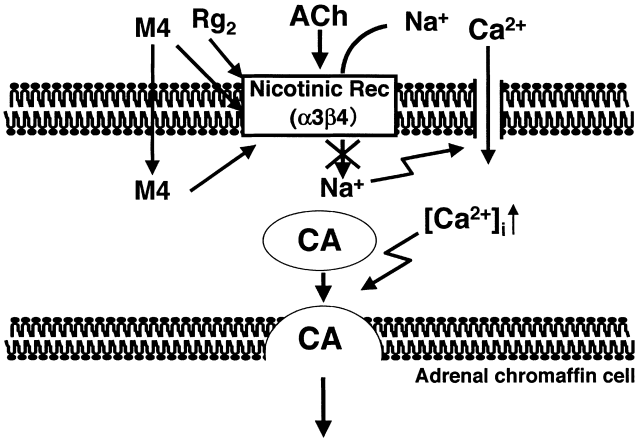

Fig. 4. Putative inhibition mechanism of M4 in catecholamine secretion from adrenal chromaffin cells. CA, catecholamine; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; Nicotinic Rec, nicotinic ACh receptor; Rg_2, ginsenoside-Rg_2.

Pharmacological significance of inhibition of catecholamine secretion by ginseng saponins and their metabolites in vivo

Stress seems to lead to the development of almost all types of diseases. The adrenal gland is one of the indispensable peripheral organs responding to stress. It secrets catecholamines and cortisol from the medulla and the cortex, respectively, and stimulates the organs to cope with stress. However, long-term or excessive stress results in the over-secretion of catecholamines and cortisol, leading to over-reaction and exhaustion of

the organs. It produces gastrointestinal symptoms (anorexia, dyspepsia, etc.) such as gastric ulcer, fatigue, psychoneurosis (anxiety, depression, amnesia, etc.), essential hypertension, and immunosuppression. Many reports have shown that ginseng and ginsenosides ameliorate stress-induced symptoms and lesions (12 – 14). It is possible that ginseng improves the phenomena induced under stressful conditions via the suppression of catecholamine secretion and cortisol production in vivo. In fact, in addition to the inhibitory effects of the saponins and their metabolites on the function of adrenal medulla, it has been reported that intraperitoneally administered ginseng total saponin and a few specific saponins suppress the corticosterone level in stressed rat plasma (15).

Ginsenosides has been reported to be only poorly absorbed from digestive tracts (16, 17). The absorption amounts of ginsenoside-Rb_1 and -Rg_1 are 0.1% and 1.9% of the dosage to rats, respectively. Furthermore, both ginsenosides are scarcely detectable in the brain, suggesting that ginsenosides can hardly penetrate the blood-brain barrier because of their high molecular weight (>600) and hydrophilicity. On the other hand, orally administered M4, which has a lower molecular weigh (477) and is less hydrophilic, has been found to be completely absorbed from digestive tracts (18). Therefore, not only both ginsenosides and their metabolites may control the functions of the peripheral organ, adrenal gland, but also the metabolites may regulate nicotinic ACh receptors in the central nervous systems. These peripheral and central actions could be, at least in part, associated with the anti-stress action of ginseng. Thus, the beneficial effects of ginseng on stress may be why ginseng has become known as an omnipotent or a prophylactic natural medicine.

References

1 Kudo K, Akasaka Y, Miyate Y, Takahashi E, Tachikawa E, Kashimoto T. Effects of red ginseng fractions on catecholamine secretion from bovine adrenal medullary cells. J Med Pharm Soc Wakan-Yaku. 1992;9:236–239.

2 Tachikawa E, Kudo K, Kashimoto T, Takahashi E. Ginseng saponins reduce acetylcholine-evoked Na^+ influx and catecholamine secretion in bovine adrenal chromaffin cells. J Pharmacol Exp Ther. 1995;273:629–636.

3 Kudo K, Tachikawa E, Kashimoto T, Takahashi E. Properties of ginseng saponins of catecholamine secretion in bovine adrenal chromaffin cells. Eur J Pharmacol. 1998;341:139–144.

4 Choi S, Jung SY, Lee JH, et al. Effects of ginsenosides, active components of ginseng, on nicotinic acetylcholine receptors expressed in *Xenopus* oocytes. Eur J Pharmacol. 2002;442:37–45.

5 Karikura M, Miyase T, Tanizawa H, Taniyama T, Takino Y. Studies on absorption, distribution, excretion and metabolism of ginseng saponins. VII. Comparison of the decomposition modes of ginsenoside-Rb_1 and -Rb_2 in the digestive tract of rats. Chem Pharm Bull. 1991;39:2357–2361.

6 Hasegawa H, Sung J-H, Matsumiya S, Uchiyama M. Main ginseng saponin metabolites formed by intestinal bacteria. Planta Med. 1996;62:453–457.

7 Tachikawa E, Kudo K, Hasegawa H, et al. In vitro inhibition of adrenal catecholamine secretion by steroidal metabolites of ginseng saponins. Biochem Pharmacol. 2003;66:2213–2221.

8 Wilkins LH Jr, Grinevich VP, Ayers JT, Crooks PA, Qwoskin LP. *N*-*n*-Alkylnicotinium analogs, a novel class of nicotinic receptor antagonists: interaction with $\alpha4\beta2$ and $\alpha7$ neuronal nicotinic receptors. J Pharmacol Exp Ther. 2003;304:400–410.

9 Campos-Caro A, Smillie FL, Domíguez del Toro E, et al. Neuronal nicotinic acetylcholine receptors on bovine chromaffin cells: cloning, expression and genomic organization of receptor subunits. J Neurochem. 1997;68:488–497.

10 Tachikawa E, Mizuma K, Kudo K, Kashimoto T, Yamato S, Ohta S. Characterization of the functional subunit combination of nicotinic acetylcholine receptors in bovine adrenal chromaffin cells. Neurosci Lett. 2001;312:161–164.

11 Léna C, Changeux J-P. Allosteric modulations of the nicotinic acetylcholine receptor. Trend Neurosci. 1993;16:181–186.

12 Attele AS, Wu JA, Yuan C-S. Ginseng pharmacology: multiple constituents and multiple actions. Biochem Pharmacol. 1999; 58:1685–1693.

13 Court WE. Ginseng, the genus *Panax*. The Netherlands: Harwood Academic Publishers; 2000.

14 Ong YC, Yong EL. *Panax* (ginseng) – panacea or placebo? Molecular and cellular basis of its pharmacological activity. Ann Acad Med Singapore. 2000;29:42–46.

15 Kim D-H, Moon Y-S, Jung J-S, et al. Effects of ginseng saponin administered intraperitoneally on the hypothalamo-pituitary-adrenal axis in mice. Neurosci Lett. 2003;343:62–66.

16 Odani T, Tanizawa H, Takino Y. Studies on the absorption, distribution, excretion and metabolism of ginseng saponin. II. The absorption, distribution and excretion of ginsenoside Rg_1 in the rat. Chem Pharm Bull. 1983;31:292–298.

17 Odani T, Tanizawa H, Takino Y. Studies on the absorption, distribution, excretion and metabolism of ginseng saponin. II. The absorption, distribution and excretion of ginsenoside Rb_1 in the rat. Chem Pharm Bull. 1983;31:1059–1066.

18 Hasegawa H, Saiki I. Cancer prevention by ginseng via its intestinal bacterial metabolites. Tokyo: Art Village Inc.; 2003.