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ACUTE GENOPROTECTIVE EFFECTS ON LYMPHOCYTIC DNA WITH GINSENG EXTRACT SUPPLEMENTATION

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Abstract: Ginseng is regarded as the 'miracle' herb or supplement in the traditional Chinese medicine. Minimizing DNA damage is thought to contribute to longevity. Previous study on ginseng aqueous extract showed in vitro DNA protection on human lymphocytes against oxidative stress. In the current study, the potential DNA protective effect on lymphocytes after supplementation of commercial ginseng extract was measured by comet assay. Seven volunteers were requested to ingest 200 mL water with five capsules of ginseng extract. On the other occasions, seven volunteers took 200 mL of water only as the control trial. Venous blood samples before ingestion were taken. Subjects were requested to keep fasting and the second blood sample was taken at 2.5 hours. Lymphocyte was isolated and subjected to comet assay. DNA damage of isolated lymphocytes was induced by 50 μM H2O2 and the level of damage of was graded under fluorescence microscope. Results showed that there was significant decrease (77% lower) in comet score in ginseng treatment group. No significant change of DNA damage in water treatment group was observed. In conclude, a normal dose of commercial ginseng extract was able to lower DNA damage of peripheral lymphocyte within 2.5 hours of ingestion.

Key words: Antioxidant, comet assay, DNA damage, ginseng extract.

Introduction

Panax ginseng is the 'miracle' herb and is native to Northeast China and Korea. The root of ginseng is widely used among Chinese medicine practitioners. Ginseng was discovered several thousand years ago in the Manchuria Mountains in China. It is a slow-growing perennial plant and is only found in the northern hemisphere. Ginseng belongs to the family Araliaceas and genus Panax. The name Panax originated from the Latin word 'panacea', i.e. 'all-healing'. Under the genus Panax, there are thirteen species: -ginseng, -japonicas, -major, -notoginseng, -omeiensis, -pseudoginseng, -quinquefoliusm, -sinensis, -stipuleanatus, -trifolius, -wangianus, -zingiberensis and -vietnamensis (1).

Ginsengs grow in cool dry climate and are mostly cultivated in the North America and the Far East (mainly northern China and Korea). Ginsengs are divided into two main types: true ginsengs and false ginsengs (2). Panax ginseng (Asian ginseng) is the most commonly used type of ginseng in Chinese medicine. It is native to China and Korea and is one of the true ginsengs. The other example of true ginseng is Panax quinquefolius. It is also known as American ginseng that is commercially grown in Wisconsin of the United States and British Columbia in Canada (2). The demand and consumption of ginseng are growing rapidly. It has been shown that about 3% of United States population uses ginseng products (3). There was a 70% growth of US herbal supplement sales within 3 years after implementation of the Dietary Supplement Health and Education Act of 1994 (DSHEA). Because of the high demand from the user of dietary supplement, wide varieties of ginseng product are available in the market. Ginseng is usually available in the form of fresh or processed (white ginseng and red ginseng respectively). Processed products are available in different forms such as ginseng tea, powder, juice, candy and extract (capsule) (4).

According to the Ban Cao Gang Mu (the Compendiumof Materia Medica) written by Li Shizhen in Ming Dynasty, ginseng is an important ingredient in Chinese herbal decoctions for treating 23 types of disease. Owing to the Qi tonic nature of ginseng, it is commonly prescribed for treating weakness and fatigue. Applications have been extended for the use on relieving women's menopause symptoms as well as men's erectile dysfunction (2). Modern evidence has also demonstrated

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improved condition among hyperglycaemic patients (5). Being an adpatogen, it is very good in supporting health and boosting the immune system (2, 6-8). This miracle herb is also used as anti-aging medicine. Antioxidant property of ginseng has been demonstrated with the discovery of ginsenosides (9, 10). The bioavailability of ginsenosides vary and pharmacokinetics are being extensively studied (11). However, the concentrations of ginsenoside are generally low in the circulation. Whether the absorbed and circulating components are still active, this remains to be investigated.

Our previous work demonstrated the in vitro DNA protecting ability of Panax ginseng aqueous extract on human lymphocyte (4). The aim of this follow-up study was to investigate whether the antioxidant activity could be extended to the in vivo environment. The DNA protecting ability of ginseng extract would be measured in a short period of time after ingestion. The decrease of DNA damage of oxidative stressed peripheral lymphocytes was taken as the end point of genoprotecting antioxidant power.

Materials and methods

Chemicals used are listed: Histopaque 1077; sodium chloride (NaCl); sodium dihydrogen phosphate dihydrate (NaH2PO4·2H2O); sodium monohydrogen phosphate (Na2HPO4¬); sodium hydroxide (NaOH) were from BDH Prolabo, Poole, Dorset, UK; disodium ethylenediaminetetraacetic acid (Na2EDTA); hydrogen peroxide (H2O2); Tris; Triton X-100; type VII low melting point agarose were from Sigma, St Louis MO, US; Standard melting point agarose was from Amresco , Solon, OH, USA.

Eleven apparently healthy individuals including six males and five females (age 29-62 years) were recruited. Three subjects attended blood taking session twice (active treatment and control) and eight attended one session only. The standard deviation of comet assay score was ~15 within our research group, 3 subjects were required to detect a difference of 45 score with 80% power. To enhance the reliability of the study, 7 subjected were tested with active ingredients and 7 with water. All volunteers were asked to fast at least 8 hours and informed consent was sought before blood sample collection. They were free from nutritional supplement and herbal medicines for at least 2 weeks before they study. And there was at least a 2-week wash-out period for those who joined both active and control treatments. Ginseng extract capsules (HKNT- 16471) were purchased from local pharmacy. The extract was emptied from 5 capsules and was mixed with about 200 mL of water. The total weight of extract was about 3250 mg. For subjects who attended only one blood taking session, either ginseng extract or water was assigned randomly. Three mL of blood was collected from each subject before and 2.5-hour after ingestion of water or ginseng extract. Subjects were asked remaining fasting between two blood samplings. EDTA blood samples collected were kept at 4°C and comet assay was performed on the same day.

Lymphocytes were harvested by mixing 100 µL of blood and 1 mL of pre-cooled PBS in micro-centrifuge tubes. One hundred µL of Histopaque 1077 was underlaid to the mixture. All tubes were centrifuged for 5 min at 1500 rpm. About 100 µL of cell suspension just above Histopaque layer was harvested and centrifugal washed twice with 1mL of PBS (12).

The washed lymphocytes were incubated with 1 mL PBS or 1 mL 50 μM H2O2 for 5 min in fridge. The cells were washed and supernatants were discarded again. The comet assay was performed following the procedures of Szeto and co-workers (12). Briefly, 1% (w/v) low melting point gel (85 μL) prepared in PBS was gently mixed with the washed cells in the microtube and added onto a glass slide pre-layered with 85 μL of 1% standard agarose. These slides were subjected to lysis after the gel solidified. Slides were put into a cool staining jar with lysis solution (pH 10) with 1% Triton X-100 and kept at 4°C for an hour in dark for cell lysis. Afterwards, these slides were put into cold electrophoresis solution (~ 40 ml in a staining jar) for 20 min DNA unwinding and alkaline-labile sites expression. The electrophoresis solution was changed once and treated for further 20 min. Fresh electrophoresis solution was cooled and poured into an electrophoresis tank. Slides were placed on the tank platform and were just covered with electrophoresis solution. Electrophoresis was run in constant voltage, 25 V, for 30 min. The current was kept at 0.3 A by adjusting the amount of electrophoresis solution in the tank. After electrophoresis, slides were placed in a staining jar with tap water for 5 minutes to remove the electrophoresis solution. This step was repeated twice and the slides were allowed to air dried.

Coded slides were stained with 40 µl ethidium bromide (2 mg/l). One hundred lymphocytes were observed under florescence microscope (Nikon Microphot-Fx with excitation filter G: Ex 510-560, Tokyo, Japan) manually by the other team member blindly. The degree of cell damage was scored according to the tails intensity which was described previously (13). Cell damage was graded into five levels (score 0, 1, 2, 3 and 4, whereas 0 represented undamaged). Comet scores were compared and analysed by Mann-Whitney U test by Prism 5.0 (GraphPad Software, CA, US).

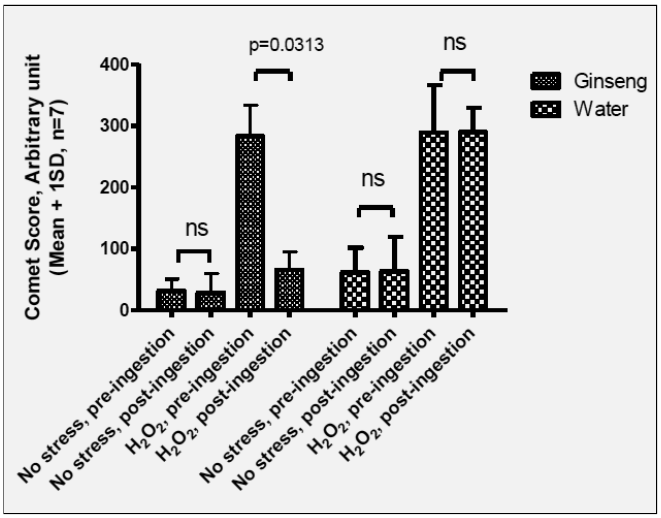
Results

A total of 7 subjects took the ginseng extract supplement while 7 subjects took the control trial (i.e. water) in different occasions. Result showed that there was no change of baseline DNA damage in two treatments over 2.5 hours. However, there was a

significant decrease of comet score on the hydrogen peroxide stressed lymphocyte after taking 5 capsules of ginseng extract (Wilcoxon signed rank test, p=0.0313). Comet scores dropped from 294 ± 45 (pre-ingestion) to 67 ± 31 (post-ingestion). It was a 77% decrease of DNA damage. On the other hand, no statistical significant change of comet score was seen in the water taking group (p>0.05). Pre- and post-ingestion comet score were 289 ± 72 and 290 ± 37 respectively. It was demonstrated that antioxidant in ginseng extract was able to be absorbed into blood in a very short period of time and conferred protection on cellular DNA.

Figure 1

Change of DNA damage before and after 5 capsules of ginseng extract in 200 mL water or just 200 mL water. Statistical significant decrease in comet scores (p=0.0313, Wilcoxon signed rank test) was seen in cell stressed with hydrogen peroxide after supplementation with ginseng capsules. While no change in DNA damage in control trial (200 mL water)



Discussion

Ginseng is one of the herbal medicines which is believed to possess anti-aging properties (14). In the modern theory of aging, DNA damage is a critical reason contributes to aging process. Minimizing cellular DNA damage is therefore a strategy to combat aging and its associated chronic diseases (15). Ginsenosides which are present in the plant genus Panax, are known to possess antioxidant power (16). Ginsenosides accumulate in ginseng and reach the peak concentration at about 6 years (17). There are thirty-eight ginsenosides found in Panax ginseng CA Meyer and are classified into three categories: 20(s)-protopanaxadiol (PPD), 20(s)-protopanaxatriol (PPT) and oleanolic acid. PPD and PPT type ginsenosides are unique in species ginseng (18). Absorption of ginsenosides is suggested to involve several steps: 1) deglycosylation by acid, 2) deglycosylation by intestinal bacteria and 3) absorption from gastrointestinal tract (19). There are a wide variety of micro-organisms present in gastrointestinal tract, especially the intestine. These bacteria play an important role in metabolism of ginsenosides (19,20).

Many of the studies have demonstrated genoprotection property of ginseng in animal model and in vitro human study (21-23). However, human supplementation data is lacking particular with the focus on cellular DNA. Medium term of supplementation of ginseng has shown the improvement of antioxidant parameters along with decreased baseline DNA damage. Up-regulated antioxidant levels might contribute to the lowered background damage (24). However, in the current study, protection was seen as quick as 2.5 hours after intake. This made modulation of antioxidant enzyme activity by ginseng to enhance DNA's resistant not very convincing. To diminish hydrogen peroxide mediated DNA damage, the active ingredients in ginseng extract had to be absorbed via gastrointestinal tract, entered into blood stream, reached the lymphocytes and remained stable. Or else the metabolites of ginseng extract had to be remained active when deglycosylation occurred. It has been reported that ginsenosides can be absorbed into circulation quickly (25). Capsule of the ginseng extract was excluded in the supplementation to eliminate its effect on absorption at the gastrointestinal tract. Besides, previous studies in our group on plant-based agents, namely orange juice (26) and grape seed extract (our unpublished result) demonstrated a rapid action (2-2.5 hours post ingestion) on cell after ingestion. Hence a relatively short period of interval was selected in the current study.

In vitro DNA protecting effect of ginseng in human cell has been demonstrated and the question on effective dosage was then emerged (4). It has to notice that a normal dose of commercial ginseng extract was consumed by healthy subjects and this sufficiently leaded to detectable and significant improvement of antioxidant status in peripheral cells. The role of ginseng as a supplement for healthy aging is now more promising.

To sum up, the protective effect of ginseng was successfully demonstrated in the in vivo setting. A normal dose of commercial ginseng extract was able to exert DNA protection on DNA of lymphocytes in circulation within 2.5 hours after ingestion.

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