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Application of Nanotechnology to Korean Black-Red Ginseng: Solubility Enhancement by Particle Size Reduction

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Abstract

In order to investigate whether the particles reduced to almost nano grade might affect the chemical and physical properties of organic materials, whole Korean Black-Red Ginseng was pulverized into almost nano size and then ginsenosides, minerals, carbohydrates, lipids and proteins in the ultrafine particles were compared with those in the regular particles as control. The mean size of the ultrafine particles was in the 350 nm range, while that of the regular particles was 127 μm. More ginsenosides, minerals, carbohydrates, lipids and proteins were detected in the ultrafine particles than in the regular particles. Interestingly, more lipids from the ultrafine particles dissolved in the water than those from the regular particles in the ethanol. Absorption and transport of carbohydrate, lipid or antioxidant activity across the intestinal wall using everted intestine sacks of mice was also enhanced by particle size reduction at the almost nano scale. More cytotoxic effect against hepatoma cell growth by ultrafine particles was also found. These results could be used as the basic data for the understanding and evaluation of the effects of organic nanomaterials on the human health.

Keywords: Nanotechnology, Ultrafine particles, Solubility, Korean Black-Red Ginseng

Nanotechnology can be defined as the control and restructuring of matter below 100 nanometers in size in order to create materials, devices, structures and functional systems. The advent of nanotechnology opens up a wealth of opportunities and applications, including medicines, food additives, cosmetics, electronics, textiles and engineering[1-3]. With the rapid growth of nanotechnology and future bulk manufacturing of nanomaterials, it is necessary to understand any negative health effects that may occur during manufacturing, during use, or by accident[4,5].

For existing substances and materials remade at the nano scale, the properties are significantly different from their larger equivalents. Chemical reactivity, color, strength, electrical and thermal conductivity, and magnetic properties may all vary in extraordinary ways[6-8]. Insoluble substances may dissolve at the nano scale[9]. Moreover, nano-sized particles have extreme mobility and are able to cross biological membranes and access cells, tissues and organs that larger-sized particles normally cannot[10-12]. Nano particles spread unhindered and pass through most available filters in water; hence, the development of new materials not feasible before is possible through nanotechnology. Paradoxically, the unique properties that are being exploited, such as high surface reactivity and ability to cross cell membranes, might have adverse influences on the health[13-15]. Especially some engineered inorganic nanoparticles may display undesirable toxicological properties, presenting potential risks to human and environmental health. Therefore, it is important to understand such relationships to enjoy the benefits of nanotechnology without being exposed to the hazards.

In spite of the increasing concern about the potential risks associated with this new technology, nanotechnology still promises to deliver significant benefits to many aspects of health care and applications. In particular, some of the most promising applications of biologically inspired nanoparticles have so far been in nanobiotechnology and in drug delivery system[16,17].

Many nanotechnological researches have so far been focused on the single inorganic nanomaterials such as carbon nanotubes, nanoscale titanium dioxide and zinc oxide etc[18,19]. However, there has been no further progress on the organic mixture nanomaterials such as foods because of its multiple constituents and

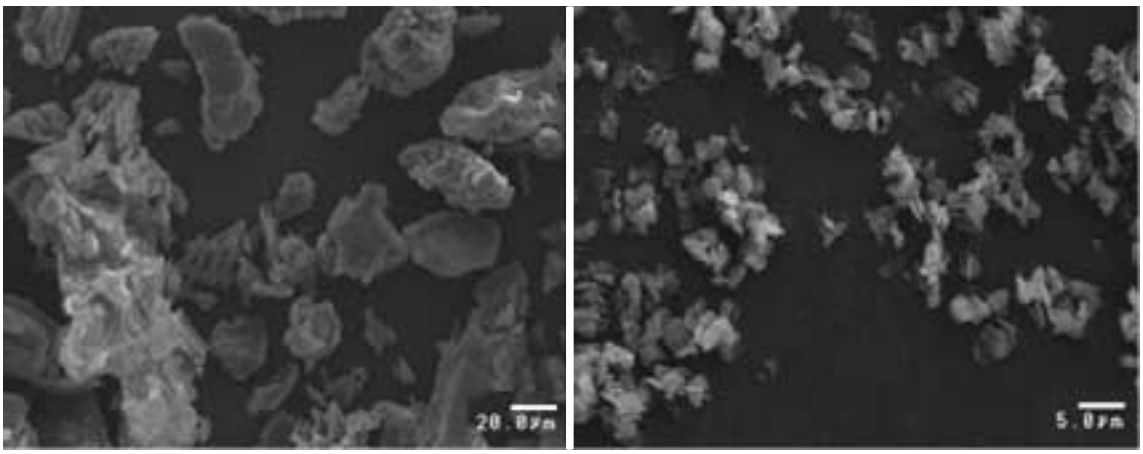

Figure 1. Scanning electron microscopic images of ultrafine particles and regular particles from Korean Black-Red Ginseng.

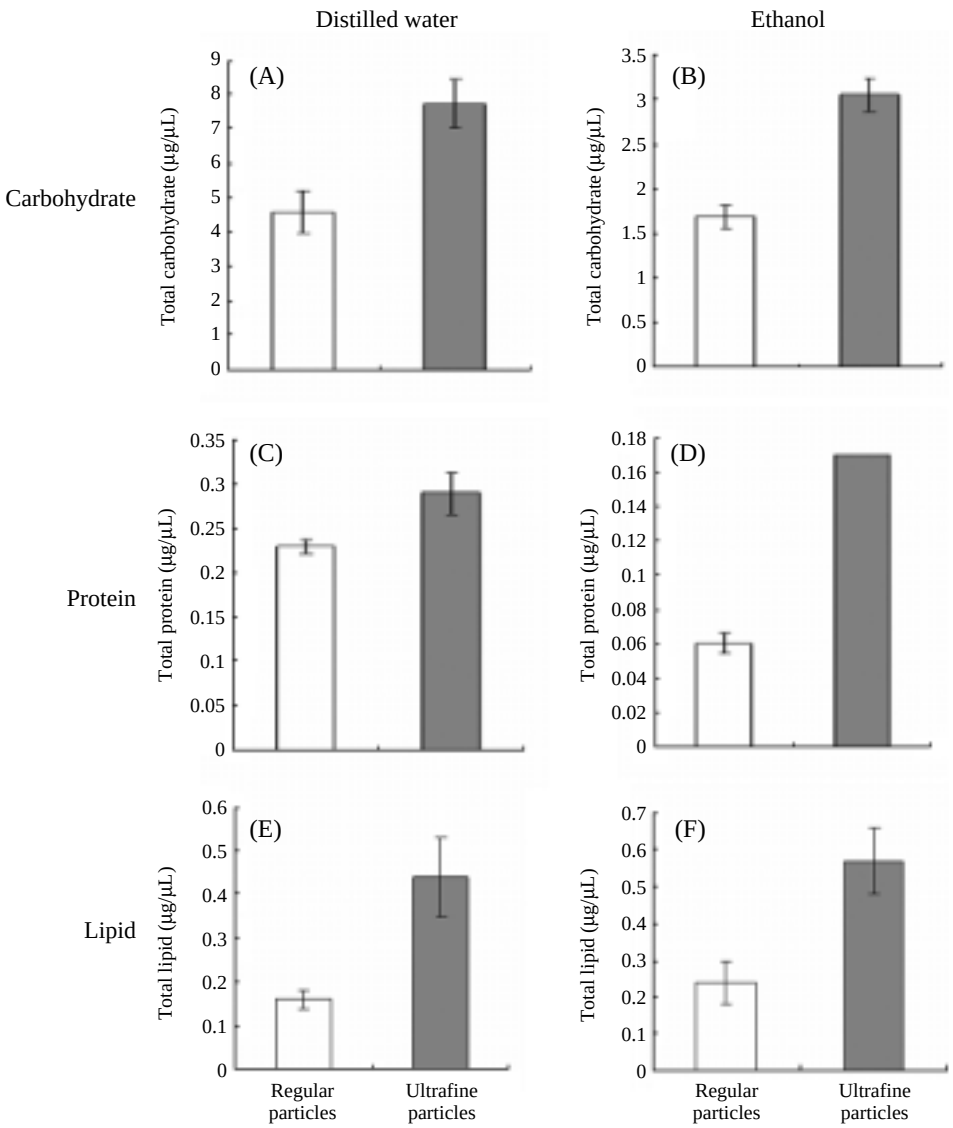

Figure 2. Biomolecule contents of ultrafine particles and regular particles from Korean Black-Red Ginseng.

the limit of analytical technology.

In this investigation, the solubility, reactivity and transport of the ultrafine particles from Korea Black-Red Ginseng were compared with those of regular particles to better understand the chemistry and functionality of the organic nanomaterials. It will be used as basic data for the evaluation of health effects of food nanomaterials.

SEM Images and Particle Size

Ultrafine particles (UFP) and regular particles (RP) in Korean Black-Red Ginseng were developed and supplied by NT & BT Co., Ltd (Hongsung, Korea). Scanning electron microscope (SEM) was used to photograph the images of ultrafine particles (UFP) and regular particles (RP) in Korean Black-Red Ginseng (Figure 1). Mean sizes of the RP and UFP were determined to be 127 μm and 3.5 μm (350 nm) through density analysis of the particles, respectively, consistent with the SEM images (data not shown).

Biomolecule Contents

Korean Black-Red Ginseng powder was fully dissolved in distilled water or 80% ethanol to measure the amounts of biomolecules, named carbohydrates, proteins, or lipids. The amounts of carbohydrates of RP and UFP dissolved in distilled water were 4.5 and 7.6 μg/μL, respectively (Figure 2A). This result showed that carbohydrates of UFP were approximately 1.7 fold higher than those of RP in distilled water. Also, the carbohydrates of UFP were approximately 1.8 fold higher than those of RP in ethanol (Figure 2B). Notably, the carbohydrates of UFP in water were about 5 fold higher than those of RP in ethanol.

Total proteins of RP and UFP were also measured as shown in Figure 2C and Figure 2D. Proteins of UFP dissolved in distilled water and ethanol were about 1.3 fold and 2.8 fold higher than RP, respectively. These results show that UFP has higher protein solubility than RP in distilled water and ethanol. For both RP and UFP, the content of proteins dissolved in distilled water was higher than in ethanol (Figure 2C, 2D).

Total lipids of RP and UFP were also measured (Figure 2E, 2F). Lipids of UFP dissolved in distilled water were about 2.8 fold higher than RP (Figure 2E). About 2.4 fold higher lipid of UFP than of RP was also found in ethanol (Figure 2F). Interestingly, lipids of UFP were higher by about 2 fold in distilled water than those of RP in ethanol (Figure 2E, 2F). These

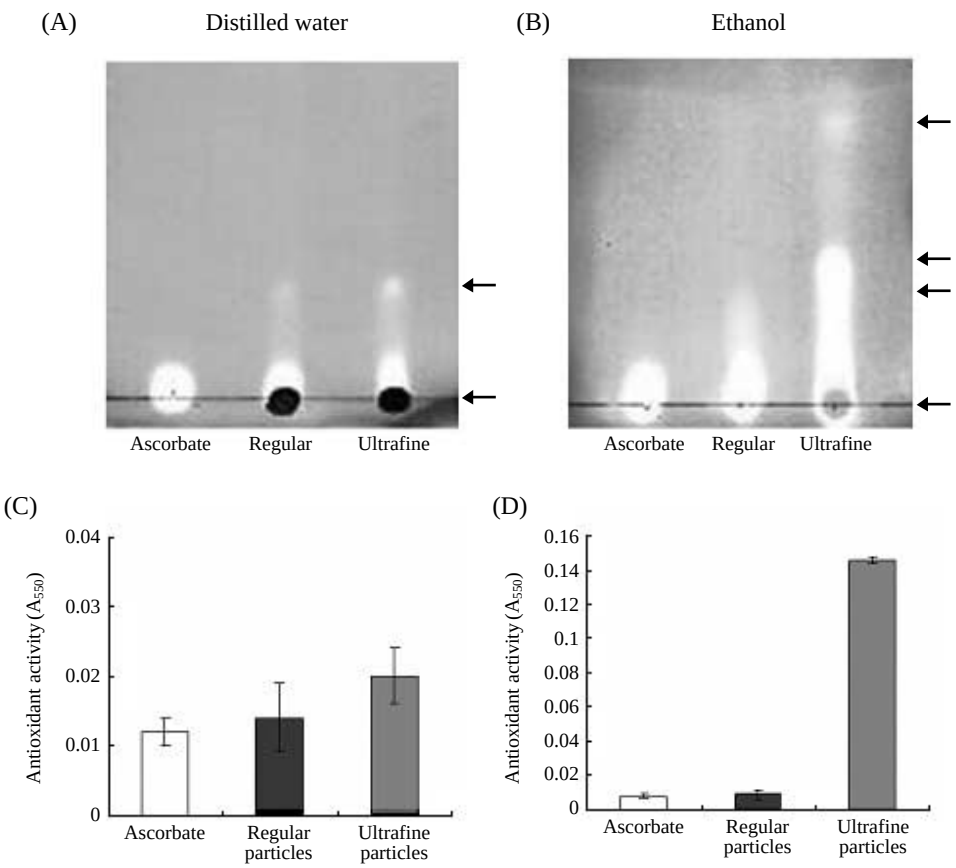

Figure 3. Antioxidant activities of ultrafine particles and regular particles from Korean Black-Red Ginseng.

results show that even for highly hydrophobic lipids, solubility in water can increase if the particles are grained to almost nano sizes. These results contradict the general notion that lipids dissolve better in organic solvent than in distilled water.

Antioxidant Activity

RP and UFP from Korean Black-Red Ginseng dissolved in water or ethanol were compared for their antioxidant activities using TLC method. It is known that browning material formed through the steaming process of the Black-Red Ginseng production has high antioxidant activity. The spots of UFP for antioxidant activity were significantly stronger and more diverse than those of RP as shown in Figure 3. Especially, UFP dissolved in ethanol shows more than four strong and diverse spots of antioxidant activity.

The antioxidant activities of RP and UFP in distilled water or organic solvents were quantitatively compared using xanthine-xanthine oxidase method. In distilled water, antioxidant activity of UFP was higher than that of RP by about 1.4 fold (Figure 3A). For organic solvent, UFP showed about 16 fold stronger antioxidant activity than RP (Figure 3B).

Transport and Absorption Tests by Using Digestive Tract

Transport and absorption of the bioactive components through digestive tract was examined using everted intestine sacks of mice as a simple active tra-

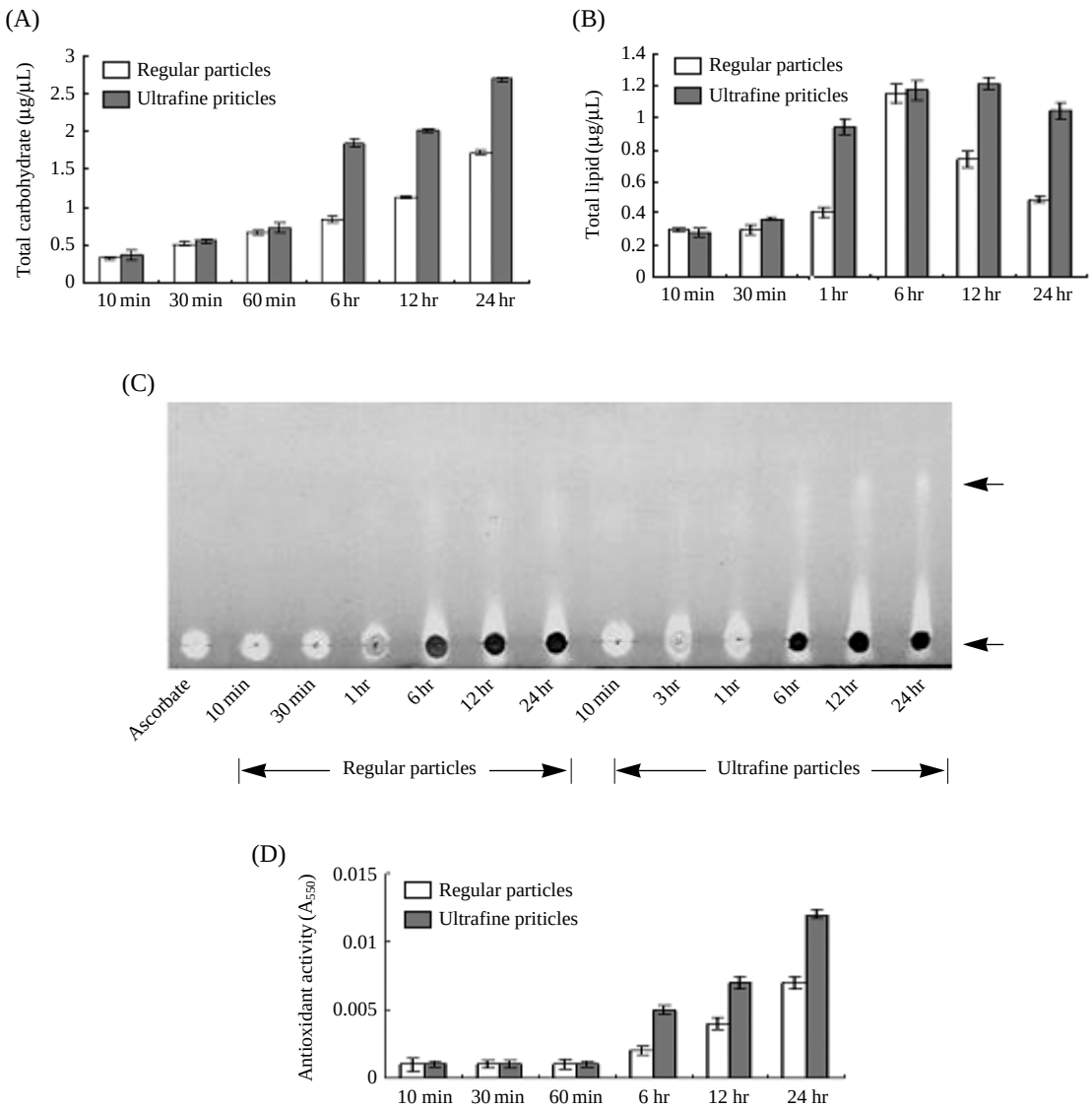

Figure 4. Transport and absorption tests by using everted intestine sacks.

nsport model. Everted sac method has been used to examine the absorption by digestive tract *in vitro*[20]. Although the everted sac absorption is different from the actual absorption because only components that have passed through the tunica serosa of digestive tract are measured, it is widely used to easily measure digestive tract absorption. The transport of carbohydrate into the everted sac increased without significant difference among RP and UFP until 1 hr, the initial absorption time (Figure 4A). However, after 6 hr, transport of UFP carbohydrates increased by 2.2 fold as compared to RP. After 12 hr and 24 hr, the absorption of carbohydrates in UFP was about 1.8 and 1.6 fold higher, respectively, than RP (Figure 4A).

The absorption of the lipids in RP and UFP by the digestive tract was also measured as shown in Figure 4B. Lipid absorption of UFP was about 2.2 fold higher than that of RP after 1 hr. In fact, absorbing lipids in UFP was remarkably higher than in RP during the entire 24 hr reaction time (Figure 4B).

Figure 4C showed the antioxidant activities absorbed by the digestive tract measured using TLC method. During entire reaction times (10 min to 24 hr), spots of antioxidant activities in UFP were stronger and more diverse than those in RP (Figure 4C). These results show that antioxidant activity of UFP absorbed by the digestive tract is significantly higher than that of RP. Quantitative measurement of antioxidant activities of the active components absorbed in digestive tract was also performed using xanthine-xanthine oxidase method[21]. Antioxidant components absorbed in the digestive tract from UFP showed about 2.5, 1.75 and 1.7 fold increase at 6, 12 and 24 hr, respectively, as compared to RP (Figure 4D), consistent well with the TLC method.

Cytotoxicity on Hepatoma Cell

Korean Black-Red Ginseng RP and UFP were each diluted to $1/2^2$-$1/2^7$, and then treated on hepatoma cell Hep3B. When RP and UFP were diluted to $1/2^3$, UFP repressed approximately 13% of hepatoma cell growth, while RP repressed approximately 8.8%. UFP repressed hepatoma cell growth more than RP at $1/2^2$-$1/2^5$ dilution. This result indicated that cytotoxicity against hepatoma cell could be enhanced when particle size was reduced to almost nano grade (Figure 5).

Comparative Analysis of Saponin Contents

Thirteen saponins dissolved in distilled water or ethanol were analyzed using HPLC analysis method. As shown in Table 1, 13 saponins from UFP were detected more than those from RP in water as well as ethanol. The sum of 13 saponins from UFP and RP dissolved in distilled water was 20.86 and 10.69 mg/g powder, respectively, showing about 2 fold more saponins from UFP than from RP in water. Moreover, the sum of 13 saponins from UFP dissolved in water was approximately 23% more than those from RP in

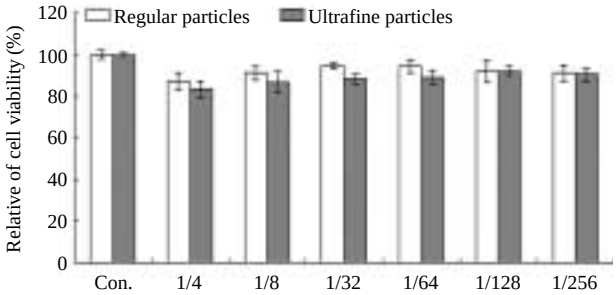

Figure 5. Cytotoxicity of ultrafine particles and regular particles on hepatoma cells.

Table 1. Comparative analysis of saponin contents dissolved in the water and ethanol from ultrafine particles and regular particles. (mg/g powder)

Saponin		Ultrafine particles		Regular particles	
		Ethanol	Distilled water	Ethanol	Distilled water
PD	Rb1	6.47	5.00	3.34	2.32
	Rb2	3.37	2.41	1.53	1.05
	Rc	3.43	2.50	1.60	1.10
	Rd	2.48	1.70	0.99	0.63
	Rg3s	2.69	1.70	1.27	0.84
	Rg3r	1.03	0.73	0.52	0.39
	Rh2s	0.67	0.28	0.34	0.14
PT	Re	2.79	2.17	1.36	1.01
	Rf	1.16	0.87	0.70	0.58
	Rg1	1.18	0.94	0.81	0.60
	Rg2s	1.86	1.49	1.08	0.83
	Rg2r	1.64	0.59	2.26	0.87
	Rh1	0.62	0.48	0.44	0.33
	Sum	29.39	20.86	16.24	10.69

Table 2. Comparative analysis of mineral contents dissolved in the water from ultrafine particles and regular particles. (mg/100 g powder in water)

Mineral	Regular particles	Ultrafine particles
Fe	0.07	0.16
Zn	0.04	0.08
Ca	1.09	1.65
K	25.54	26.87
Na	2.02	2.81
Al	0.12	0.16
Mg	2.24	3.28
Mn	0.08	0.11

organic solvent ethanol.

The PD/PT ratios from RP and UFP were also calculated. Represented by Rb1/Rg1, PD/PT radio is currently used as an important quality index of ginseng. PD/PT ratio for UFP is 2.18-2.19, higher than 1.44-1.53 for RP.

Comparative Analysis of Mineral Contents

Korean Black-Red Ginseng RP and UFP were dissolved in distilled water, and then the amounts of minerals were analyzed. As indicated in Table 2, 8 minerals were detected more in UFP than in RP. Especially, Fe and Zn in UFP were approximately 2 fold more than in RP. These results suggest that the solubility of minerals from UFP in water increased by particle size reduction.

Discussion

Nanotechnology is expanding rapidly and will affect many aspects of everyday life[22]. Nanomaterials in the form of nanoscale powders and fibers are already being used in sunscreens, cosmetics, food additives, disinfectants, fuel additives, batteries and a range of other products. Nanotechnology has also provided a theoretical basis and technical support for the improvement of the solubility and bioavailability of the bioactive components in drugs and foods[23,24]. However, the exciting production of nanomaterials is not yet matched by the issues regarding its safety and health effect[25-28].

The novel characteristics of nanomaterials arise from the complex interplay between quantum physics and classical mechanics that occur in the nano realm[23]. Therefore, the properties and effects of nanomaterials are often highly unpredictable[29]. The unique and diverse properties of nanomaterials which, at the nanoscale, are quite different from those of the same compounds on a larger scale[30,31]. For example, insoluble substances may easily dissolve at the nano scale. Moreover, nano-sized particles have extreme mobility and are able to cross biological membranes that larger-sized particles normally cannot[29-31].

Ginseng, the root of *Panax ginseng* C.A. MEYER, has been used as a representative tonic for two thousand years in the Far East countries. Although ginseng exhibits multiple pharmacological actions *in vitro* or *in vivo*, its mechanisms on the various efficacies are still elusive. The main molecular components responsible for the action of ginseng are the ginsenoside, which are also known as ginseng saponins, and about 30 different forms have been isolated and identified[32-34]. Ginsenoside is one of the derivatives of the triterpenoid dammarane consisting of 30 carbon atoms; hence ginsenoside has a four-ring, steroid-like structure with sugar moieties attached[35, 36].

This is the first investigation on the properties of ultrafine particles from whole Ginseng in detail. The mean size of the ultrafine particles from Black-Red Ginseng, as a model organic nanosystem, was in the 3.5 μm range, almost nano grade. Notably, about 2 fold more lipids from UFP could dissolve in the water than those from RP in the ethanol, contradicting the general idea that lipid dissolves better in organic solvent than in distilled water. The chemical and physical properties of the biomaterials at the nano scale seem to be significantly different from their larger equivalents in Black-Red Ginseng powder.

The ultrafine particles had much higher contents of ginsenosides, named Re, Rf, Rg1, Rg2s, Rh1, Rb1, Rb2, Rc, Rd, Rg3s, Rg3r and Rh2s than regular particles in ethanol as well as water. Especially, the sum of all the saponins detected from UFP in water was about twice more than from RP in water. The sum of 13 saponins from ultrafine particles in the water was also greater than that from regular particles in the ethanol.

Moreover, the transport and absorption of total carbohydrates or lipids in ultrafine particles across the intestinal wall were better than those in regular particles. Much stronger antioxidant activity, evaluated using TLC and enzymatic detection method was also found in ultrafine particles. Even more cytotoxic effect on the hepatoma cell growth also occurred in the ultrafine particles of Black-Red Ginseng. Taken together, enhanced solubility and cellular transport of the bioactive components could occur by particle size reduction. Pulverization of the whole Korean Black-Red Ginseng into almost nano grade might improve it into a better functional nanofood with higher pharmacological and physical activities. However, it is still unclear how ultrafine particles exert their effects on humans with higher reactive surface area per unit mass, along with unique chemistry and functionality.

Methods

Measurement of Total Carbohydrates

100 mg of Korean Black-Red Ginseng regular particles (RP) as control and the ultrafine particles (UFP) as sample were fully dissolved in 5 mL of distilled water or 80% ethanol. After centrifugation at 5,000 × g for 10 min, the supernatant was used as a sample for this investigation.

Total carbohydrates of RP and UFP in distilled water or in ethanol were calculated using the phenol-

sulfate method[37]. 10 μL of the solutions were diluted with distilled water until the final volumes were 2 mL. After adding 1 mL of 5% phenol, 5 mL of 95.5% H_2SO_4 was added and left at room temperature for 10 min. After cooling in iced-water bath for 15 min, the amounts of carbohydrates were calculated at 492 nm based on the glucose standard curve.

Measurement of Total Proteins

The amounts of proteins were determined according to the Bradford method[38]. 100 mg each RP and UFP were fully dissolved in 5 mL distilled water or 80% ethanol. 50 μL of the dissolved solutions were each adjusted until final volumes were 800 μL, and then 200 μL of the solutions were each added to the Bradford solution. The amounts of proteins were calculated at 595 nm based on the bovine serum albumin concentration curve.

Measurement of Total Lipids

Total lipid content dissolved in 100 mg each of RP and UFP fully dissolved in 5 mL distilled water or 80 % ethanol were calculated using the sulfo-phospho-vanillin method[39]. 50 μL of the dissolved solutions were each adjusted to final volume of 800 μL, then 200 μL of sulfuric acid was added and reacted at 100 ˚C for 10 min. The solutions were cooled in cold water bath for 5 min; thereafter, 10 mL of phospho-vanillin reagents were added at 37˚C for 15 min. After 5 min cooling, total lipid content was calculated at 540 nm using a standard curve with olive oil.

Measurement of Antioxidant Activities Using TLC Method

Korean Black-Red Ginseng RP and UFP (1 g each) were dissolved in 5 mL of distilled water or 80% ethanol to compare their antioxidant activities using TLC method. 0.625 ppm ascorbate was used as a control spot for antioxidant activity. 5 μL of each solution was used to make as identical size spots as possible on TLC plate. Development solution for TLC plate was hexane : acetone with a 6 : 4 ratio. The TLC plate was submerged in 0.15 mM DPPH reactant dissolved in 100% ethanol, and reacted for 10 sec after complete drying. TLC plate was taken out and dried when spots indicating antioxidant activity emerged.

Measurement of Antioxidant Activities Using Xanthine-Xanthine Oxidase Method

The antioxidant activities of RP and UFP were compared using xanthine-xanthine oxidase method. The solution used was 0.75 mM xanthine, 1.5 mM EDTA, 3 mM NBT in 50 mM potassium phosphate buffer (pH 7.8). 50 mU xanthine oxidase was added to 20 μL of each solution, and the absorbance increase at 550 nm was measured.

Construction of Everted Intestine Sac and Digestive Tract Absorption

Everted sac was prepared according to the Chan method[20]. After overnight fasting, Sprague-Dawley rats (150-200 g) were anesthetized by diethyl ether. Lumen of entire intestine was washed with Krebs-Ringer solution (pH 7.4) containing 1 mg/mL glucose; thereafter, a long and thin tube was used to evert the intestine. Of the everted intestine, jejunum and ileum portions were cut into 2-3 cm pieces. For each piece, one end was tied with string, filled with 500 μL of Krebs-Ringer solution (pH 7.4) containing 1 mg/mL glucose, and then the opposite end was tied to make an everted intestine sac.

Everted sacs were put in the 7.5% Korean Black-Red Ginseng RP and UFP solution at 25˚C for 10 min, 30 min, 1 hr, 6 hr, 12 hr and 24 hr. The sacs were quickly washed with Krebs-Ringer solution (pH 7.4) containing 1 mg/mL glucose thereafter. The absorbed biomolecules inside the intestine sacs were used to measure the transport and absorption through everted intestine sac.

Measurement of Ginsenoside Contents

1 g each of RP and UFP were added to 50 mL of distilled water or 80% ethanol, and then ginsenosides were detected following hot water reflux extraction for 1 hr at 80˚C. After filtering with Whatman paper W41, hot water reflux extraction was performed repeatedly. The distilled water and ethanol extracts were extracted with 50 mL of water saturated n-butanol three times using separatory funnel. After washing with 30 mL of distilled water three times the extracts were concentrated, and the final analysis sample was dissolved in high quality methanol. HPLC analysis was carried out using Waters 717 Plus attached with an autosampler and C18 column.

Measurement of Mineral Contents

10 g each of Korean Black-Red Ginseng RP and NP were dissolved in 500 mL of distilled water. After centrifugation at 5,000 × g for 10 min, the supernatant was used for the determination of mineral concentration. Analysis was performed according to the Micronutrient Experiment Method described in Food Code.

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