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—Full Paper—

Effects of Ginsenosides on Organogenesis and Expression of Glutathione Peroxidase Genes in Cultured Rat Embryos

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Abstract. Ginseng has been extensively used around the world for several thousand years as a food or drug. However, recently, several reports have indicated that the organogenesis of cultured embryos is inhibited by treatment with ginsenoside, the principal component of ginseng. In this study, we evaluated the morphological changes of embryos and the gene expression patterns of antioxidant enzymes, 3 types of glutathione peroxidases [*GPx*; cytosolic (*cGPx*), plasma (*pGPx*) and phospholipid hydroperoxide (*phGPx*) forms], in cultured rat embryos (embryonic days 9.5–11.5) exposed to ginsenosides Rb1, Rg1, Re and Rc at levels of 5, 50 and 100 μg/ml. With regard to total morphological scores, no significant differences were noted in the embryos exposed to all doses of ginsenosides, with the exception of 50 μg/ml of Rc. In the cultured embryos exposed to Rg1, a majority of the developmental parameters were normal, but growth of the hind- and mid- brains and the caudal neural tube was significantly increased compared with that observed in the control group ($P<0.05$). Furthermore, Rc significantly enhanced the growth of a variety of developmental parameters in the cultured embryos, with the exception of the hindlimbs. According to the results of our semiquantitative RT-PCR analysis, the levels of *cGPx* and *phGPx* mRNA in the cultured embryos were unaffected by treatment with the ginsenosides. However, the levels of *pGPx* mRNA increased significantly in the embryos treated with ginsenosides Re, Rc and Rb1 compared with the control group ($P<0.05$). These findings indicate that ginsenosides may exert a stimulatory effect on the growth of embryos via differential expression of *GPx* genes.

Key words: Ginsenoside, Glutathione peroxidase (*GPx*), Reverse transcription-polymerase chain reaction (RT-PCR), Rat, Whole embryo culture

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Ginseng, the root and rhizome of *Panax ginseng* C.A. Meyer (*Araliaceae*), is one of the most popular herbs and is utilized in a variety of ways in traditional and herbal medicine to extend the human lifespan. The majority of pharmacological actions of ginseng have been attributed to ginsenosides and have been previously demonstrated in the central nervous, cardiovascular, endocrine and immune systems. In humans, ginseng and its constituents have been demonstrated to exert anti-neoplastic, anti-stress and antioxidant effects [1].

Ginsenosides are generally divided into two groups on the basis of the type of aglycone, the panaxadiol and panaxatriol groups. Whereas the ginsenosides of the panaxadiol group with 20S-protopanaxadiol as a glycone consist principally of Ra, Rb1, Rb2, Rc and Rd, those of the panaxatriol group with 20S-protopanaxatriol as an aglycone consist primarily of Re, Rf, Rg1 and Rg2 [2, 3].

Ginseng is commonly used during pregnancy in humans as a nutritive supplement. In a recent survey, it was reported that 9.1% of pregnant women utilize herbal supplements, including ginseng [4, 5]. However, there have only been a few studies conducted concerning the toxic effects of ginseng during early embryonic development. In those reports, exposure of the embryos of mice and rats to 30–50 μg/ml of ginsenosides Rg1, Rb1 and Re, inhibited, to a significant degree, development of total morphological scores, yolk sac, midbrain, forebrain, etc. [6–9]. Therefore, it should be determined whether or not ginsenosides exert detrimental effects on embryogenesis.

Under normal conditions, oxygen metabolism in aerobic organisms results in generation of dangerous and very reactive oxygen compounds, referred to as free radicals or reactive oxygen species (ROS) [10, 11]. The physiological level of oxidants is generally regulated via antioxidant defense mechanisms, both endogenous and exogenous, which maintain the flux of ROS in circulating blood and organ tissues via specific scavenger reactions and detoxification pathways [12, 13]. Recently, mouse embryos exposed to ROS for short periods were shown to be impaired in terms of embryo differentiation and morphogenesis as a result of oxidative stress [14].

The family of glutathione peroxidases (*GPx*) contains selenocysteine in the active region and ultimately removes organic hydroperoxides using reduced glutathione. Classical or cytosolic GPx (*cGPx*), the first discovered mammalian selenoprotein, is expressed in almost all tissues, including the erythrocytes, kidney, liver, heart, lung, brain, lens, etc. [15–19]. Plasma GPx (*pGPx*) has been detected in milk, plasma and lung alveolar fluid [20]. Phospholipid hydroperoxide GPx (*PHGPx*), an intracellular enzyme,

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Table 1. Primer list of glutathione peroxidase genes

Gene name	Primer	Annealing temperature	Gene accession No.
cGPx	Forward: 5'-TACATTGTTTTGAGAAGTGCG-3', Reverse: 5'-GACAGCAGGGTTTCTATGTC-3'	57 C	NM-008160
pGPx	Forward: 5'-CCTTTTAAGCAGTATGCAGG-3', Reverse: 5'-CTTTCTCAAAGAGCTGGAAA-3	57 C	NM-008161
phGPx	Forward: 5'-ATGCACGAATTCTCAGCCAAG-3', Reverse: 5'-GGCAGGTCCTTCTCTAT-3'	57 C	NM-008162
β-actin	Forward: 5'-CGTGACATCAAAGAGAAGCTGTGC-3', Reverse: 5'-GCTCAGGAGGAGCAATGATCTTGAT-3'	57 C	NM-007393

cGPx: Cytosolic glutathione peroxidase, *pGPx*: Plasma glutathione peroxidase, *phGPx*: Phospholipid hydroperoxide glutathione peroxidase.

can directly reduce lipid hydroperoxide levels in the cell membrane and is also abundantly expressed in the testes [21].

In this study, the whole embryo culture technique [22] was applied to determine the effects of each of the ginsenosides (Rg1, Re, Rb1 and Rc) in rat embryos during the critical period of organogenesis [embryonic days (EDs) 9.5–11.5], and semiquantitative RT-PCR analysis was conducted to determine the gene expression patterns of the *GPx* genes as an antioxidant enzyme.

Materials and Methods

Chemicals and animals

Ginsenosides Rg1, Re, Rb1, and Rc were purchased from the Chromatex Chemicals (Pakistan) and were diluted with dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA). Virgin female Sprague-Dawley (SD) rats were mated overnight in our facilities in an environment maintained at 21 ± 2 C and a relative humidity of 55 ± 10% with a 12-h light/dark cycle. Pregnancy was confirmed the following morning by the presence of vaginal plugs or detection of spermatozoa in a vaginal smear; this day was considered to be ED 0.5. All procedures were conducted in compliance with the "Guidelines for the care and use of animals" (Chungbuk National University Animal Care Committee, according to NIH # 86-23).

Rat serum preparation

The SD rat serum was prepared as follows. After collection, blood samples were immediately centrifuged for 10 min at 3,000 rpm and 4 C to clear the plasma fractions of the cells. Then, the upper fluid was transferred to new tubes. The tubes were recentrifuged for 10 min at 3,000 rpm and 4 C in order to separate the blood cells again. The clear serum supernatant was decanted and pooled. The pooled serum was then heat-inactivated for 30 min at 56 C in a water bath and either used immediately or stored at –70 C. The serum was incubated at 37 C and filtered through a 0.2 μm filter prior to use in culture.

Whole embryo culture

The whole embryo culture system was based on a previously described model [22]. The animals were sacrificed via cervical dislocation at ED 9.5 between 9 and 10 o'clock in the morning, and only embryos with crown-rump lengths of 1.5 ± 0.3 mm were utilized in this experiment. After the removal of decidua and Reichert's membranes, those embryos evidencing intact visceral yolk sacs and ectoplacental cones were placed randomly into sealed culture bottles (3 embryos/bottle) containing 3 ml of culture medium and different concentrations (0, 5, 50 and 100 μg/ml) of ginsenosides Rg1, Re, Rb1 and Rc. The cultures were incubated at 37 ± 0.5 C and rotated at 25 rpm. The culture bottles were initially gassed at 150 ml/min with a mixture of 5% O_2, 5% CO_2 and 90% N_2 over a 17-h period. Subsequent gassing at 150 ml/min occurred over 7 h (20% O_2, 5% CO_2 and 75% N_2) and 24 h (40% O_2, 5% CO_2 and 55% N_2). All embryos were cultured for 48 h using a whole embryo culture system (Ikemoto Rika Kogyo, Tokyo, Japan).

Morphologic scoring

At the end of the 48-h culture period, the embryos were evaluated in accordance with the morphologic scoring system developed by Van Maele-Fabry *et al*. [23]. Only viable embryos evidencing yolk sac circulation and heartbeat were utilized in morphological scoring, and all embryos were alive during the experimental period. Measurements of each viable embryo were obtained with the 17 standard scoring items, yolk sac diameter, crown-rump length and head length. The morphological features assessed principally included the embryonic flexion; heart; neural tube; cerebral vesicles (forebrain, midbrain, and hindbrain); otic, optic and olfactory organs; branchial arch; maxilla; mandible; limb buds (forelimb and hindlimb buds); yolk sac circulation; allantois; and somites.

RT-PCR analysis

Total RNA was extracted from the rat embryos using a Trizol reagent kit (Invitrogen, Carlsbad, CA, USA). Two micrograms of total RNA were utilized for reverse transcription (RT) to generate cDNA using a cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA, USA). The cDNA was employed as a template for PCR reactions with *GPx* or *β-actin* specific primers (Table 1). Each cDNA was amplified with an initial denaturation at 94 C for 2 min; 94 C for 20 sec, 57 C for 10 sec, and 72 C for 30 sec at 25, 30 or 35 cycles; and a final 5-min elongation step at 72 C. The amplified cDNA was then separated on agarose gel. The relative intensities of the *GPx* bands were normalized to the corresponding *β-actin* band intensities. The results were analyzed using an AlphaEase V

Table 2. Growth and development of rat embryos exposed to ginsenoside Rg1 on embryonic day 9.5 for 48 h *in vitro*

Group	Control	Rg1		
Dose (μg/ml)	0	5	50	100
Number of embryos	33	30	30	26
Yolk sac diameter (mm)	3.80 ± 0.26	3.92 ± 0.41	3.85 ± 0.39	3.93 ± 0.56
Yolk sac circulatory	4.44 ± 0.40	4.52 ± 0.39	4.54 ± 0.41	4.57 ± 0.33
Allantois	2.90 ± 0.18	2.97 ± 0.09	2.98 ± 0.08	3.00 ± 0.00*
Flexion	4.93 ± 0.18	4.99 ± 0.05	5.00 ± 0.00	4.94 ± 0.22
Crown-rump length (mm)	3.21 ± 0.23	3.45 ± 0.28	3.29 ± 0.28	3.22 ± 0.39
Head length (mm)	1.68 ± 0.14	1.72 ± 0.20	1.64 ± 0.17	1.64 ± 0.21
Heart	3.55 ± 0.30	3.82 ± 0.29	3.68 ± 0.36	3.71 ± 0.34
Hindbrain	4.61 ± 0.42	4.96 ± 0.19*	4.91 ± 0.21*	4.94 ± 0.15*
Midbrain	4.63 ± 0.37	4.96 ± 0.19*	4.88 ± 0.27*	4.92 ± 0.16*
Forebrain	4.70 ± 0.40	4.97 ± 0.21	4.88 ± 0.38	4.96 ± 0.18
Otic system	4.64 ± 0.34	4.79 ± 0.35	4.65 ± 0.43	4.80 ± 0.31
Optic system	4.17 ± 0.26	4.12 ± 0.17	3.94 ± 0.65	4.08 ± 0.15
Branchial bar	2.12 ± 0.29	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
Maxillary process	2.86 ± 0.18	2.91 ± 0.24	2.89 ± 0.19	2.89 ± 0.16
Mandibular process	2.88 ± 0.18	2.88 ± 0.17	2.87 ± 0.24	2.77 ± 0.30
Olfactory system	2.09 ± 0.40	1.88 ± 0.38	1.72 ± 0.46*	1.90 ± 0.34
Caudal neural tube	4.63 ± 0.47	5.00 ± 0.00*	5.00 ± 0.00*	4.92 ± 0.23*
Forelimb	2.36 ± 0.38	2.24 ± 0.26	2.26 ± 0.19	2.14 ± 0.20
Hindlimb	1.51 ± 0.39	1.47 ± 0.35	1.31 ± 0.30	1.23 ± 0.23
Somites	4.50 ± 0.51	4.67 ± 0.48	4.47 ± 0.51	4.65 ± 0.49
Total score	95.80 ± 3.61	98.39 ± 3.42	95.93 ± 4.15	96.97 ± 3.72

Data represent means ± SD. Asterisks (*) indicate the significant differences compared with the control group (P<0.05).

5.5 analyzer system (Alpha Innotech Corporation, San Leandro, CA, USA).

Statistical evaluation

Group differences were assessed via one-way ANOVA/ Scheffé's test. All analyses were conducted using the Statistical Package for Social Sciences for Windows software, version 10.0 (SPSS, Chicago, IL, USA). Statistical significance was assessed at P<0.05. All data were expressed as means ± SD.

Results

Effects of ginsenosides on organogenesis

The results are shown in Tables 2–5. In the total morphological scores, no significant differences were observed in the embryos exposed to any of the doses of ginsenosides, with the exception of a significant increase induced by treatment with 50 μg/ml of Rc, compared with the control group (P<0.05).

In the embryos exposed to ginsenoside Rg1 (Table 2), the majority of morphological parameters in the developing embryos were within the normal range. However, the scores for the hindbrain, midbrain and caudal neural tube exposed to any dose of Rg1 and the allantois exposed to 100 μg/ml of Rg1 were significantly increased compared with those of the control group. At a concentration of 50 μg/ml, only the olfactory system evidenced a significant reduction (P<0.05).

All organogenic parameters remained unaffected in the embryos exposed to the ginsenoside Re at all concentrations (Table 3).

In the embryos treated with ginsenoside Rb1 (Table 4), a majority of the tested morphological parameters in the developing embryos were within the normal range. However, the score for yolk sac circulation was significantly increased at an Rb1 concentration of 50 μg/ml, whereas the score for the mandibular process was significantly decreased at Rb1 concentrations of 5 and 100 μg/ml (P<0.05).

In the embryos exposed to the ginsenoside Rc (Table 5), the scores for yolk sac circulation, allantois, hindbrain, midbrain, forebrain, otic system and caudal neural tube at all doses and the crown-rump length at a concentration of 50 μg/ml were significantly increased. However, the scores for the optic system, forelimb, and hindlimb at all doses and for the olfactory system and branchial bar at a concentration of 5 μg/ml were significantly decreased (P<0.05). However, the other parameters in the embryos exposed to ginsenoside Rc were within the normal range.

Effects of ginsenosides on the expression of GPx genes

Effects of ginsenoside Rg1 (Fig. 1): In the embryos exposed to ginsenoside Rg1, the expression levels of *cGPx*, *pGPx* and *phGPx* mRNAs were similar to those of the control group.

Effects of ginsenoside Re (Fig. 2): In the embryos exposed to ginsenoside Re, the expression levels of *cGPx* and *phGPx* mRNA did not differ significantly from those of the control group. However, the *pGPx* mRNA level was increased at all concentrations of Re, and there was a significant difference at concentrations of 5 and 50 μg/ml Re (P<0.05).

Effects of ginsenoside Rb1 (Fig. 3): In the embryos exposed to

Table 3. Growth and development of rat embryos exposed to ginsenoside Re on embryonic day 9.5 for 48 h *in vitro*

Group	Control	Re		
Dose (μg/ml)	0	5	50	100
Number of embryos	33	32	28	30
Yolk sac diameter (mm)	3.80 ± 0.26	3.70 ± 0.40	3.77 ± 0.33	3.80 ± 0.37
Yolk sac circulatory	4.44 ± 0.40	4.25 ± 0.37	4.56 ± 0.33	4.50 ± 0.31
Allantois	2.90 ± 0.18	2.87 ± 0.20	2.99 ± 0.06	2.97 ± 0.13
Flexion	4.93 ± 0.18	4.89 ± 0.25	5.00 ± 0.00	5.00 ± 0.00
Crown-rump length (mm)	3.21 ± 0.23	3.13 ± 0.29	3.21 ± 0.26	3.27 ± 0.38
Head length (mm)	1.68 ± 0.14	1.58 ± 0.18	1.61 ± 0.16	1.63 ± 0.21
Heart	3.55 ± 0.30	3.70 ± 0.30	3.81 ± 0.32	3.76 ± 0.32
Hindbrain	4.61 ± 0.42	4.52 ± 0.43	4.60 ± 0.35	4.88 ± 0.18
Midbrain	4.63 ± 0.37	4.58 ± 0.43	4.64 ± 0.37	4.90 ± 0.18
Forebrain	4.70 ± 0.40	4.56 ± 0.44	4.66 ± 0.36	4.87 ± 0.29
Otic system	4.64 ± 0.34	4.26 ± 0.58	4.36 ± 0.41	4.54 ± 0.46
Optic system	4.17 ± 0.26	4.00 ± 0.41	4.23 ± 0.25	4.19 ± 0.24
Branchial bar	2.12 ± 0.29	2.02 ± 0.09	2.03 ± 0.28	2.01 ± 0.23
Maxillary process	2.86 ± 0.18	2.82 ± 0.28	2.78 ± 0.27	2.71 ± 0.36
Mandibular process	2.88 ± 0.18	2.80 ± 0.29	2.77 ± 0.29	2.77 ± 0.35
Olfactory system	2.09 ± 0.40	2.19 ± 0.39	2.05 ± 0.27	2.16 ± 0.39
Caudal neural tube	4.63 ± 0.47	4.22 ± 0.80	4.56 ± 0.61	4.83 ± 0.36
Forelimb	2.36 ± 0.38	2.44 ± 0.45	2.33 ± 0.46	2.22 ± 0.29
Hindlimb	1.51 ± 0.39	1.47 ± 0.33	1.90 ± 2.99	1.38 ± 0.32
Somites	4.50 ± 0.51	4.25 ± 0.44	4.32 ± 0.48	4.50 ± 0.57
Total score	95.80 ± 3.61	93.39 ± 5.07	95.25 ± 4.91	96.12 ± 4.27

Data represent means ± SD.

Table 4. Growth and development of rat embryos exposed to ginsenoside Rb1 on embryonic day 9.5 for 48 h *in vitro*

Group	Control	Rb1		
Dose (μg/ml)	0	5	50	100
Number of embryos	33	26	28	22
Yolk sac diameter (mm)	3.80 ± 0.26	3.63 ± 0.37	3.86 ± 0.29	3.82 ± 0.31
Yolk sac circulatory	4.44 ± 0.40	4.50 ± 0.39	4.81 ± 0.26*	4.56 ± 0.35
Allantois	2.90 ± 0.18	2.80 ± 0.21	2.95 ± 0.12	2.76 ± 0.21
Flexion	4.93 ± 0.18	4.69 ± 0.62	4.98 ± 0.09	4.81 ± 0.38
Crown-rump length (mm)	3.21 ± 0.23	3.05 ± 0.40	3.33 ± 0.36	3.04 ± 0.62
Head length (mm)	1.68 ± 0.14	1.61 ± 0.15	1.71 ± 0.19	1.64 ± 0.16
Heart	3.55 ± 0.30	3.55 ± 0.21	3.74 ± 0.26	3.50 ± 0.30
Hindbrain	4.61 ± 0.42	4.46 ± 0.39	4.83 ± 0.29	4.54 ± 0.35
Midbrain	4.63 ± 0.37	4.49 ± 0.41	4.91 ± 0.17	4.57 ± 0.33
Forebrain	4.70 ± 0.40	4.48 ± 0.43	4.98 ± 0.21	4.61 ± 0.34
Otic system	4.64 ± 0.34	4.42 ± 0.45	4.75 ± 0.37	4.68 ± 0.25
Optic system	4.17 ± 0.26	4.20 ± 0.28	4.17 ± 0.27	4.32 ± 0.27
Branchial bar	2.12 ± 0.29	2.38 ± 0.49	2.15 ± 0.26	2.46 ± 0.39
Maxillary process	2.86 ± 0.18	2.72 ± 0.19	2.80 ± 0.24	2.72 ± 0.20
Mandibular process	2.88 ± 0.18	2.61 ± 0.36*	2.84 ± 0.23	2.62 ± 0.26*
Olfactory system	2.09 ± 0.40	2.12 ± 0.41	2.21 ± 0.43	2.26 ± 0.44
Caudal neural tube	4.63 ± 0.47	4.50 ± 0.45	4.79 ± 0.37	4.44 ± 0.45
Forelimb	2.36 ± 0.38	2.23 ± 0.45	2.31 ± 0.34	2.44 ± 0.43
Hindlimb	1.51 ± 0.39	1.44 ± 0.25	1.36 ± 0.27	1.31 ± 0.27
Somites	4.50 ± 0.51	4.50 ± 0.51	4.75 ± 0.43	4.41 ± 0.59
Total score	95.80 ± 3.61	93.92 ± 4.55	98.40 ± 2.76	94.56 ± 4.54

Data represent means ± SD. Asterisks (*) indicate the significant differences compared with the control group ($P<0.05$).

Table 5. Growth and development of rat embryos exposed to ginsenoside Rc on embryonic day 9.5 for 48 h *in vitro*

Group	Control	Rc		
Dose (μg/ml)	0	5	50	100
Number of embryos	33	40	34	39
Yolk sac diameter (mm)	3.80 ± 0.26	3.98 ± 0.35	4.05 ± 0.37	3.96 ± 0.36
Yolk sac circulatory	4.44 ± 0.40	4.92 ± 0.17*	4.87 ± 0.19*	4.83 ± 0.30*
Allantois	2.90 ± 0.18	3.00 ± 0.00*	2.99 ± 0.05*	3.00 ± 0.00*
Flexion	4.93 ± 0.18	4.99 ± 0.08	4.99 ± 0.05	5.00 ± 0.00
Crown-rump length (mm)	3.21 ± 0.23	3.41 ± 0.28	3.48 ± 0.29*	3.35 ± 0.31
Head length (mm)	1.68 ± 0.14	1.68 ± 0.17	1.82 ± 0.52	1.70 ± 0.14
Heart	3.55 ± 0.30	3.78 ± 0.30	3.75 ± 0.32	3.78 ± 0.27
Hindbrain	4.61 ± 0.42	4.99 ± 0.08*	4.99 ± 0.09*	5.00 ± 0.00*
Midbrain	4.63 ± 0.37	4.91 ± 0.22*	4.99 ± 0.09*	4.98 ± 0.07*
Forebrain	4.70 ± 0.40	4.96 ± 0.14*	5.02 ± 0.13*	5.02 ± 0.10*
Otic system	4.64 ± 0.34	4.93 ± 0.22*	4.97 ± 0.11*	4.89 ± 0.22*
Optic system	4.17 ± 0.26	4.02 ± 0.09*	4.01 ± 0.05*	3.99 ± 0.08*
Branchial bar	2.12 ± 0.29	1.98 ± 0.09*	2.00 ± 0.00	2.00 ± 0.00
Maxillary process	2.86 ± 0.18	2.89 ± 0.18	2.94 ± 0.17	2.89 ± 0.19
Mandibular process	2.88 ± 0.18	2.77 ± 0.28	2.86 ± 0.23	2.79 ± 0.25
Olfactory system	2.09 ± 0.40	1.70 ± 0.28*	1.89 ± 0.30	2.01 ± 0.23
Caudal neural tube	4.63 ± 0.47	4.90 ± 0.20*	4.96 ± 0.14*	5.00 ± 0.00*
Forelimb	2.36 ± 0.38	2.09 ± 0.16*	2.05 ± 0.13*	2.05 ± 0.12*
Hindlimb	1.51 ± 0.39	1.11 ± 0.18*	1.16 ± 0.18*	1.13 ± 0.17*
Somites	4.50 ± 0.51	4.45 ± 0.50	4.76 ± 0.43	4.74 ± 0.44
Total score	95.80 ± 3.61	96.85 ± 2.97	98.98 ± 2.71*	98.08 ± 3.07

Data represent means ± SD. Asterisks (*) indicate the significant differences compared with the control group (P<0.05).

ginsenoside Rb1, there were no significant differences in the *cGPx* and *phGPx* mRNA levels compared with those of the control group. However, the *pGPx* mRNA levels were increased significantly to 172 and 150% at concentrations of 50 and 100 μg/ml of Rb1, respectively (P<0.05).

Effects of ginsenoside Rc (Fig. 4): In embryos exposed to ginsenoside Rc, the expression levels of *cGPx* and *phGPx* mRNAs did not differ significantly from those of the control group. However, the *pGPx* mRNA level was increased at all Rc concentrations, and there was a significant difference at Rc concentrations of 5 and 50 μg/ml (P<0.05).

Discussion

In the present study, we evaluated the developmental and antioxidative effects of ginsenosides Rg1, Re, Rb1 and Rc on cultured rat embryos. It has been demonstrated that ginseng and ginsenosides exert antioxidant effects against a variety of ROSs. They can enhance immunity and central cholinergic system function, inhibit the generation of free radicals and nitrogen oxide and promote the proliferation of neural progenitor cells *in vitro* and *in vivo* [24]. Ginsenosides Rb1, Rb2, Re and Rg1 have been proven effective in reducing cell death due to H_2O_2 treatment and reduced ROS formation in astrocytes [25]. Ginsenoside Rd protects cultured proximal tubule cells against destruction of the cell membrane resulting from hypoxia-reoxygeneration [26, 27]. These findings indicate that ginsenosides can inhibit the lipid peroxidation mediated by free radicals and are useful for the maintenance of cell function.

In previous studies, total morphological scores were significantly reduced at concentrations of 50 μg/ml of Rg1, 30 and 50 μg/ml of Rb1 and 50 μg/ml Re in mice and at 30 μg/ml Re in rats [6–9]. However, Chan *et al*. (2004) reported that ginsenoside Rc caused no differences in the biometric and morphological parameters of the control groups and embryos exposed to 5 and 50 μg/ml [7]. In this study, we demonstrated that most morphological scores were normal or increased significantly in the rat embryos as result of treatment with ginsenosides Rb1, Rg1, Re and Rc, although some of the morphological parameters in the embryos were reduced significantly as a consequence of ginsenoside treatment. Moreover, previous studies have shown that ginsenoside Rg1 promotes proliferation of hippocampal progenitor cells and that ginsenoside Rb2 enhances epidermal cell proliferation by upregulating the expressions of proliferation-related factors, suggesting that ginsenosides have an influence on cellular proliferation [28, 29]. These findings indicate that the effects of ginsenosides on the organogenesis of embryos may remain controversial.

ROS production can be increased within cultured embryos under a variety of conditions, and the overproduction of ROS in cultured mammalian embryos *in vitro* is usually considered to be detrimental to embryonic development [14, 30, 31]. Oxidative stress in embryos influences the gene expression of antioxidant enzymes, which could protect embryos in the early stage of development against peroxidative damage [32]. Ginsenosides exert an antioxidant ability that prevents the decrease of antioxidant enzymes and acts as a free-radical scavenger [33–35]. The regulations of *GPx* mRNA level and activity by ginseng treatment are responsible for

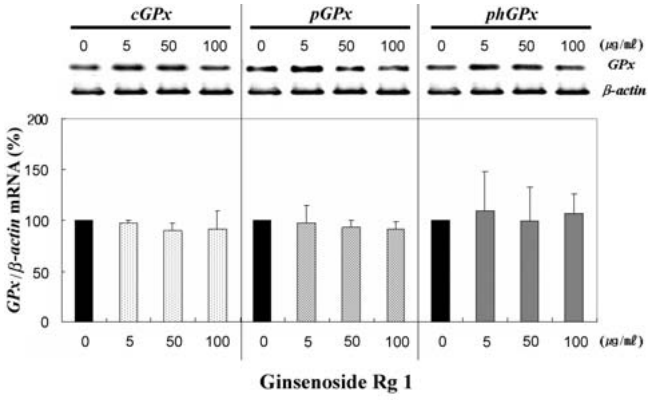

Fig. 1. RT-PCR analysis of glutathione peroxidase (*GPx*) mRNAs in rat embryos exposed to ginsenoside Rg1 on embryonic day 9.5 for 2 days *in vitro*. *cGPx*: cytosolic *GPx*. *pGPx*: plasma *GPx*. *phGPx*: phospholipid hydroperoxide *GPx*.

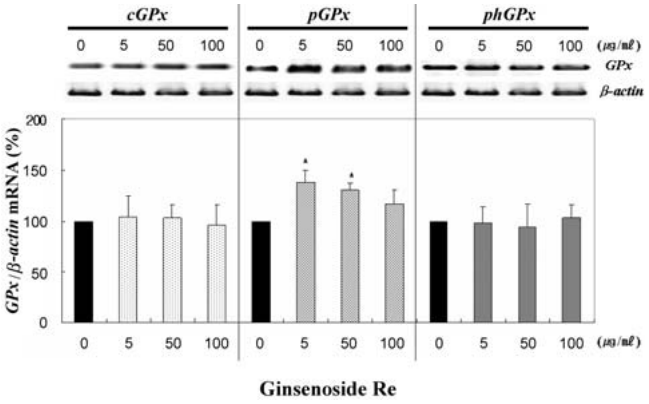

Fig. 2. RT-PCR analysis of glutathione peroxidase (*GPx*) mRNAs in rat embryos exposed to ginsenoside Re on embryonic day 9.5 for 2 days *in vitro*. The mRNA level of plama GPx (*pGPx*) was significantly increased by treatment with 5 and 50 μg/ml of ginsenoside Re (*P<0.05). *cGPx*: cytosolic *GPx*. *phGPx*: phospholipid hydroperoxide *GPx*.

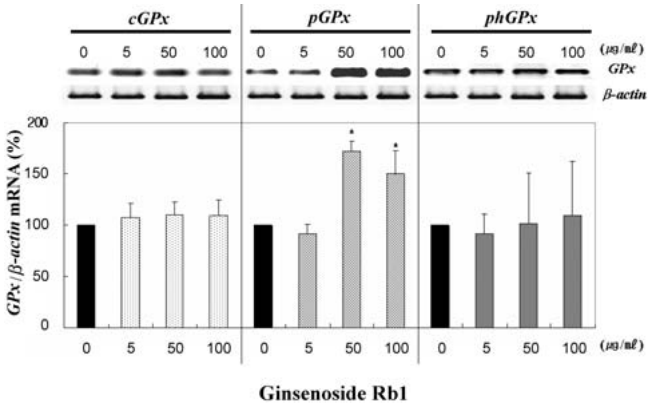

Fig. 3. RT-PCR analysis of glutathione peroxidase (*GPx*) mRNAs in rat embryos exposed to ginsenoside Rb1 on embryonic day 9.5 for 2 days *in vitro*. The mRNA level of plama GPx (*pGPx*) was significantly increased by treatment with 50 and 100 μg/ml of ginsenoside Rb1 (*P<0.05). *cGPx*: cytosolic *GPx*. *phGPx*: phospholipid hydroperoxide *GPx*.

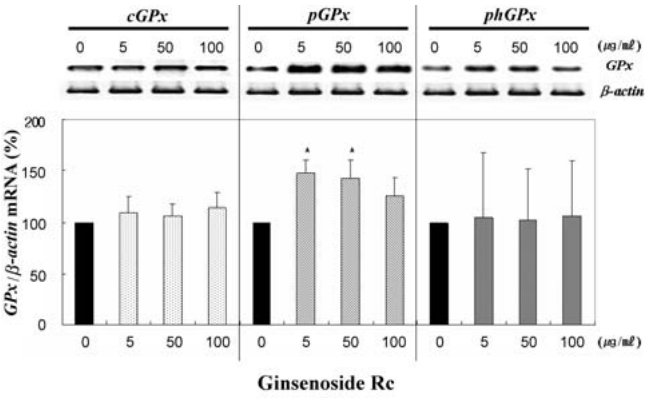

Fig. 4. RT-PCR analysis of glutathione peroxidase (*GPx*) mRNAs in rat embryos exposed to ginsenoside Rc on embryonic day 9.5 for 2 days *in vitro*. The mRNA level of plama GPx (*pGPx*) is significantly increased by treatment with 5 and 50 μg/ml of ginsenoside Rc (*P<0.05). *cGPx*: cytosolic *GPx*. *phGPx*: phospholipid hydroperoxide *GPx*.

protecting the animal against ROS-inducing radiation and can be applied to therapeutic remedies for a variety of ROS-associated diseases [36]. In our previous study, *cGPx* mRNA was shown to be expressed during mouse embryogenesis, distributed in specific cells and organs, and to be abundantly expressed in active differentiation sites [37]. The mRNA of *phGPx* is expressed ubiquitously during embryogenesis. Early embryonic lethality has been previously observed in *phGPx* homozygous mice [38, 39]. *GPx* activity is reduced by fetal dysmorphogenesis in diabetic rats and by aluminum-induced developmental toxicity in the rat brain [40, 41]. These results show that *GPx* may be crucial to embryogenesis.

In accordance with the previous results, ginsenosides Rb1, Rb2, Rd, Re and Rg1 improve *GPx* activity significantly in astrocytes and ginsenoside Rd increases *GPx* activity in aging mice, indicating that ginsenosides attenuate oxidative damage and reduce ROS formation via *GPx* activity [27, 42]. As shown in Figs. 1–4, the *cGPx* and *phGPx* mRNA levels were similar to those of the control group in embryos exposed to ginsenosides Rg1, Re, Rc and Rb1. However, the *pGPx* mRNA levels increased significantly in the groups treated with Rb1, Re and Rc. Although the various roles of *pGPx* have yet to be fully elucidated, *pGPx* has been identified as an important extracellular antioxidant [43]. Mork *et al*. (2003) reported that the *pGPx* mRNA levels were reduced markedly in the Barrett's epithelium (pre-carcinogenic state), which were associated with insufficient secretion of *pGPx* for local antioxidative requirements [44]. Following implantation, rodent embryos obtain oxygen and nutrients via diffusion through the chorion, allantois and amnion [45]. This suggests that ginsenosides may exert an

antioxidative effect during embryogenesis via expression of *pGPx*.

Ji *et al*. (2004) previously reported that following administration of a commercial ginseng capsule (1,500 mg) to healthy men, the maximum Rb1 concentration was 15.9 ng/ml in the plasma, as demonstrated via the LC/MS method [46]. Therefore, in this study, 5 μg/ml of ginsenoside Rb1, as a low dose in the cultured embryos (about 10 mg body weight), was 300 times higher than the human plasma absorption concentration. These data may suggest that ginsenosides at clinical concentrations exert no detrimental effects on embryonic organogenesis because humans ingest ginseng as a total extract form, rather than as a specific ginsenoside form.

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