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Antioxidant Effects of Pyrogallol in Neural Cells and Mice Brain

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마우스 뇌조직과 신경세포에서 pyrogallol의 항산화 효과

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<ABSTRACT>

Polyphenols can easily react with reactive oxygen species (ROS) resulting in powerful antioxidant activity. Pyrogallol is a polyphenol found in a variety of fruits and vegetables. Therefore, plants containing pyrogallol can be used for their antioxidant, anti-bacterial, anti-fungal, anti-allergic, and anti-inflammatory properties. However, its effectiveness as an antioxidant is still a matter of debate because pyrogallol is also a superoxide anion generator and induces superoxide anion-mediated death of several types of cells. In this study, we tried to confirm the antioxidant effects and mechanisms of pyrogallol in mouse neuroblastoma (N2A), human neural stem cells (F3), and mouse brain. Treatments with various concentrations of pyrogallol significantly increased the free radical scavenging ability and reduced lipid peroxidation in N2A and F3 cells in a concentration-dependent manner. In the cytotoxicity test, low concentrations of pyrogallol (0–10 μg/mL) did not significantly influence cell viability, while high concentrations (125-1000 μg/mL) induced death in N2a and F3 cells. Furthermore, treatment with pyrogallol(1.0, 3.2, and 10.0 μg/mL) up-regulated the mRNA expression of superoxide dismutases 1, 2, and 3, glutathione peroxidase (GPx)1, and phospholipid hydroperoxide GPx in N2A and F3 cells, in the brains of mice that were orally administered pyrogallol (1.0, 3.2, and 10.0 mg/kg/day) for 7 days. The mice did not exhibit any toxic effects due to this treatment. Taken together, although pyrogallol induced cell death at high concentrations, it might be a good candidate antioxidant for neurodegenerative disease at low concentrations.

Key words : Pyrogallol, antioxidant, reactive oxygen species, free radical, neuroprotection

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I. Introduction

Polyphenols are naturally occurring phenolic compounds found largely in vegetables, fruits, seeds, nuts, and flowers (Spencer et al., 2008). Fruits like grapes, apples, pears, cherries, and berries contain up to 200–300 mg polyphenols per 100 g fresh weight (Pandey & Rizvi, 2009). Typically, a glass of red wine, or a cup of tea or coffee, contain about 100 mg polyphenols (Pandey & Rizvi, 2009). Cereals, dry legumes, and chocolate also contribute to the polyphenolic intake of humans (Pandey & Rizvi, 2009).

Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens (Beckman, 2000). Especially, they can easily react with reactive oxygen species (ROS) resulting in powerful antioxidant activity (Gu¨lcin et al., 2011). For this reason, diets rich in plant polyphenols offer some protection against the development of cancers, cardiovascular diseases, and neurodegenerative diseases (Arts & Hollman, 2005; Graf, Milbury & Blumberg, 2005). Based on these findings indicating possible beneficial effects on human health, polyphenols are the subject of increasing scientific interest.

Pyrogallol is a polyphenol commonly found in avocados and apricots (Ozturk, 2016). It is used as an active reducer for gold, silver, and mercury salts, a chemical reagent for antimony and bismuth, and a developer in photography and holography (Budavari, 1996; McCann, 1992; Zeiger, 1998). Additionally, pyrogallol is used in the manufacture of pharmaceuticals and pesticides, and has been used for medicinal purposes as a topical anti-psoriatic (Bianco, Handaji & Savolainen, 1998; Budavari, 1996; Grayson, 1985). Owing to its antioxidant properties, pyrogallol is used as a corrosion inhibitor (i.e., oxygen scavenger) in boilers (Zupanovich et al., 1987). Previously, we reported that white rose petal extract (WRPE) displayed a broad spectrum of antioxidant, anti-bacterial, anti-fungal, anti-allergic, and anti-inflammatory properties (Choi et al., 2015; Jeon et al., 2009, Park et al., 2016). We suggested that these effects of WRPE resulted from pyrogallol, a major substance in this extract.

The value of pyrogallol as a therapeutic agent is still a matter of debate. In humans, long-term use of drinking water containing a high pyrogallol content can cause gastrointestinal tract irritation, hemolysis, renal injury, uremia, methemoglobinemia, and death (Zeiger et al., 1998). Chronic application of a topical cream containing pyrogallol resulted in the formation of ulcerated lesions on the hands of one psoriatic individual (Mercado-Feliciano et al., 2013). Also, pyrogallol causes oxidative damage and induces mutagenesis, carcinogenesis, and hepatotoxicity (Mercado-Feliciano et al., 2013; Upadhyay et al., 2008). These pyrogallol-mediated toxicities are due to its oxygen radical generating property (Gupta, Sharma & Chaudhary, 2002; Upadhyay et al., 2008). Specifically, pyrogallol is a superoxide anion generator (Saeki et al., 2000). ROS such as the superoxide anion, hydrogen peroxide, and the hydroxyl radical have been implicated in the regulation of many important cellular events including transcription factor activation, gene expression, cell differentiation, and cell proliferation (Baran et al., 2004; Gonzalez et al., 2002). Excessive production of ROS can initiate events leading to death in several cell types (Simon, Haj-Yehia & Levi-Schaffer, 2000; Wallach-Dayan et al., 2006), and pyrogallol induces superoxide anion-mediated death of mesangial, human lymphoma, human glioma, and Calu-6 lung cancer cells (Han et al., 2009, Moreno-Manzano et al., 2000; Saeki et al., 2000; Sawada et al., 2001). Based on such results, the current study was designed to confirm the antioxidant effects and mechanisms of pyrogallol in mouse neuroblastoma (N2A), human neural stem (F3) cells, and mouse brain.

II. Materials and Methods

1. Measurement of Radical Scavenging Activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich, St. Louis, MO, USA) radical scavenging activity of pyrogallol was measured as described previously (Olney, Rhee & Ho, 1974). Specifically, 10 μL of a solution containing various concentrations of pyrogallol (0-5000 μg/mL), dissolved in distilled water, were placed in a 96-well plate, then 190 μL of 200 mM DPPH, dissolved in methanol, was added. The mixture was incubated at room temperature for 30 min and the absorbance of the reaction mixture was measured at 517 nm with a microplate reader (Molecular Devices, San Jose, CA, USA). DPPH radical scavenging activity was expressed as follows: % scavenging activity = (control absorbance - sample absorbance)/ control absorbance × 100. Ascorbic acid (Sigma-Aldrich) was used as a reference control. All samples were analyzed in triplicate.

2. Measurement of Lipid Peroxidation

Lipid peroxidation was measured by determining the formation of thiobarbituric acid-reactive substances (TBARS) in the brain. Briefly, the brains of ICR mice were dissected immediately after intracardial perfusion with cold phosphate-buffered saline. Brain tissue was homogenized in 10 volumes of cold phosphate-buffered saline and centrifuged at 3000 rpm for 20 min at 4°C to obtain the supernatant. To induce lipid peroxidation, the brain homogenate (450 μL) was mixed with 50 μM ferric chloride (25 μL) in the presence or absence of pyrogallol (25 μL) and incubated for 30 min at 37°C. Sodium dodecyl sulfate (500 µL of an 8.1% w/v solution) and 1 mL of 20% acetic acid (pH 3.5) were added to the brain homogenate and centrifuged. Aliquots of the clear supernatant were mixed with an equal volume of thiobarbituric acid solution (0.8% w/v) and heated in a glass tube capped with aluminum foil at 95°C for 30 min. Samples were cooled on ice, 100 µL of each sample pipetted into 96-well plates, and the absorbance was read at 532 nm with a microplate reader.

3. Cell Culture

N2A (a mouse neuroblastoma cell line) and HB1.F3 (F3; a human neural stem cell) were cultured in Dulbecco's modified Eagle's medium (DMEM; Biowest, Nuaille´, Cholet, France) containing antibiotics (100 IU/mL penicillin and 100 μg/mL streptomycin) plus 10% heat-inactivated fetal bovine serum (Biowest) at 37°C in a 5% CO2/95% air atmosphere. In all experiments, cells were grown until more than 90% confluent and subjected to no more than 20 passages.

4. 3-(4,5–Dimethylthiazol–2–yl)-2,5-Diphenyltetrazolium Bromide (MTT) Assay

The cytotoxicity of pyrogallol was determined via the MTT assay. Briefly, N2A and F3 cells (1 × 106 cells/mL) were seeded into each well of a 96-well plate. After a 24 h incubation, the cells were treated with various concentrations (0-1000 μg/mL) of pyrogallol for 24 h. The cells were then washed twice with fresh medium, 10 µL of MTT (5 mg/mL in DMEM) were added, and incubated for an additional 2 h. The medium was discarded and the formazan formed in the living cells was dissolved in 50 µL of dimethyl sulfoxide. The absorbance was measured at 570 nm within 30 min using a microplate reader. The experiments were performed in triplicate, and mean values are presented.

5. Animals and Treatments

Five-week-old male ICR mice were purchased from Daehan Biolink (Eumseong, Korea). They were housed in an environmentally-controlled room with constant temperature (23 ± 3°C), relative humidity (50 ± 10%), and a 12 h light/dark cycle. The animals were fed standard rodent chow and purified water ad libitum. After acclimation to the laboratory environment for one week, the mice were assigned to treatment groups (n = 6/group). Pyrogallol at 1.0, 3.2, or 10 mg/kg/day, or vehicle (purified water), were administered orally for 7 days. All of experimental procedures were carried out in accordance with the Standard Operating Procedures of the Laboratory Animal Center, Chungbuk National University (CBNU), Korea. This protocol was approved by the Institutional Animal Care and Use Committee of CBNU (approval #: CBNUA-1239-19-02).

6. Hematology and Blood Biochemistry

Blood samples were collected from the caudal vena cava after a 16 h fast, and hematological parameters were measured with a Hemavet 850 (CDC Technologies, Dayton, OH, USA). Blood biochemistry parameters were measured in serum obtained following centrifugation of the blood samples at 3000 rpm for 20 min using a Hitachi 747 blood chemistry analyzer (Hitachi Korea, Seoul, Korea).

7. Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from cells and brain tissue using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. qRT-PCR was performed according to previously described methods (Yon, Kim & Park, 2018). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal standard to normalize expression of the target transcripts. The primer sets used to amplify superoxide dismutase 1 (SOD1), SOD2, SOD3, glutathione peroxidase 1 (GPx1), and phospholipid hydroperoxide GPx (phGPx) are shown in Table 1. Triplicate data were analyzed by five independent assays using the comparative Ct method (Yon, Kim & Park, 2018).

<Table 1> Primer sequences used in the current study

Gene Name	Accession No.	Mouse primers	
		Forward (5' - 3')	Reverse (5' - 3')
GPx1	NM_008160	tgtttgagaagtgcgaagtg	gtgttggcaaggcattcc
phGPx	NM_008162	taagaacggctgcgtggt	gtaggggcacacacttgtagg
SOD1	NM_011434	tgcgtgctgaagggcgac	gtcctgacaacacaactggttc
SOD2	NM_013671	ggagcaaggtcgcttacaga	gtgctcccacacgtcaatc
SOD3	NM_011435	ctcttgggagagcctgaca	cccctggatttgacatggt
GAPDH	NM_008084	cgtgccgcctggagaaacc	tggaagagtgggagttgctgttg
Gene Name	Accession No.	Human primers	
		Forward (5' - 3')	Reverse (5' - 3')
GPx1	NM_000581	acacccagatgaacgagctg	caaactggttgcacgggaag
phGPx	NM_002085	cagtgaggcaagaccgaagt	ccgaactggttacacgggaa
SOD1	NM_000454	atgacttgggcaaaggtgga	gggcgatcccaattacacca
SOD2	NM_000636	ggagaacccaaaggggagttg	gccgtcagcttctccttaaac
GAPDH	NM_001289746	aagaaggtggtgaagcag	gtcaaaggtggaggagtg

8. Statistical Analyses

Statistical comparisons between groups were performed using a one-way analysis of variance followed by Tukey's multiple comparison test. All analyses were conducted using Statistical Package for Social Sciences for Windows software, version 12.0 (IBM, Chicago, IL, USA). Statistical significance was assessed at $p < 0.05$. All data are expressed as means ± standard deviation.

III. Results

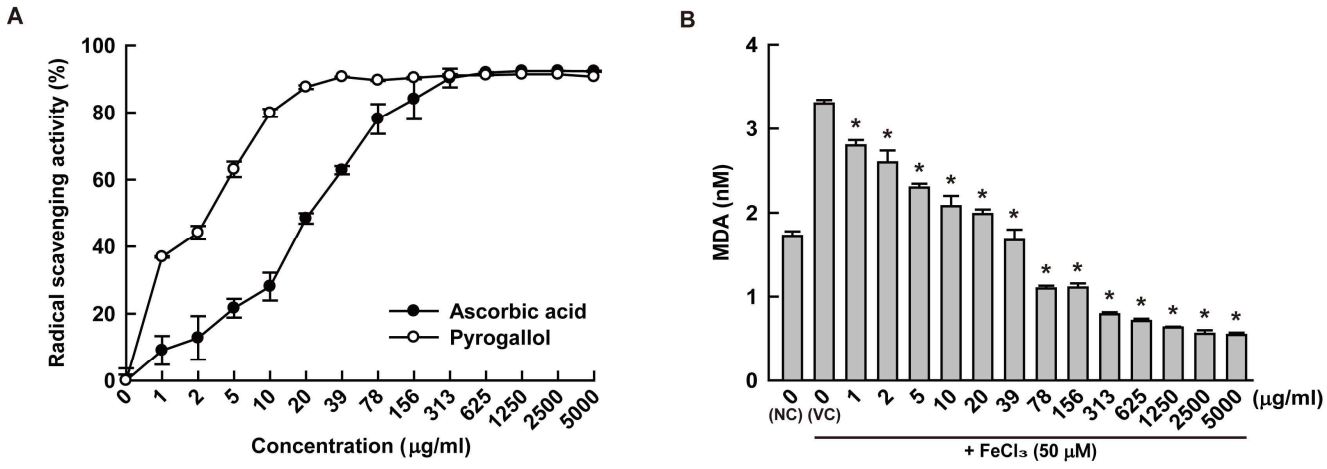
1. Antioxidant Activity In Vitro

The free radical scavenging activities of pyrogallol and ascorbic acid were evaluated by recording the changes of absorbance produced by the reduction of DPPH (Fig. 1A). The effect of pyrogallol plateaued at 20 μg/mL, whereas ascorbic acid reached a plateau at 315 μg/mL in 20 min. Thus, pyrogallol was more potent in scavenging free radicals than ascorbic acid.

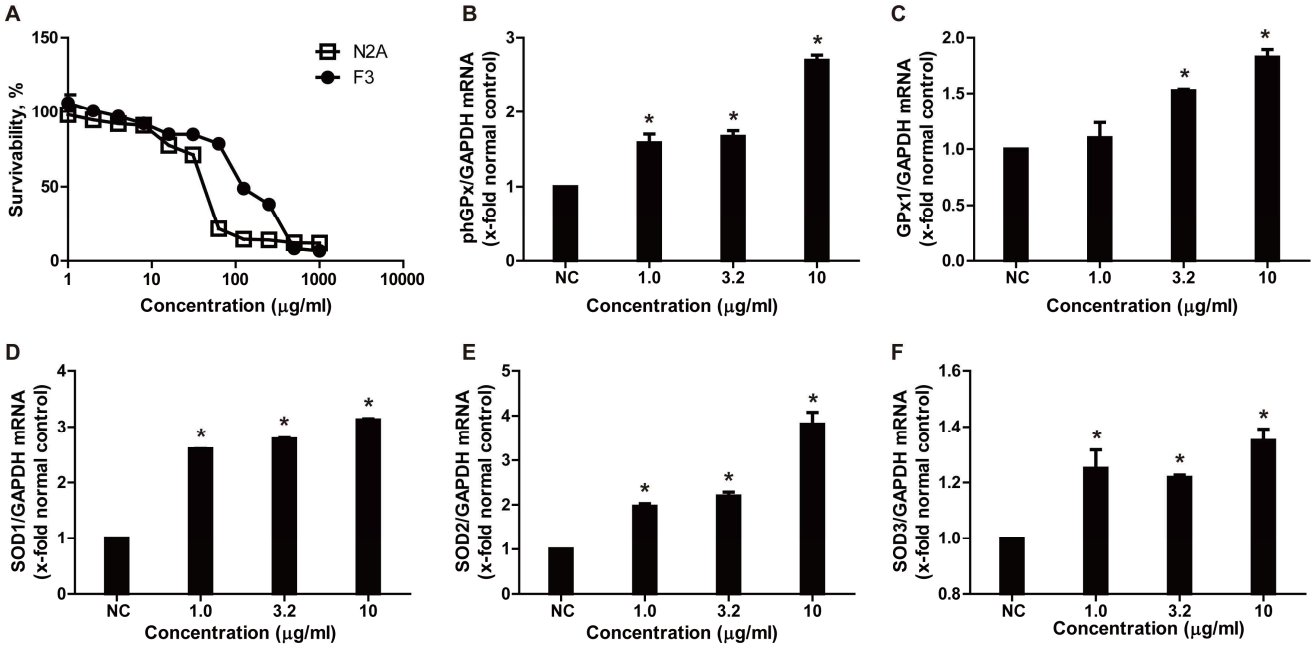
The lipid peroxidation assay showed a 178% increase in the TBARS level after exposure to 50 μM FeCl3 compared with normal control (Fig. 1B). Treatment with various concentrations (0 - 5000 μg/mL) of pyrogallol significantly reduced lipid peroxidation in a concentration-dependent manner.

2. In Vitro Cytotoxicity and Antioxidant Enzyme Induction of Pyrogallol in N2A and F3 cells

To evaluate cytotoxicity, N2A and F3 cells were treated with various concentrations (0-1000 μg/mL) of pyrogallol. As shown Figure 2A, low concentrations (0-10 μg/mL) did not significantly influence cell viability. The concentrations required to inhibit cell viability by 50% were about 35 and 65 μg/mL in N2A and F3 cells, respectively. At concentrations of 125 and 500 μg/mL, pyrogallol almost completely inhibited the viability of N2A and F3 cells, respectively. Thus, we analyzed the antioxidant effects of pyrogallol at concentrations of 1.0, 3.2, and 10.0 μg/mL.



[Figure 1] Antioxidant activities of pyrogallol. (A) 1,1-Diphenyl-2-picrylhydrazyl radical-scavenging activity in 30 min. Results are expressed as a percentage. (B) Inhibitory activity of pyrogallol on FeCl3-induced lipid peroxidation expressed as nM of malondialdehyde (MDA) equivalents of thiobarbituric acid reactive substances. Data are expressed as means ± SD. n = 3 #Significantly different from normal control (NC) ($p < 0.05$). *Significantly different from vehicle control (VC) ($p < 0.05$).



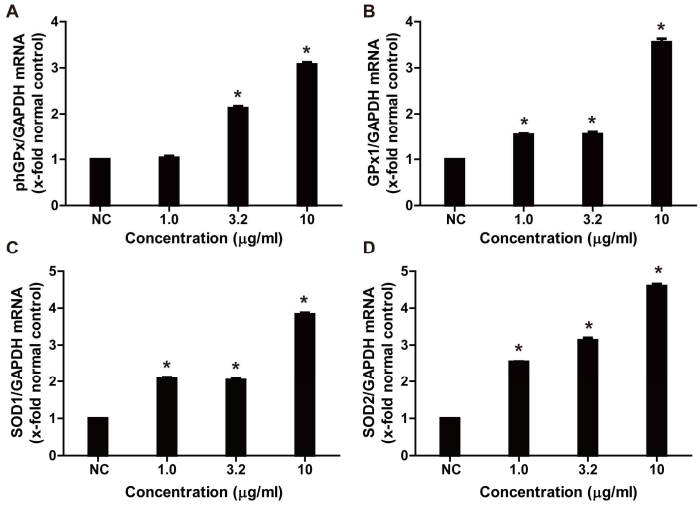
[Figure 2] Toxicity of pyrogallol and its effect on antioxidant enzyme mRNAs. (A) Cytotoxicity of pyrogallol to N2A mouse neuroblastoma and F3 cells using the MTT assay. Real-time polymerase chain reaction analyses of (B) phospholipid hydroperoxide glutathione peroxidase (phGPx), (C) GPx1, (D) superoxide dismutase 1 (SOD1), (E) SOD2, and (F) SOD3 mRNAs in N2A cells normalized to GAPDH mRNA. Data are expressed as means ± SD. n = 3 *Significantly different from normal control (NC) ($p < 0.05$).

As mentioned above, pyrogallol was a more potent antioxidant than ascorbic acid, and lipid peroxidation was reduced by pyrogallol in concentration-dependent manner. Because pyrogallol exhibited a potent antioxidant effect, we investigated its effect on the mRNA levels of SOD1, SOD2, SOD3, GPx1, and phGPx by qRT-PCR. As shown in Figure 2B-F, treatment of N2A cells with pyrogallol significantly enhanced expression of these enzyme genes above their control levels in a concentration- dependent manner. Treatment of F3 cells with pyrogallol for 24 h resulted in similar significant increases in mRNA levels of these antioxidant enzymes (Fig. 3).

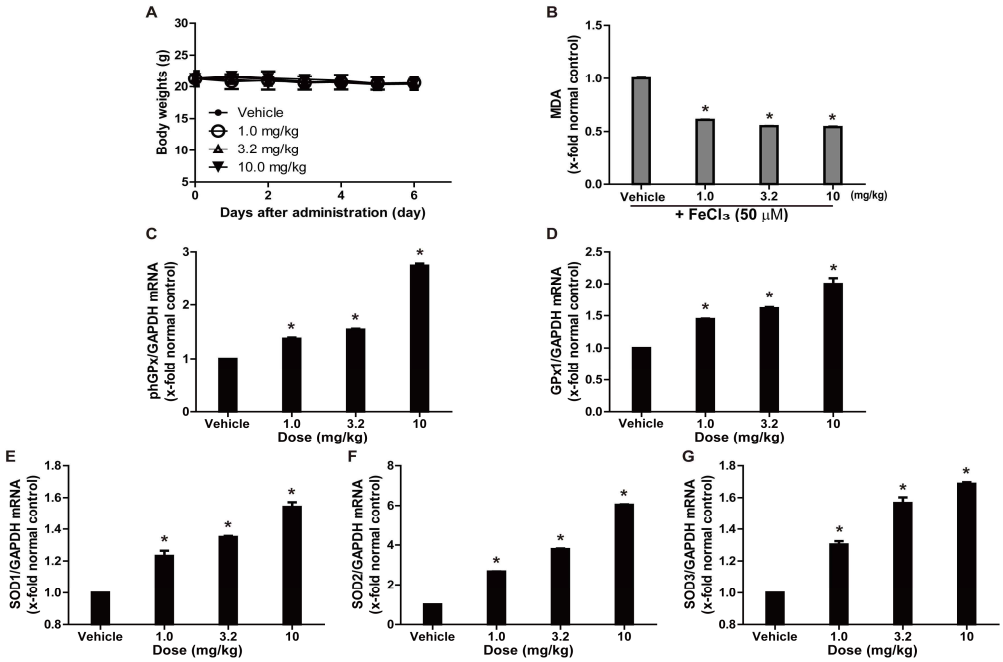
3. Toxicity and Antioxidant Enzyme Induction of Pyrogallol in Vivo

To assess the in vivo toxicity and antioxidant effect of pyrogallol, mice were administered vehicle or pyrogallol orally at 0, 1.0, 3.2, or 10 mg/kg/day for 7 days. These treatments had no effect on body weights (Fig. 4A), nor water and feed consumption (data not shown). Hematology (Table 2) and serum biochemistry (Table 3) analyses showed decreases only in white blood cells, triglycerides, and lactate dehydrogenase in the pyrogallol compared with vehicle group.

To measure the antioxidant effect of pyrogallol in the brain, lipid peroxidation was induced by 50 μM FeCl3. The TBARS concentration was reduced in mice administered pyrogallol in dose-dependent manner to about 0.60, 0.55, and 0.54 fold of control at 1.0, 3.2, and 10.0 mg/kg/day, respectively (Fig. 4B). Coincidentally, the mRNA levels of the antioxidant enzymes GPx1, phGPx, SOD1, SOD2, and SOD3 were up-regulated dose-dependently by administration of pyrogallol (Fig. 4C-G).



[Figure 3] Up-regulation of antioxidant enzyme mRNAs in F3 human neural stem cells treated with pyrogallol. Real-time polymerase chain reaction analyses of (A) phospholipid hydroperoxide glutathione peroxidase (phGPx), (B) GPx1, (C) superoxide dismutase 1 (SOD), and (D) SOD2 normalized to GAPDH mRNA. Data are expressed as means ± SD. n = 3 *Significantly different from vehicle control ($p < 0.05$).



[Figure 4] Antioxidant effects of pyrogallol administered orally for 7 days to male ICR mice. (A) Changes in body weights of control and treated mice. (B) FeCl3-induced lipid peroxidation expressed as nmol of malondialdehyde (MDA) equivalents of thiobarbituric acid reactive substances in brain tissue from mice treated with pyrogallol for 7 days. Real-time polymerase chain reaction analyses of (C) phospholipid hydroperoxide glutathione peroxidase (phGPx), (D) GPx1, (E) superoxide dismutase 1 (SOD1), (F) SOD2, and (G) SOD3 normalized to GAPDH mRNA. Data are expressed as means ± SD. n = 6 *Significantly different from vehicle control ($p < 0.05$).

<Table 2> Hematology of mice treated with or without pyrogallol for 7 days

	Vehicle	Pyrogallol 1.0 mg/kg	Pyrogallol 3.2 mg/kg	Pyrogallol 10.0 mg/kg
WBC (10^3/μL)	2.0 ± 0.3	2.1 ± 0.3	1.4 ± 0.2	1.1 ± 0.0
Neutrophils (%)	46.0 ± 10.1	40.1 ± 0.4	48.2 ± 9.5	46.3 ± 0.3
Eosinophils (%)	2.7 ± 0.7	2.0 ± 0.4	1.4 ± 0.8	1.7 ± 0.4
Basophils (%)	0.3 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.5
Lymphocytes (%)	50.1 ± 10.3	57.8 ± 0.3	49.7 ± 10.3	50.9 ± 0.2
Monocytes (%)	1.0 ± 0.1	0.2 ± 0.3	0.8 ± 0.1	0.9 ± 0.2
RBC (10^6/μL)	10.8 ± 0.1	11.1 ± 0.4	11.0 ± 0.6	10.8 ± 0.4
Hematocrit (%)	60.3 ± 0.6	60.2 ± 1.9	60.4 ± 3.5	59.0 ± 4.0
Hemoglobin (g/dL)	15.9 ± 0.3	16.5 ± 0.8	16.1 ± 1.0	15.7 ± 0.7
MCV (fL)	55.6 ± 1.1	54.4 ± 0.2	55.1 ± 0.4	54.5 ± 1.5
MCH (pg)	14.7 ± 0.1	14.9 ± 0.3	14.7 ± 0.1	14.5 ± 0.1
MCHC (g/dL)	26.4 ± 0.7	27.4 ± 0.6	26.7 ± 0.1	26.6 ± 0.6
Platelets (10^3/μL)	724 ± 10	693 ± 11	740 ± 14	722 ± 9

<Table 3> Serum biochemistry of mice treated with or without pyrogallol for 7 days

	Vehicle	Pyrogallol 1.0 mg/kg	Pyrogallol 3.2 mg/kg	Pyrogallol 10.0 mg/kg
ALT (U/L)	45.1 ± 2.5	44.8 ± 1.4	46.6 ± 3.4	41.8 ± 2.1
AST (U/L)	58.6 ± 5.5	66.5 ± 3.0	68.3 ± 7.3	74.0 ± 18.5
T-proteins (g/dL)	4.9 ± 0.2	5.0 ± 0.3	5.3 ± 0.8	5.0 ± 0.7
Albumin (g/dL)	3.1 ± 0.1	3.2 ± 0.1	3.3 ± 0.4	3.2 ± 0.2
Cholesterol (mg/dL)	133 ± 4	132 ± 7	128 ± 6	134 ± 2
Triglycerides (mg/dL)	79.5 ± 28.7	88.7 ± 13.7	63.5 ± 25.4	33.2 ± 8.3
HDL (mg/dL)	76.5 ± 2.9	77.8 ± 4.3	73.1 ± 1.3	75.1 ± 2.1
LDL (mg/dL)	5.2 ± 0.5	4.7 ± 0.3	4.6 ± 0.3	4.7 ± 0.8
BUN (mg/dL)	22.8 ± 2.1	26.3 ± 4.6	24.7 ± 2.5	27.4 ± 1.8
Uric Acid (mg/dL)	2.3 ± 0.5	2.2 ± 0.7	1.9 ± 0.3	1.8 ± 0.4
Creatinine (mg/dL)	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
LDH (U/L)	470 ± 81	350 ± 80	321 ± 84	282 ± 114

Generally, phenolic and flavonoid compounds are secondary metabolites with high antioxidantand anti-inflammatory properties (Kumar & Pandey, 2013). The in vitro and in vivo activities of phenolic compounds are related to the number of hydroxyl functional groups in their structures (Kumar & Pandey, 2013). Pyrogallol is a benzenetriol carrying hydroxyl groups at positions 1, 2, and 3 (Budavari, 1996). In our previous studies, we reported that WRPE had potent antioxidant and neuroprotective activities against ROS damage (Yang et al., 2013). In addition, WRPE improves allergic dermatitis, skin aging and, especially, ischemic stroke, andkainic acid-induced brain damage via its antioxidant and anti-inflammatory properties (Choi et al., 2015; Jeon et al., 2009; Park et al., 2016; Yang et al., 2013; Yon, Kim & Park, 2018). Based on these characteristics, although WRPE contains other phenolic compounds such as gallic acid, gentisic acid, (+)-catechinic acid, caffeic acid, veratric acid, hesperidin, and cinnamic acid, we assumed that the antioxidant activity of WRPE was caused primarily by pyrogallol (Yon,

Kim & Park, 2018) which is a major substance (43.6%) in the butanol fraction of this extract (Yang et al., 2013). Indeed, in the present study, treatment with pyrogallol decreased free radical production and lipid peroxidation in vivo and in vitro. Furthermore, at low concentrations, it increased the mRNA expression of the antioxidant enzymes SOD1, SOD2, SOD3, phGPx, and GPx1.

Oxygen free radicals contribute to enhanced oxidative stress, leading to subcellular changes including oxidation of membrane phospholipids and proteins (Visavadiya et al., 2016). Pyrogallol autoxidizes rapidly in aqueous solution (Gao et al., 1998) and has been used extensively as a superoxide generator through this autoxidation mechanism (Gupta, Sharma & Chaudhary, 2002; Saeki et al., 2000; Upadhyay et al., 2008). The rate of pyrogallol autoxidation strongly depends on pH as well as its concentration, and the solution forms several intermediate products (Gao et al., 1998; Marklund & Marklund, 1974). Thus, the solution becomes yellow-brown with an absorbance spectrum showing a shoulder between 400 and 425 nm (Marklund & Marklund, 1974). In the current study, 125 μg/mL pyrogallol almost totally inhibited the viability of N2A cells, and 500 μg/ml induced almost complete cell death in F3 cells. Interestingly, at concentrations initiating cell death, the color change of pyrogallol was evident. Thus, it is likely that ROS generated by high concentrations of pyrogallol exceeded its antioxidant effects and induced cell death.

The oral LD50 for pyrogallol is 300 mg/kg in mice (Zeiger, 1998). When mice were treated acutely with pyrogallol by intraperitoneal injection, only near-lethal doses caused convulsions, which were accompanied by distinct cyanosis and occurred only after a sustained period of central nervous system depression (somnolence) (Zeiger, 1998). Also, a short-term dermal study found that repeated pyrogallol treatment sensitized the skin of guinea pigs (CIR, 1991). Administration of pyrogallol in the diet in combination with sodium nitrite in drinking water for 4 weeks induced cell proliferation of the forestomach in rats (Yoshida et al., 1994). However, lifetime dermal exposure of mice and rabbits to low doses of pyrogallol did not induce toxic effects, including carcinogenicity (Stenbäck & Shubik, 1974). Similarly, no toxicity was observed in our study in mice treated orally with pyrogallol (1.0, 3.2, 10.0 mg/kg) for 7 days, though this treatment up-regulated the mRNA expression of antioxidant enzymes in brain tissue.

IV. Conclusions

In the present study, we demonstrated that pyrogallol, a polyphenol, up-regulated antioxidant activity in neuroblastoma and human neural stem cells in vitro, and mouse brain tissue in vivo. The antioxidant activity of phenolic compounds results mainly from their redox properties, which permit them to act as reducing agents, singlet oxygen quenchers, or hydrogen donors. Although pyrogallol is used as a superoxide generator for the SOD activity assay, United States Food and Drug Administration regulations allow for its use as a color additive. However, the results of the current study show that pyrogallol can also up-regulate antioxidant enzyme mRNA levels (and presumably activities) and is relatively non-toxic, thus potentially useful to treat neurodegenerative diseases.

Acknowledgments

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