

Comparison of Cytotoxic Effects of Pentagalloylglucose, Gallic Acid, and its Derivatives Against Human Cancer MCF-7 and MDA MB-231 Cells

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Received November 21, 2013, Accepted December 1, 2013

Key Words : Pentagalloylglucose, Gallic acid, Gallic acid methyl ester, MCF-7 cells, MDA MB-231 cells

1,2,3,4,6-Penta-*O*-galloyl- β -D-glucose (pentagalloylglucose, PGG, **1**) is a naturally occurring hydrolysable tannin that can be found in many medicinal plants, such as *Paeoniae radix* and *Rhus typhina* (Fig. 1).^{1,2} PGG has been found to exhibit a wide range of biological and pharmacological activities, such as anti-diabetic,³ anti-oxidant,⁴ anti-cancer,⁵ anti-inflammatory,⁶ and anti-allergic effects⁷ *in vitro* and *in vivo*. Although promising *in vivo* data has been accumulated, detailed pharmacokinetic studies on PGG might be required to define its exact mechanism, toxicity, or effective dosage for use as a therapy or chemoprevention in several human diseases. On the other hand, cell permeability studies using human intestinal epithelial Caco-2 cells have suggested that PGG has poor bioavailability due to limited transport through the cell membrane, and in part by degradation into tri- and tetragalloyl glucose.⁸

Gallic acid (**2**, GA) and its methyl ester (**3**, MG1) are also common natural products and are known to have anti-inflammatory,⁹ anti-oxidant,¹⁰ and anti-tumor activity.¹¹ We thought that GA could be another possible metabolite of PGG which might be generated by the hydrolysis of PGG in the gastrointestinal tract when it taken orally.¹² Accordingly, we wondered whether the *in vivo* activities of PGG are mediated by direct actions or through its metabolite GA.

Breast cancer is a heterogeneous group of diseases. A large proportion of breast cancer (60-70%) is characterized by high expression of estrogen receptor (ER) or progesterone receptor (PR) or both, indicating that estrogen is required for

tumor growth. 20 to 30 percent of breast cancers express a high level of HER-2. Other breast cancers (15-20%), known as triple-negative breast cancer, do not express ER, PR, and high levels of HER-2 protein.¹³

In the present work, the cytotoxic effects of PGG and GA against breast cancer cells, MCF-7 and MDA-MB231, were examined to compare their activities and to gain insight on the role of GA in the anticancer activity of PGG. We also included GA methyl ester, MG1 and its three semi-synthetic methyl ethers (**4-6**), which were modified by methylation at the phenolic part of GA.

Methyl gallate (**3**, MG1) and its three methyl ethers **4-6** (MG2-4) were synthesized from GA (**2**), as shown in Scheme 1. GA was heated at reflux in methanol in the presence of a catalytic amount of *c*-H₂SO₄ to afford MG1 in 86% yield. MG1 was transformed into its three methyl ethers, MG2-4, by using excess MeI in DMF.¹⁴

To test the effect of ER on the growth inhibitory activities, PGG, GA, and its derivatives MG1-4 were treated with MCF-7 cells in the presence or absence of estrogen, and their cell growth inhibition effects were evaluated by MTT assay (Fig. 2).^{17,18} Among the compounds tested, GA exhibited cytotoxicity most potently in a dose-dependent manner, followed by MG1, PGG, and MG4. The cytotoxic effects of GA and MG1 showed 50% growth inhibition at concentrations of 15.7 \pm 0.6 and 19.2 \pm 0.8 μ M, respectively, in the presence of estrogen. In the same assay system, PGG showed only 36.7 \pm 1.2% inhibition of cell growth at

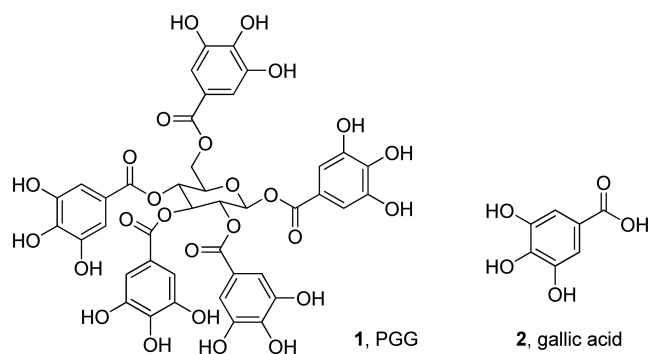
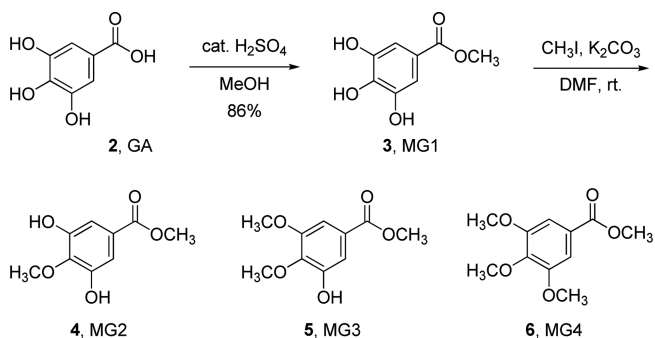


Figure 1. Structures of pentagalloylglucose (**1**) and gallic acid (**2**).



Scheme 1. Synthesis of gallic acid derivatives **3-6**.

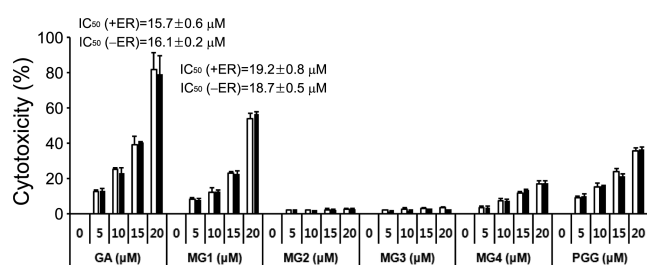


Figure 2. The cytotoxicity of PGG, GA and its derivatives against MCF-7 cells with (unfilled bars) or without estrogen (filled bars).

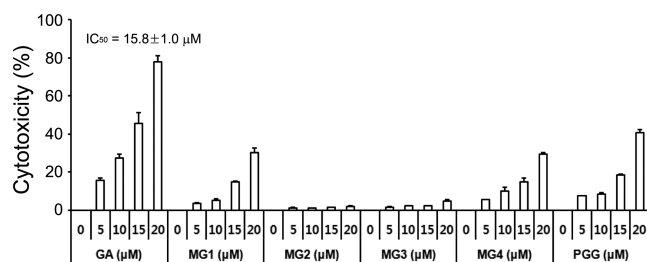


Figure 3. The cytotoxicity of PGG, GA, and its derivatives against MDA-MB231 cells.

20 μM. The cytotoxic effects of GA and its derivatives against MCF-7 cells in the presence and absence of estrogen were not different indicating that estrogen does not influence the growth inhibitory activities of GA, MG1, and PGG against MCF-7 cells.

Next, we measured the cytotoxic effects of GA and its derivatives against MDA-MB231, a prognosis-poor triple-negative breast cancer cell (ER-, PR- and HER-2 negative) (Fig. 3). Among tested compounds, GA again exhibited cytotoxicity most potently with an IC₅₀ at 15.8 ± 1.0 μM, followed by PGG and MG1. PGG and MG1 showed 40.9 ± 1.3 and 30.1 ± 2.6% inhibitions of cell growth, respectively, at 20 μM. The cytotoxic effects of MG2-4, which are *O*-methyl ether derivatives of MG1, against MCF-7 in the presence or absence of estrogen and MDA-MB231 cells were quite weak when compared to those of GA, PGG, and MG1, indicating the important role of hydroxyl groups on the cytotoxic effects against breast cancer cells.

In summary, PGG, GA, and its derivatives MG1-4 were tested for their cell growth inhibitory activities against MCF-7 cells in the presence or absence of estrogen and MDA-MB231 cells. GA showed more potent cytotoxicities than PGG against both human breast cancer cells. MG1, a GA methyl ester, also exhibited comparable cytotoxicities to PGG. Estrogen did not influence the cytotoxic effects of PGG, GA, and MG1. Based on the observed data, it is supposed that GA might play some role in the cytotoxic effect of a PGG *in vivo* model. A detailed study for the comparison of the mechanism of action of PGG, GA, and MG1 is under way.

Acknowledgments. This work was supported by the Basic Science Research Program of the Korean National

Research Foundation (NRF) funded by the Ministry of Education, Science and Technology of Korean Government (#2012-006431).

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- Spectral data of compounds. **Compound MG1 (3)**: ¹H NMR (400 MHz, CD₃OD) δ 7.04 (s, 2H), 3.81 (s, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 167.7, 145.1(2C), 138.4, 120.1, 108.8(2C), 51.0. **Compound MG2 (4)**: ¹H NMR (400 MHz, CDCl₃) δ 7.26 (s, 2H), 3.98 (s, 3H), 3.89 (s, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 167.2, 150.3(2C), 139.8, 125.1, 108.8(2C), 59.4, 51.2. **Compound MG3 (5)**: ¹H NMR (400 MHz, CDCl₃) δ 7.31 (d, 1H, *J* = 2.0 Hz), 7.20 (d, 1H, *J* = 2.0 Hz), 3.96 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 166.8, 152.1, 149.1, 139.7, 125.5, 110.1, 105.6, 60.9, 56.0, 52.2. **Compound MG4 (6)**: ¹H NMR (400 MHz, CDCl₃) δ 7.30 (s, 2H), 3.91 (s, 12H); ¹³C NMR (100 MHz, CDCl₃) δ 166.5, 152.9(2C), 142.1, 125.1, 106.7(2C), 60.8, 56.1(2C), 52.1.
- MCF-7 and MDA-MB231 cell lines were purchased from the Korea Cell Collection Bank (Seoul, Korea). MCF-7 cells were grown in RPMI-1640 medium supplemented with estrogen-free 10% fetal bovine serum (FBS) and 1% antibiotics in the absence or presence of estrogen in an incubator at 37 °C with 5% CO₂. MDA-MB231 cells were grown in RPMI-1640 medium supplemented with 10% FBS and 1% antibiotics in an incubator at 37 °C with 5% CO₂. At 24 h after plating, the medium was changed before starting the treatment with test agents.
- The cytotoxicity test: the cytotoxicity against breast cancer cells was conducted according to experimental procedures described in detail earlier.¹⁷ For cytotoxicity evaluation, the breast cancer cells, MCF-7 (estrogen receptor-positive) and MDA-MB231 (estrogen receptor-negative) in exponential growth phase, were seeded and separately incubated with different concentrations (0-50 μM) for 40 h, and the viability of cells was assessed by the trypan-blue staining method. For trypan-blue staining, the cells (0.1 mL) were suspended in phosphate-buffered saline solution and stained with 0.1 mL of 0.25% trypan-blue solution. The blue-stained dying and non-blue viable live cells were counted. All data are expressed as the mean ± S.D. as the percent viability of cells (*n* = 3). *P* values of ≤ 0.05 were evaluated as significant.
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