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1,2,3,4,6-Penta-*O*-galloyl-β-D-glucose suppresses colon cancer through induction of tumor suppressor

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ABSTRACT

Colon cancer is the third most common malignancy in both sexes of Korea. Here, we investigated anti-colorectal cancer effects of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (PGG), a gallotannin from *Galla rhois,* and its possible mechanisms. PGG induced cytotoxicity and decreased proliferation of colon cancer cells without affecting normal colon fibroblasts. PGG inhibited clonogenic ability and induced apoptosis in cancer cells. One of the underlying mechanisms of the anti-cancer effect exerted by PGG, was owing to the induction p53 expression, a well-known tumor suppressor, and increased in P21, the representative target gene of p53. PGG affected cell-cycle- or apoptosis-related proteins such as cyclin E, CDK2, and Bcl-2, cleaved caspase-3. Also, PGG induced caspase-3/7 activity. These data suggest that PGG exerts anti-colorectal cancer effects.

Currently, there is a strong interest in the development of new anti-cancer agents from natural resources. As part of our ongoing screening program, we are evaluating the anti-cancer potentials of several compounds. One of them, 1,2,3,4,6-penta-*O*-galloyl-β-d-glucose (PGG, Fig. 1) is a gallotannin and a polyphenolic compound present in several medicinal herbs such as *Galla rhois*,[1] *Rhus chinensis Mill*,[2] and *Paeonia suffruticosa*.[3] Many studies have shown that PGG possesses various medicinal properties such as anti-diabetic effect,[4] anti-allergic activity,[5,6] anti-inflammatory activity,[7,8] and anti-cancer effect in prostate cancer,[9–11] breast cancer,[2,12,13] glioma,[14] and hepatocellular carcinoma.[15,16] Although Shaikh's research group showed that PGG was more active in HCT-116 cells, the human colorectal carcinoma cell line (IC_{50} of 1.61 ± 0.36 μM) than in HT-29 cells, the human colon adenocarcinoma cell line, (IC_{50} of 4.46 ± 1.16 μM),[17] the anti-cancer effect of PGG on colon cancer cells has been relatively less studied and its underlying mechanisms remain unclear.

Cancer is a major public health problem and the leading cause of death in Korea in the last decade. In 2016, deaths from cancer constituted 27.8% of the total deaths. The death rate because of cancer was 153.0 per 1000 individuals in 2016, rising by 1.4% from 2015.[18] Among 10 major cancers except for thyroid cancer, colon cancer is the third most common malignancy in both sexes in Korea.[19] For the first time, in 2016 the death rate for colon cancer was higher than that for stomach cancer after the recording of statistical data started in 1983.[18] Until now, the main treatment for localized early-stage colon cancer is surgical resection. In order to augment the chances of cure in high-risk colon cancer patients, adjuvant therapy such as chemotherapy and radiotherapy in combination with surgical resection has been conducted.[20,21] However, chemical treatment and radiation have harmful effects on normal cells because these therapies affect all proliferative cells containing normal cells. Therefore, it is necessary to provide new agents that selectively kill cancer cells without affecting normal cells. From this point of view, there is an increasing need to search for bioactive compounds from natural products having anti-cancer effect with relatively low side effects.

Therefore, in this study we investigated anti-colorectal cancer effects of PGG, a bioactive compound rich in nature, and possible mechanisms.

PGG used in this study was extracted from *Galla rhois* as described in a previous study.[1] The purity was ~98%. Firstly, CytoTox96® Non-Radioactive Cytotoxicity Assay (Promega, Madison, WI, USA) was employed to investigate the optimum time at which PGG induced cytotoxicity, according to the manufacturer's instructions. We found that PGG exerted cytotoxicity at 48 h in both types of colon cancer cells (Fig. 2A). Therefore, all subsequent experiments investigated the effect of PGG in colon cancer cell at 48 h unless mentioned. Next, to verify dose dependency of PGG, HCT-116 and HT-29 cells were treated with different doses of PGG (Fig. 2). As shown in Fig. 2B and C, PGG induced cell cytotoxicity and inhibited proliferation of colon cancer cells; HCT-116 cells being more sensitive to PGG than HT-29 cells. Lu's study

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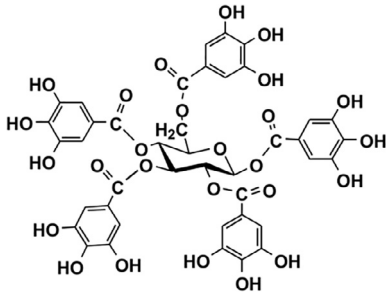

Fig. 1. Structure of PGG.

showed that *Oldenlandia diffusa* extracts (ODE) had low IC_{50} in p53-wild HCT-116 cells,[22] while it was relatively high in p53-mutant HT-29 cells. In addition, PGG was more active in HCT-116 cells than in HT-29 cells.[17] These results are consistent with our result. In addition, PGG did not exert cytotoxicity or inhibition of proliferation in colon fibroblast normal cell line, CCD-18 Co cells (Fig. 2D). These results suggest that PGG selectively exerts cytotoxic and anti-proliferative activity against colon cancer cells without affecting the normal cells.

Clonogenic potential assay or colony formation assay is used to assess *in vitro* cell survival based on the ability of a single cell to grow into a colony. It is used to investigate the ability of every cell in a population to undergo "unlimited" divisions and is essentially a suitable system to estimate long-term cytotoxic drug effects *in vitro*.[23] To demonstrate the inhibitory effect of PGG on clonogenic potential of colon cancer cells, we performed the colony formation assay with crystal violet staining (Sigma-Aldrich, St. Louis, MO, USA). PGG treatment led to decreased colony formation in a dose dependent manner in both the cancer cells (Fig. 3).

To confirm the anti-proliferative activity and verify the cell killing mechanism(s) of PGG, we conducted cell cycle analysis using the Muse™ Cell Cycle Kit (Merk Millipore, Darmstadt, Germany). The percentage of S-phase cells and G_2/M-phase cells remained relatively steady in both PGG treated cell lines (Fig. 4). On the other hand, PGG treatment was shown to decrease G_0/G_1 population. PGG appeared to

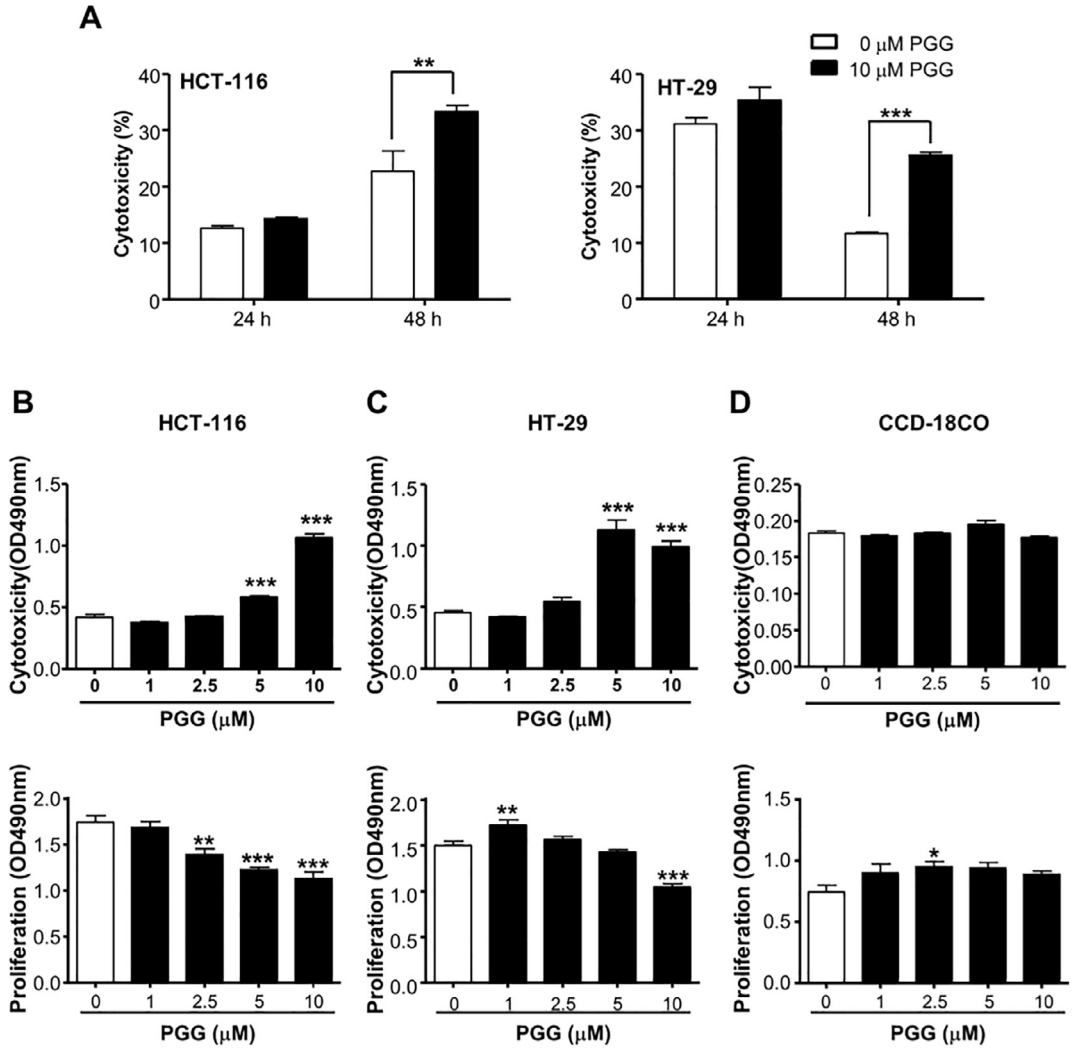

Fig. 2. Induction of cytotoxicity and decreases in proliferation of colonic cancer cells by PGG. (A) HCT-116 and HT-29 cells treated with PGG for 24 h or 48 h each. (B) HCT-116 cells, (C) HT-29 cells, and (D) CCD-18Co cells treated with indicated concentrations of PGG for 48 h. Cytotoxicity and proliferation were determined by the methods described in the main text. The results are reported as the mean ± S.E.M. of three independent experiments (n = 4). Statistical significance is based on the difference when compared with 0 μM PGG by (A) two-way ANOVA followed by Bonferroni test or by (B–D) one-way ANOVA followed by Dunnett's test. ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).