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Research article

Increase in apoptotic effect of *Panax ginseng* by microwave processing in human prostate cancer cells: *in vitro* and *in vivo* studies

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ABSTRACT

Background: Ginseng, which is widely used in functional foods and as an herbal medicine, has been reported to reduce the proliferation of prostate cancer cells by mechanisms that are not yet fully understood.
Methods: This study was designed to investigate the changes in ginsenoside content in ginseng after treatment with a microwave-irradiation thermal process and to verify the anticancer effects of the extracts. To confirm the anticancer effect of microwave-irradiated processed ginseng (MG), it was tested in three human prostate cancer cell lines (DU145, LNCaP, and PC-3 cells). Involvements of apoptosis and autophagy were assessed using Western blotting.
Results: After microwave treatment, the content of ginsenosides Rg1, Re, Rb1, Rc, Rb2, and Rd in the extracts decreased, whereas the content of ginsenosides 20(S)-Rg3, 20(R)-Rg3, Rk1, and Rg5 increased. Antiproliferation results for the human cancer cell lines treated with ginseng extracts indicate that PC-3 cells treated with MG showed the highest activity with an half maximal inhibitory concentration of 48 μg/mL. We also showed that MG suppresses the growth of human prostate cancer cell xenografts in athymic nude mice as an *in vivo* model. This growth suppression by MG is associated with the inductions of cell death and autophagy.
Conclusion: Therefore, heat processing by microwave irradiation is a useful method to enhance the anticancer effect of ginseng by increasing the content of ginsenosides Rg3, Rg5, and Rk1.

1. Introduction

Prostate cancer is a commonly diagnosed tumor in men that represents a broad spectrum of severity, ranging from indolent to highly lethal [1]. Because prostate cancer cannot grow or differentiate without androgens, hormone therapy has become the standard treatment for prostate cancer [2]. However, cancer recurrence normally develops in years when the patient with prostate cancer no longer responds to hormone therapy. Therefore, chemotherapy with cytotoxic agents has been suggested as an alternative growth inhibitor for hormone-refractory prostate cells [3]. However, the effectiveness of cytotoxic agents against prostate cancer cells is markedly diminished due to the slow proliferation of these cells [4].

Several anticancer agents inhibiting proliferation of cancer cells, inducing apoptosis, or modulating signal transductions are currently used for the treatment of cancers, and a combination of multiple chemopreventive agents with multiple targets is considered to be more effective [5]. Therefore, herbal therapy has been suggested, partly because herbal medicines consist of several constituents with multiple targets, and partly because there is a long history of using herbal medicines in Asian and European countries [6]. Natural products have appreciably contributed to the development of a large number of anticancer drugs. Approximately 50%

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of all anticancer drugs approved internationally are either natural products or natural product mimics and were developed based on the knowledge obtained from small molecules or macromolecules existing in nature [7–9].

Ginseng, generally the root of *Panax ginseng* Meyer, has been used in Oriental medicine and is now used widely around the world. There are a variety of commercial ginseng products available, such as white, red, sun, and black ginsengs [10,11]. More than 30 different ginsenosides have been identified and isolated, all with several pharmacological effects. Ginsenosides are divided into 20(S)-protopanaxadiols (ginsenoside Rb1, Rb2, Rc, Rd, and Rg3) and 20(S)-protopanaxatriols (ginsenoside Re and Rg1) groups on the basis of their aglycone moieties (Fig. 1) [11,12].

Recently, our group developed a novel ginseng extract by microwave-assisted processing. This novel microwave-irradiated processed ginseng (MG) extract has increased content of ginsenosides Rg3, Rg5, and Rk1 [13]. In this study, we demonstrate that MG inhibits prostate cancer cell growth in three human prostate cancer cell lines (DU145, LNCaP, and PC-3 cells). Furthermore, we sought to investigate the anticancer efficacy of MG on the growth of prostate cancer cells *in vivo* (as xenografts in athymic nude mice).

2. Materials and methods

2.1. Chemicals and reagents

Dulbecco modified Eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen Co. (Grand Island, NY, USA). EZ-Cytox enhanced cell viability assay kit was purchased from ITSBIO (Seoul, Korea). Ginsenoside standards Rb1, Rb2, Rc, Rd, Re, 20(S)-Rg3, 20(R)-Rg3, Rk1, and Rg5 were purchased from Ambo Institute (Seoul, Korea). Monoclonal antibodies against cleaved caspase-8 and β-actin and polyclonal antibodies against cleaved caspase-3, cleaved caspase-9, Bcl-2, Bax, and poly (ADP-ribose) polymerase (PARP) were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). The water and acetonitrile used were of HPLC grade from Fisher Scientific

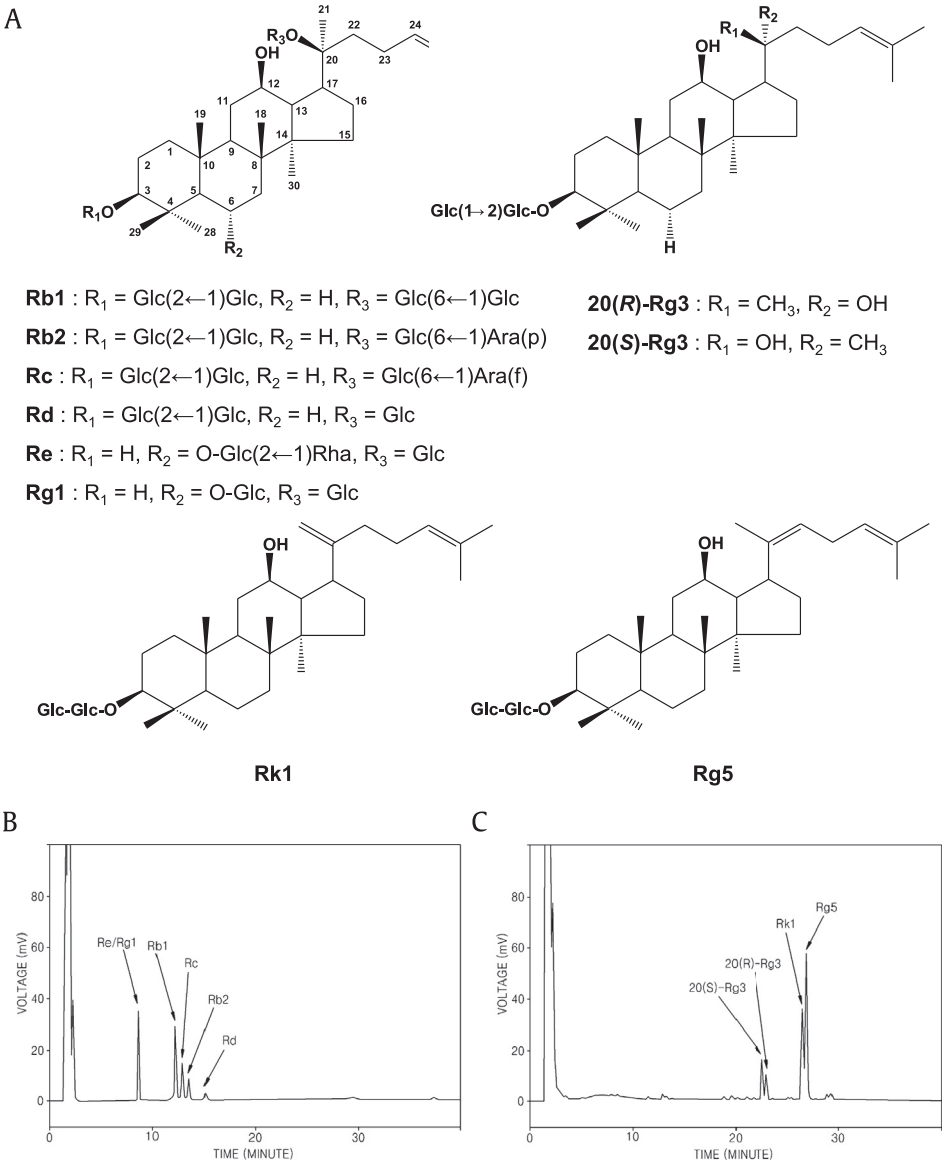

Fig. 1. Structures and analysis of ginsenosides. (A) Structures of ginsenosides contained in *Panax ginseng*. (B) HPLC chromatogram analyzing ginsenosides (Re/Rg1, Rb1, Rc, Rb2 and Rd) in processed ginseng by microwave. (C) HPLC chromatogram analyzing ginsenosides (20(S)-Rg3, 20(R)-Rg3, Rk1, and Rg5) in processed ginseng by microwave. -Glc, D-glucopyranosyl; -Rha, L-rhamnopyranosyl; -Ara(f), L-arabinofuranosyl; -Ara(p), L-arabinopyranosyl.