

A novel radiolytic rotenone derivative, rotenoisin A, displays potent anticarcinogenic activity in breast cancer cells

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ABSTRACT

Chemotherapy for cancer treatment has therapeutic limitations, such as drug resistance, excessive toxic effects and undesirable adverse effects. Therefore, efforts to improve the safety and efficacy of chemotherapeutic agents are essential. Ionizing radiation can improve physiological and pharmacological properties by transforming structural modifications of the drug. In this study, in order to reduce the adverse effects of rotenone and increase anticancer activity, a new radiolytic rotenone derivative called rotenoisin A was generated through radiolytic transformation. Our findings showed that rotenoisin A inhibited the proliferation of breast cancer cells and increased the rate of apoptosis, whereas it had no inhibitory effect on primary epidermal keratinocytes compared with rotenone. Moreover, rotenoisin A-induced DNA damage by increasing reactive oxygen species (ROS) accumulation. It was also confirmed not only to alter the composition ratio of mitochondrial proteins, but also to result in structural and functional changes. The anticancer effect and molecular signalling mechanisms of rotenoisin A were consistent with those of rotenone, as previously reported. Our study suggests that radiolytic transformation of highly toxic compounds may be an alternative strategy for maintaining anticancer effects and reducing the toxicity of the parent compound.

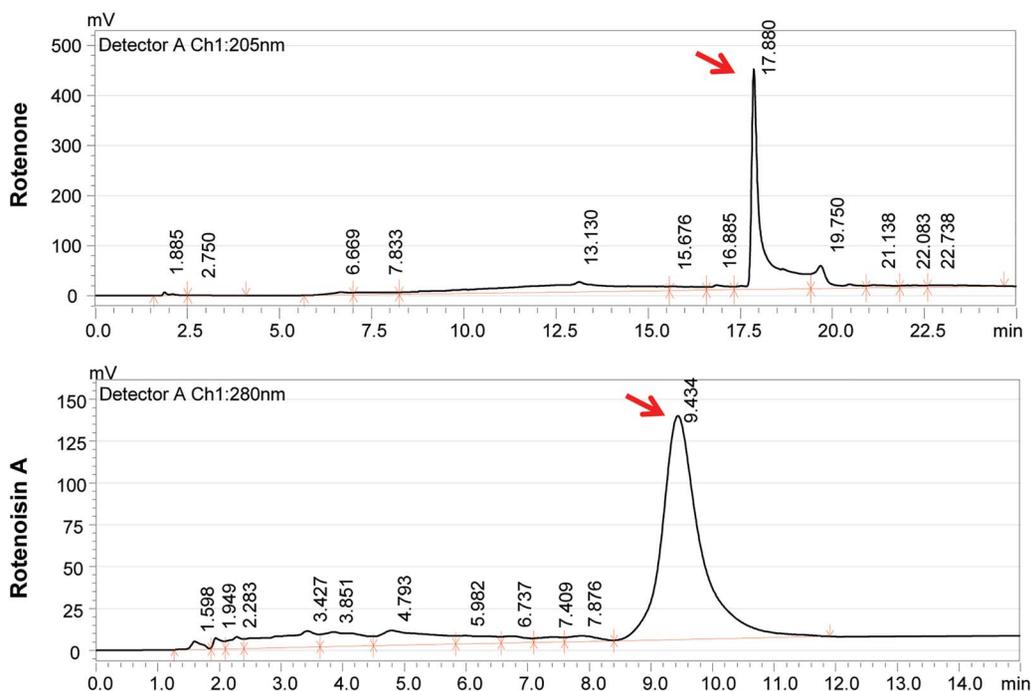
Keywords: rotenone; rotenoisin A; breast cancer; radiolytic transformation; mitogen-activated protein kinase (MAPK)

INTRODUCTION

Breast cancer is the most commonly diagnosed cancer in women worldwide, after non-melanoma skin cancer [1]. Breast cancer is classified into three main subtypes depending on the presence of molecular markers for oestrogen and progesterone receptors and human epidermal growth factor 2 (ERBB2; formerly HER2): hormone receptor-positive/ERBB2-negative (70% of patients), ERBB2-positive (15–20%) and triple-negative (tumour without all three standard molecular markers: 15%) [2]. Breast cancer treatment combines surgery, radiation therapy, chemotherapy and hormonal therapy, depending on the patient's condition, grade, stage and molecular tumour subtype [3]. Among these, chemotherapy is routinely administered

to early-stage breast cancer patients as adjuvant therapy to reduce tumour size or cancer recurrence before and after mastectomy. Representative chemicals currently used to treat early breast cancer include doxorubicin, cyclophosphamide, paclitaxel, trastuzumab, carboplatin, fluorouracil and epirubicin [4, 5]. Various combinations of these chemicals are generally recommended for early-stage breast cancer patients and play an important role in the direction of tumour treatment and patient survival. However, the typical drawbacks of these drugs, such as drug resistance and unnecessary adverse effects, remain important challenges to be addressed. In particular, due to adverse effects such as nausea, vomiting, fatigue and joint pain, 28% of breast cancer patients did not continue with their prescribed chemotherapy [6].

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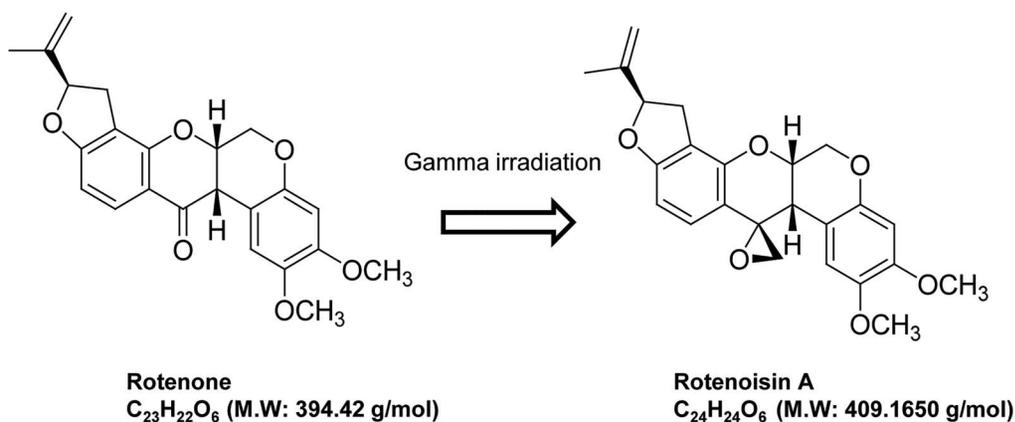


Fig. 1. Ionizing radiation induced changes in the molecular properties of rotenone. (A) Chromatograms of rotenone (top) and rotenoisin A (bottom). (B) Structure of rotenone and rotenoisin A. These data we modified from Park *et al.* 2013 [15].

Most chemotherapeutic drugs cause DNA damage during replication or destroy rapidly growing cancer cells by other mechanisms [7–9]. However, the drug can damage fast-growing normal cells, causing severe adverse effects. Additionally, in the case of tumours that are resistant to chemotherapeutic drugs, a higher dose is required, which increases cytotoxicity to normal tissues and increases the incidence of multiple drug resistance. Indeed, the severe adverse effects of chemotherapy drugs in healthy tissues and organs are a major cause of increased mortality in cancer patients, limiting chemotherapy. However, limiting the concentration of the drug can minimize adverse effects and damage to normal cells, but the therapeutic efficacy on tumour cells is also significantly reduced. Therefore, it is desirable to

develop chemotherapeutic agents with enhanced therapeutic effects and minimal toxicities to normal tissues.

Recently, ionizing radiation has attracted attention as an alternative to improve the bioactivity of drugs and improve their therapeutic efficacy in tumours. Ionizing radiation can enhance pharmacological properties and anticancer activity by inducing structural modifications to the drug [10, 11]. However, research related to radiation conversion is still very limited. We irradiated dexamethasone, a representative anticancer agent in previous studies, to produce a dexamethasone derivative (Dex-IR) with new chemical characteristics [12]. Dex-IR effectively induced apoptosis through caspase–poly(ADP-ribose) polymerase (PARP)-dependent pathways in non-small cell lung

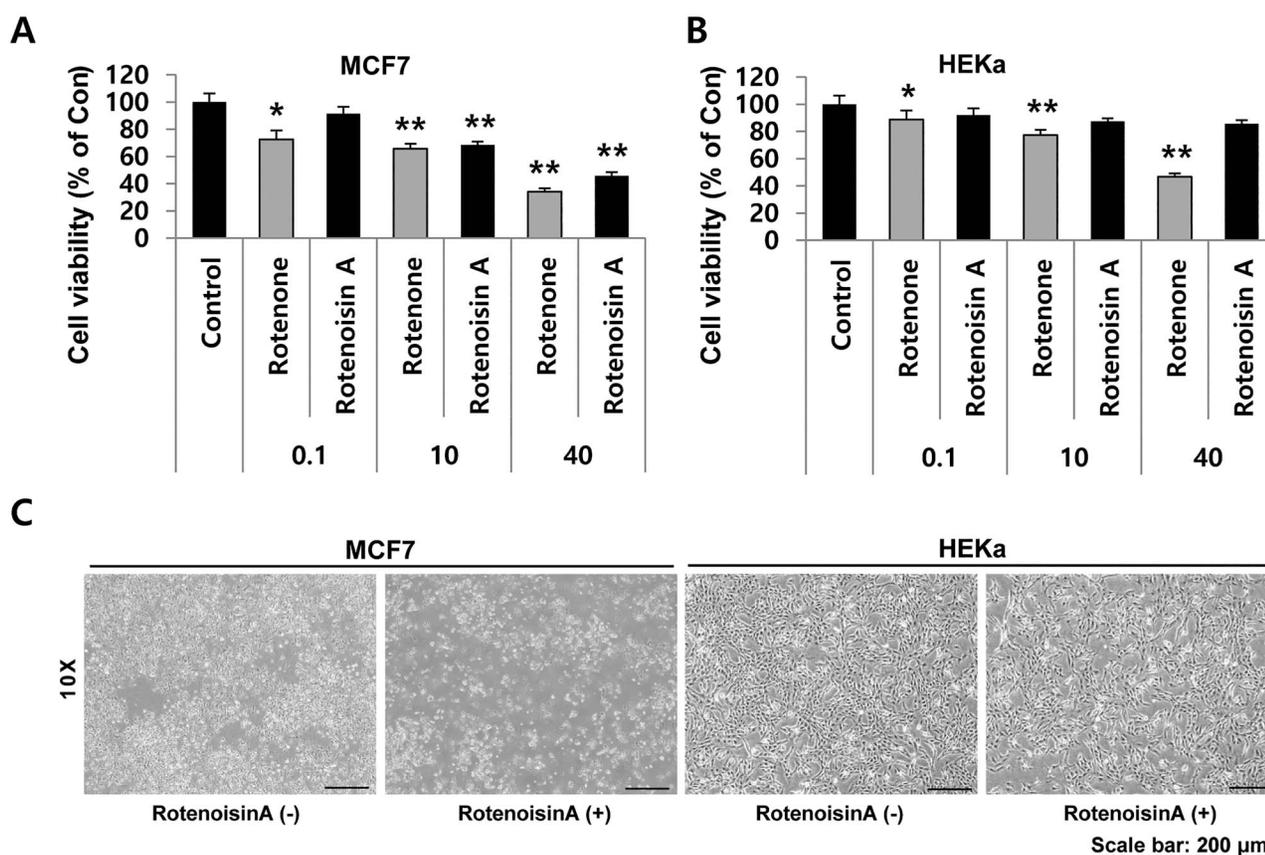


Fig. 2. Rotenoisin A inhibited the proliferation of breast cancer (MCF-7) cells but not primary epidermal keratinocytes (HEKa). (A) The MCF-7 cell line was treated with increasing concentrations of rotenone and rotenoisin A for 24 h. The effects of rotenoisin A on the viability of MCF-7 cells at the indicated concentrations were determined using the CCK-8 assay and compared with non-treated cells. (B) HEK293 cells were treated with increasing concentrations of rotenone and rotenoisin A for 24 h. The effects of rotenoisin A on the viability of HEK293 cells at the indicated concentrations were determined using the CCK-8 assay and compared with those of the rotenone-treated cells. Data are presented as the means \pm SEM of three independent experiments ($P \leq 0.05$). (C) Representative images of the morphological changes in MCF-7 and HEK293 cells were observed at 24 h after rotenone and rotenoisin A treatments, respectively. Scale bar: 200 μ m. Con, control.

cancer (NSCLC). In addition, we confirmed that new compounds are induced by a radiolytic transformation in the isoflavonoid, rotenone. In particular, it has been demonstrated that rotenoisin B, a radiolytic rotenone derivative, effectively inhibits the proliferation of liver cancer cells while reducing adverse effects. Rotenone is an anticancer substance known to induce apoptosis through the activation of reactive oxygen species (ROS) and JNK/p38 mitogen-activated protein kinase (MAPK) in the human breast cancer line MCF-7 [13]. However, low doses of rotenone (<10 nM) cause oxidative damage and death of dopaminergic neurons, and long-term intake has been reported to induce Parkinson's disease [14]. Recently, we demonstrated the radiolysate, rotenoisin A, using the radioactive transformation method of the isoflavonoid rotenone field by γ -irradiation [15]. After material modification with irradiation in the liquid chromatography analysis, the retention time was reduced from 17.88 min (rotenone) to 9.434 min (rotenoisin A). The secondary product was found to have a different mass (Fig. 1A), and the modified structure was elucidated using spectroscopy (Fig. 1B), adopted from Chul-Hong Park's study

and modified [15]. In this study, we attempted to generate new radiolytic rotenone derivatives through radiolytic transformations to reduce the adverse effects of rotenone and increase its anticancer activity. This study aimed to evaluate the effect of radiolytic rotenone, rotenoisin A, on human breast cancer MCF-7 cells and determine its utility as a novel anticancer target for breast cancer.

MATERIALS AND METHODS

Materials and reagents

Rotenone, Cell Counting Kit-8 (CCK-8) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anti-Bax (#5023), anti-Bcl2 (#2870), anti-caspase 9 (#95020), anti-PARP (#9532), anti-cleaved PARP (#9541), anti-ERK (#4695), anti-p-ERK (#4377), anti-p38 (#9212), anti-p-p38 (#9215), anti-JNK (#9252), anti-p-JNK (#9251), anti-H2AX(#9718) and anti-GAPDH (#2118) were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). RPMI 1640, penicillin/streptomycin and

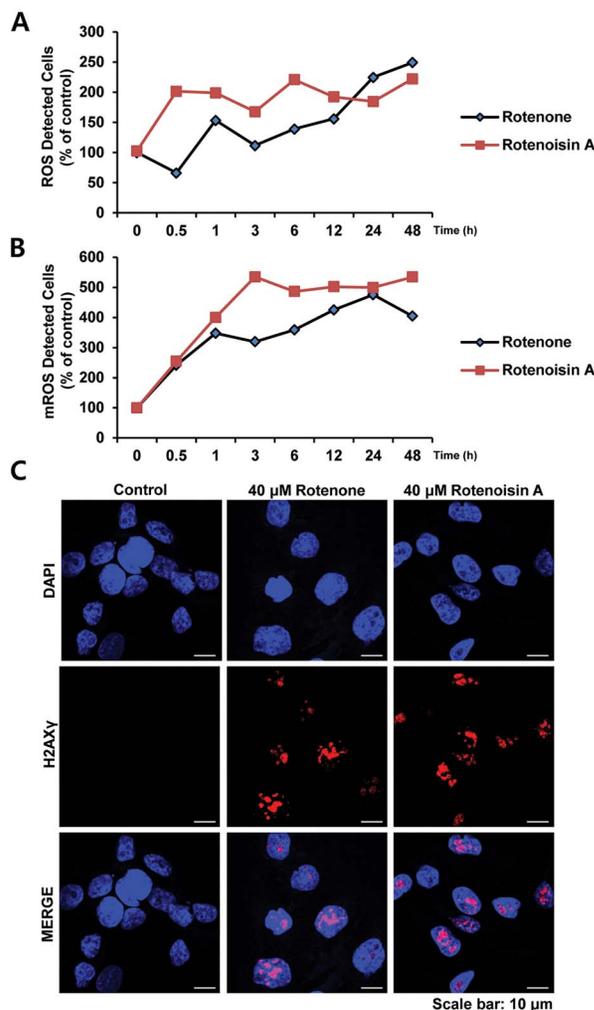


Fig. 3. Rotenoinis A increased reactive oxygen species (ROS) accumulation and induced DNA damage. (A) Intracellular ROS detection using the Muse™ Cell Analyser. (B) mROS detection using MitoSOX™. (C) γ H2AX and MCF-7 cells were cultured on glass coverslips, and the cells were fixed and stained with γ H2AX primary antibodies and Alexa Fluor 555-conjugated secondary antibodies. Then, the nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Representative images are shown for each control and treatment group, with γ H2AX foci indicating double-strand DNA breaks in the nuclei. Scale bar: 10 μ m.

foetal bovine serum (FBS) were purchased from Lonza (Walkersville, MD, USA). MitoTracker™ and MitoSOX™ Red were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

γ -Irradiation and high-performance liquid chromatography (HPLC)

Rotenoinis A was prepared using the method described in our previous report [15]. Briefly, a sample solution of rotenone (0.5 g) in MeOH (200 ml) in capped vials was exposed to 50 kGy (absorbed

dose) of radiation. Irradiation was carried out at ambient temperature using a cobalt-60 irradiator (point source AECL, IR-79, MDS Nordion International Co. Ltd, Ottawa, ON, Canada) at the Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute (Jeongup, Korea). The source strength was ~ 320 kCi, with a dose rate of 10 kGy h^{-1} at the location of the sample. The irradiated methanolic solution was immediately evaporated to remove the solvent and lyophilized. The dried irradiated solution was directly subjected to column chromatography over a silica gel column (2.5 cm i.d. \times 32 cm) with $CHCl_3$ -MeOH-MeOH to yield pure rotenoinis A (129.1 mg, retention time 4.5 min). HPLC analysis was carried out on a YMC-Pack ODS A-302 column (4.6 mm i.d. \times 150 mm; YMC Co., Ltd) and was developed at 40°C with 1% HCOOH/MeCN (1:1, flow rate 1.0 min^{-1} , detection 280 nm).

Cell culture

Human breast cancer MCF-7 cells and primary epidermal keratinocytes (HEKa) were purchased from the American Type Culture Collection (Rockville, MD, USA). MCF-7 cells were cultured in RPMI 1640 medium supplemented with FBS (10%), L-glutamine (4 mM), penicillin (100 U ml^{-1}) and streptomycin (100 μg ml^{-1}). HEKa cells were cultured with Dermal Cell Basal Medium supplemented with a Keratinocyte Growth Kit (ATCC PCS-200-040™), penicillin (100 U ml^{-1}) and streptomycin (100 μg ml^{-1}). The cells were incubated under sterile conditions at 37°C in a humid environment containing CO_2 (5%).

Viability assay and morphological analysis

Cell viability was measured using the CCK-8 assay. The cells were seeded in 96-well plates (1×10^4 cells per well) and incubated overnight. The cells were treated with rotenone or rotenoinis A at the indicated concentrations and incubated for 24 h. A solution of CCK-8 was added to each well, and the plates were incubated for 1 h at 37°C to allow the reaction to take place before removal of the culture medium. Cell viability was determined using a spectrophotometer, and the absorbance was measured at 450 nm (Tecan, Männedorf, Switzerland). The cell viability for each group was expressed as a percentage of the control group. Cell morphology was monitored using an Olympus IX71 fluorescence microscope (Olympus Corporation, Tokyo, Japan).

Oxidative stress assay

MCF-7 cells (2×10^6 cells per well) grown in 12-well plates were incubated with 40 μ M rotenone or rotenoinis A for 24 h at 37°C. The oxidative stress assay was conducted by carrying out quantitative measurement of cellular populations undergoing oxidative stress using the Muse™ Cell Analyzer and Muse™ Oxidative Stress Kit (EMD Millipore, Billerica, MA, USA). According to the manufacturer's protocol, the cells were detached, resuspended to obtain 1×10^6 cells ml^{-1} , and incubated at 37°C for 30 min with the Muse™ Oxidative Stress working solution. The number of oxidized cells was counted using the Muse™ Cell Analyzer based on the intensity of red fluorescence. The results were obtained from four independent experiments. Mitochondrial ROS (mROS) levels were measured using the mitochondrion-specific fluorescent hydroethidine-derivative dye, MitoSOX Red (10 μ M; Thermo Fisher Scientific), as previously

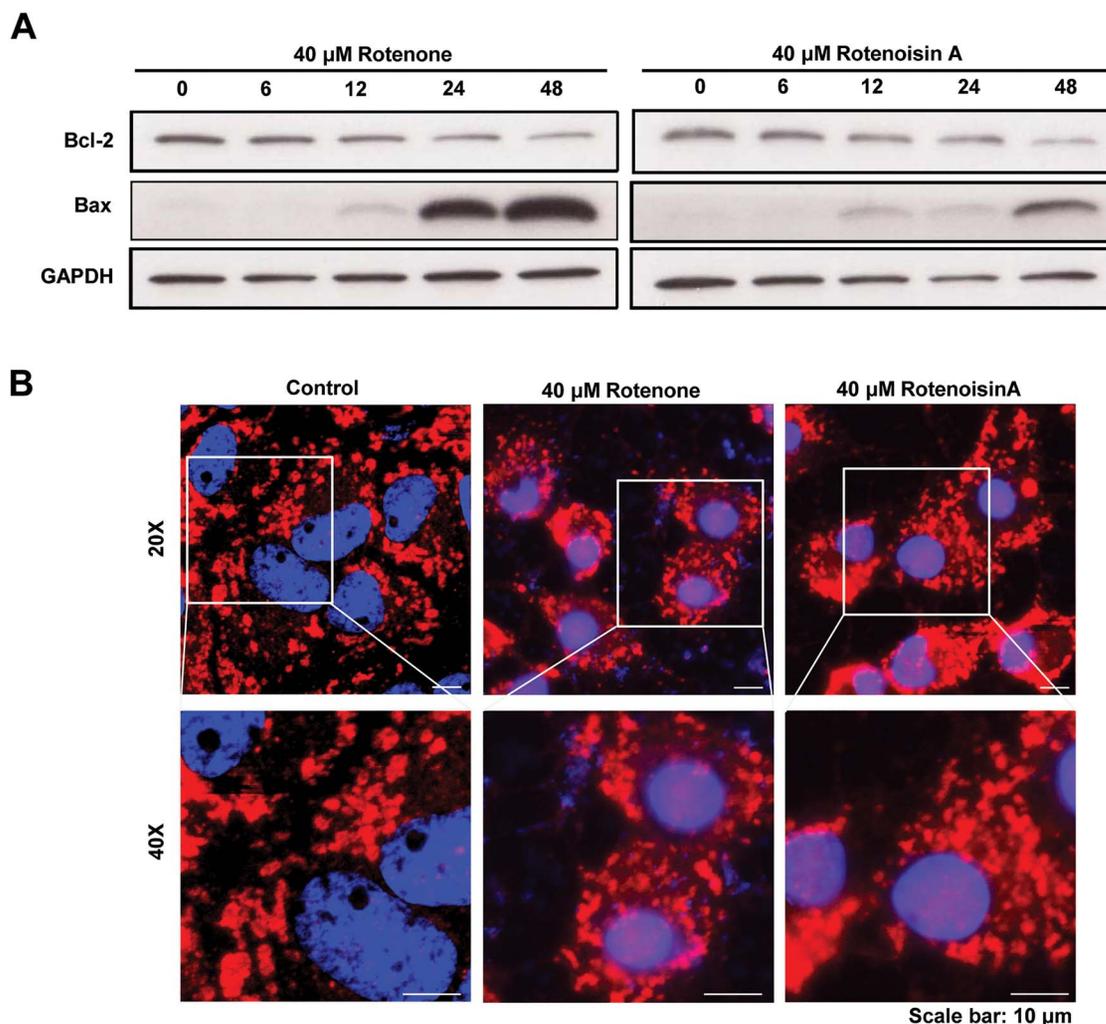


Fig. 4. Rotenoisin A decreased Bcl-2/Bax and disrupted the mitochondrial network in MCF-7 cells. (A) Representative images of the western blot analysis. After rotenoisin A treatment, the expressions of Bcl-2 and Bax in MCF-7 cells shifted in a time-dependent manner. (B) Representative images of MitoTracker™ staining. The nuclei were counterstained with DAPI. Scale bar: 10 μ m.

described [16]. Cells were incubated in 96-well black plates with MitoSOX Red for 30 min at 37°C in 5% CO₂. The cells were washed twice with phosphate-buffered saline (PBS). MitoSOX Red was measured using a spectrophotometer (Tecan, Männedorf, Switzerland) at excitation and emission wavelengths of 510 and 580 nm, respectively.

Evaluation of apoptosis by annexin-V FITC/propidium iodide (PI)

MCF-7 cells (2×10^6 cells per well) grown in 12-well plates were incubated with 40 μ M rotenone or rotenoisin A for 24 h at 37°C. The cells were harvested and washed with Dulbecco's PBS. The cells were stained with annexin V and the Dead Cell reagent (MCH100105; EMD Millipore) for 20 min, and the flow cytometric assessment was performed using the Muse™ Cell Analyser. The number of apoptotic cells was expressed as the percentage of the live, early/late apoptotic

and dead cells, which were determined using the Muse analysis software (Muse 1.1.2; EMD Millipore).

Immunofluorescence staining

Cells (1×10^5 cells per well) were prepared on sterilized glass coverslips (BD Biosciences, Franklin Lakes, NJ, USA) in triplicate. The cells were incubated with rotenone or rotenoisin A for 24 h at 37°C. After incubation, the cells were fixed in 2% paraformaldehyde and 2% glutaraldehyde in PBS for 15 min and permeabilized with 0.25% Triton X-100 in PBS for 10 min. Next, the following procedures were performed according to the experiments. (i) Mitochondria network imaging: cells were stained with 50 nM MitoTracker™ (Thermo Fisher Scientific) for 1 h at 37°C in the dark. (ii) γ H2AX staining: cells were incubated with the γ H2AX primary antibody for 2 h at room temperature. The cells were washed to remove excess primary antibodies and incubated with the appropriate fluorescently labelled secondary antibodies for 1 h

at room temperature. The nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI; D9542, Sigma-Aldrich, St. Louis, MO, USA) and incubated for 5 min. After mounting, fluorescence images were captured using a confocal microscope (LSM 700; Carl Zeiss, Oberkochen, Germany). To quantify the immune-reacted cells, the fluorescence intensity was measured in 10 randomly selected images.

Western blot analysis

MCF-7 cells (1×10^7 cells per well) grown in 6-well plates were incubated with 40 μM rotenone or rotenoisin A for 24 h at 37°C. The cells were washed with PBS and lysed with radioimmunoprecipitation assay (RIPA) buffer. The proteins (30–50 μg) were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% non-fat dry milk for 1 h at room temperature and then incubated overnight with a 1:1000 dilution of primary antibodies at 4°C. The membranes were washed with Tris-buffered saline and incubated with horseradish peroxidase-conjugated secondary antibodies (anti-rabbit IgG, HRP-linked antibody, #7074; Cell Signaling Technology, Inc.) for 2 h at room temperature. The proteins were then visualized using an enhanced chemiluminescence reagent (ECL; EMD Millipore) and exposure to an X-ray film.

Statistical analysis

Statistical differences were evaluated using Student's *t*-test. The results were considered statistically significant when the *P* value was <0.05 . All experiments were performed at least three times independently.

RESULTS

Cytotoxicity induced by rotenoisin A compared with rotenone

To explore the inhibitory effect of rotenoisin A on cell growth, MCF-7 cells were treated with various concentrations of rotenoisin A for 24 h. The results from the CCK-8 assay showed that rotenoisin A reduced MCF-7 cell viability in a concentration-dependent manner (Fig. 2A). The reduction reached $34.13 \pm 2.68\%$ after 40 μM rotenone treatment, whereas 40 μM rotenoisin A treatment resulted in a reduction in viability to $45.82 \pm 2.13\%$ in MCF-7 cells compared with the control. Although the inhibitory effect of rotenoisin A was somewhat weaker than that of rotenone in the MCF-7 cell line, the inhibitory effect of rotenoisin A on HEK293T was decreased relative to that of rotenone (Fig. 2B). In previous reports, rotenone used as an organic pesticide has been reported to have neurotoxic effects that could play a role in the development of Parkinson's disease [17, 18]. Therefore, these results suggest that rotenoisin A may be a new antiproliferative candidate in breast cancer with lower toxicity in normal cells.

Rotenoisin A induced accumulation of ROS in MCF-7 cells, resulting in DNA damage

Previous studies have shown the irreversible binding of rotenone to complexes of mitochondrial electron transport chains, followed by the disruption of oxidative phosphorylation by blocking the transfer of electrons from complexes to ubiquinone, resulting in the accumulation

of ROS [19, 20]. The Muse™ Cell Analyzer and Mitosox™ assay revealed that rotenoisin A induces the accumulation of cytosolic and mitochondrial ROS in MCF-7 cells. Interestingly, the number of cells with high cytosolic and mitochondrial ROS-positive fractions increased after treatment with 40 μM rotenoisin A, and this trend was maintained over time (Fig. 3A and B).

Oxidative stress and DNA damage are associated with various human pathological conditions, including ageing and cancer. We monitored γH2AX expression as a measure of DNA damage in MCF-7 cells treated with rotenoisin A for 24 h. Similar to rotenone, an increased γH2AX intensity was observed following treatment with rotenoisin A compared with the control (Fig. 3C). Overall, the results confirmed that increased ROS accumulation due to rotenoisin A treatment results in DNA damage.

Effects of rotenoisin A on disruption of the mitochondrial protein Bcl2/Bax ratio in MCF-7 cells

In many previous studies, rotenone has been identified as an inhibitor of the mitochondrial electron transport chain complex I and is activated by a disrupted electron transport system that results in high ROS accumulation [19, 21, 22]. Mitochondria are not only involved in caspase-dependent apoptosis but also significantly affect the Bcl2 pathway during caspase-independent apoptosis [23, 24]. In this study, we investigated changes in the Bcl2/Bax ratio following rotenone or rotenoisin A treatment. Bcl2 exists in the mitochondrial outer membrane and plays a role in cell survival as well as inhibition of proapoptotic proteins. In addition, Bcl2 also prevents the release of mitochondrial contents, including ROS and cytochrome *c*, which induces caspase activation. The expression of Bax protein is found in the cytosol, but Bax changes its steric structure when apoptosis signalling begins. During the induction of apoptosis, Bax is associated with cell membranes, specifically mitochondrial membranes [25, 26]. The Bcl2/Bax ratio as a potential molecular marker of the apoptosis pathway is reliable [27, 28]. We found that the expression of Bcl2 in the whole protein decreased 48 h after treatment with 40 μM rotenoisin A. However, Bax protein expression appeared to increase. This tendency is similar to that of natural rotenone. Eventually, it was confirmed that the Bcl2/Bax ratio was decreased by rotenoisin A treatment (Fig. 4A).

Additionally, we observed mitochondria located around the cell nucleus without rotenone or rotenoisin A treatment. After the addition of either substance, dense deposits were observed in the mitochondria, and apoptotic cells displayed shrunken nuclei but increased and swollen mitochondria. The overall distribution also changed irregularly (Fig. 4B). These results suggest that treatment with rotenoisin A not only altered the composition ratio of mitochondrial proteins but also resulted in structural and functional changes.

Rotenoisin A induced phosphorylation of JNK, p38 and ERK MAPKs

To explore the stress index, the levels of the active form of stress-activated MAPKs were measured. Among the MAPKs, JNK and ERK MAPKs act as damaging circuits in the cellular stress environment, inducing excessive oxidative stress [22, 29]. In particular, the activation of p38 MAPK promotes mitochondrial translocation of Bax proteins and plays an important role in intrinsic apoptosis [30, 31]. In this study,

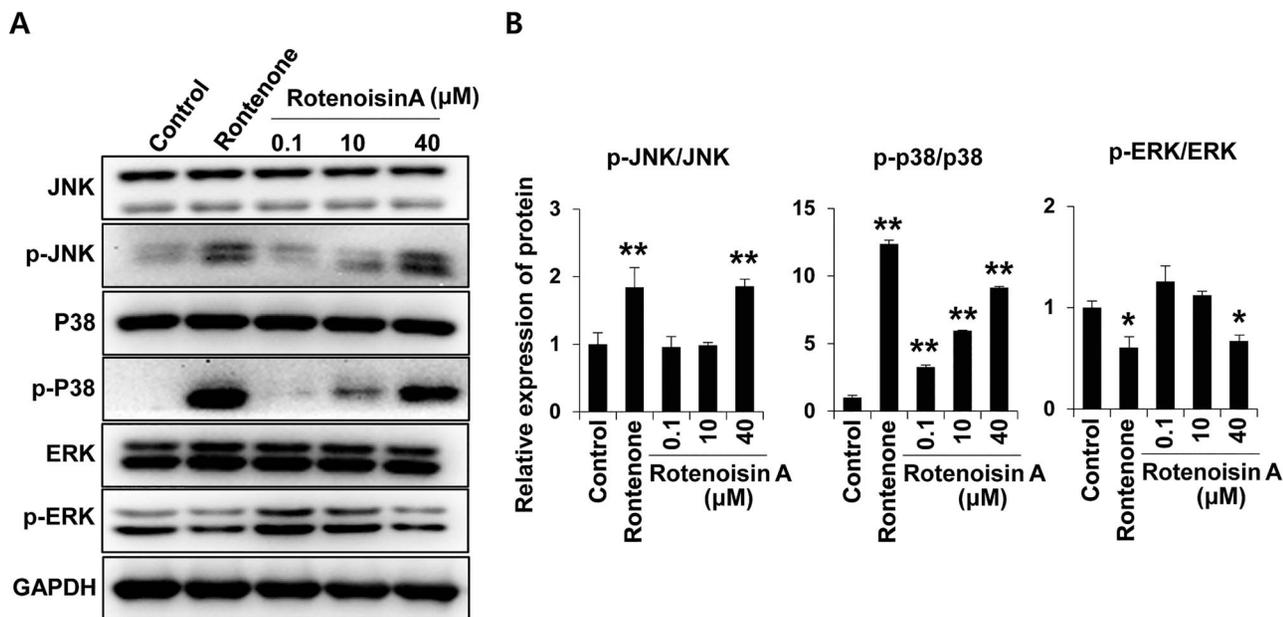


Fig. 5. Rotenoisin A stimulated the mitogen-activated protein kinase (MAPK) pathway in MCF-7 cells. (A) Representative images of the western blot analysis for the protein expression of JNK, p-JNK, p38, p-p38, ERK, p-ERK and GAPDH antibodies. (B) Densitometry analysis. The data are presented as the means \pm SDs. * $P \leq 0.05$ and ** $P \leq 0.01$ versus control.

rotenoisin A treatment triggered the phosphorylation of JNK and p38, but phosphorylation of ERK was down-regulated in a concentration-dependent manner. These results suggest that damage to the rotenoisin A pathway occurs via MAPKs in MCF-7 cells (Fig. 5A and B).

Rotenoisin A induced apoptosis in the MCF-7 cell line

In addition to changes in MAPK proteins, the changes in apoptotic proteins were significant. The collapsed mitochondria secrete cytochrome *c* into the cytoplasm, and this secreted 'apoptotic cell death factor' promotes caspase activity [32]. The activity of caspase-9 promoted by apoptosomes eventually leads to DNA repair defects due to PARP dysfunction [33, 34]. In this study, rotenoisin A not only increased cleaved caspase-9 but also increased cleaved PARP expression in a time-dependent manner following treatment (Fig. 6A). Furthermore, apoptosis was confirmed by Annexin/PI staining, which is a commonly employed approach for studying apoptotic cells. The total percentage of apoptosis in vehicle control cells was $12.09 \pm 2.24\%$, which significantly changed to $33.75 \pm 3.74\%$ in 40 μM rotenoisin A and $32.74 \pm 3.66\%$ in 40 μM rotenone (Fig. 6B). Taken together, it was confirmed that cell damage caused by rotenoisin A resulted in cell death through the apoptosis pathway.

DISCUSSION

Rotenone, the isoflavone, naturally protects plants against insects [35, 36]. It is produced from the leaves, seeds or stems of Mexican turnip (*Pachyrhizus erosus*) plants, or the roots of the Leguminosae family [37]. The insecticidal effect of rotenone has been used since it was observed that insects that ate the plants that produced it died. However, its commercial use has been increasingly discouraged or banned by

several reports of concerns about the neurophysiological side effects of rotenone [38, 39].

However, no attempt has been made to utilize the rotenone-induced mitochondrial disruption in mammalian cells and take advantage of this property in human healthcare. In a previous report, we modified rotenone with ionizing radiation and confirmed the changes in chemical properties via nuclear magnetic resonance (NMR) analysis [15]. In this study, we observed the potential of rotenones as antiproliferative candidates for breast cancer. Rotenoisin A, the modified form induced by ionizing radiation, has shown not only similar efficiency in the inhibition of cell growth in MCF-7 cell lines but also decreased toxicity in HEK293 cell lines, the normal human keratinocyte, compared with rotenone.

Ionizing radiation allows the introduction of energy into substances to cause favourable chemical changes [40, 41]. Sufficiently irradiated materials can decompose to yield daughter molecules or form new ones. This platform is an advanced technology to transform various materials usefully. Although technology using exposure to ionizing radiation to transform materials is not common, it is physically accurate and has excellent reproducibility. For example, our researchers have confirmed that the reproducibility of rotenoisin A production is consistent when various concentrations and volumes are exposed at a calculated exposure dose. This suggests that γ -irradiation is an economical and innovative way to produce rotenoisin A, a derivative of rotenone.

In this study, although we demonstrated the inhibitory effects of rotenoisin A in a breast cancer cell line, we did not use animal bearing models to identify the systemic efficiency and safety of rotenoisin A. Previous reports have shown that the toxicity of rotenone is less when it is exposed to the skin directly (absorption capacity of <10%) and

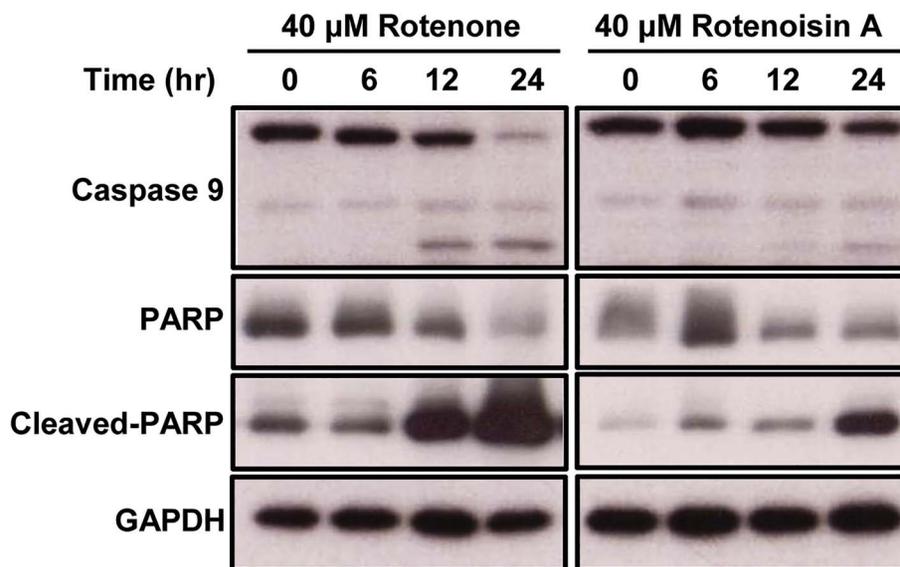
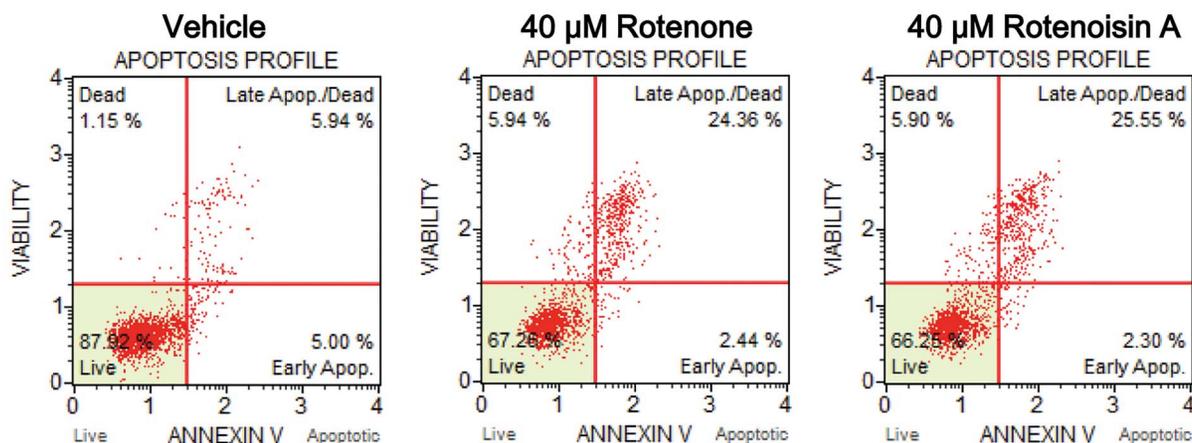
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Fig. 6. Rotenoinis A induced apoptotic cell death in MCF-7 cells. (A) Representative images of western blot analysis for the protein expression of caspase-9, PARP, cleaved PARP and GAPDH antibodies. (B) Representative images of annexin-V/propidium iodide (PI) measured using the Muse™ Cell Analyser.

occurs through ingestion, inhalation or intradermal delivery [42]. We demonstrated the toxicity of rotenone in human keratinocytes, which is counteracted by modification by ionizing radiation. In this context, we believe that rotenoinis A potentially plays a novel role in the inhibition of breast cancer with less toxicity in human organisms. We will continue to systematically explore this advantage using experimental animal studies.

In conclusion, rotenoinis A, a radiolytic rotenone derivative, showed decreased cytotoxicity compared with rotenone in HEK293 cells and higher anticancer effects in MCF-7 cells. This modified reagent was able to inhibit cancer cell proliferation via oxidative stress, up-regulation of the MAPK pathway and ultimately apoptotic cell death.

In this study, we suggest that the improved efficacy of rotenoinis A establishes it as an anticancer drug with low reversible effects. However, the mechanism underlying rotenoinis A function in complex organs, such as the human body, remains unclear and needs further investigation.

COMPETING INTERESTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Hyoungh-woo Bai: project administration, supervision. Chul-Hong Park and Byung Yeoup Chung: writing—original draft, writing—review and editing. Dong Ho Bak and Seong Hee Kang: methodology.

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