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Anticancer effects of quercetin and rutin on KB human oral cancer cells

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사람 구강암세포 KB에서
quercetin과 rutin의 항암효과

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사람 구강암세포 KB에서 quercetin과 rutin의 항암효과

김 서 윤

지도교수 : 김 도 경

조선대학교 대학원 치의학과

양파, 과일, 메밀 및 약초 등에서 유래한 플라보노이드의 유효성분 중 하나인 quercetin(3,5,7,3′,4′-tetrahydroxyflavone)은 항염증, 항동맥경화증, 항산화 및 항암효과 등을 포함한 여러 유익한 생물학적 활성이 있다고 보고되어져 왔으며, 특히 특정 암세포 성장을 억제시킬 수 있다고 보고되었다. Rutin(quercetin-3-O-rutinoside)은 식물 이차대사물로서 quercetin의 글리코시드 유도체 중 하나이다. 그러나 구강암과 관련된 quercetin과 rutin에 관한 자료는 거의 없다. 따라서 본 연구에서는 사람 구강암 세포주를 이용하여 quercetin과 rutin의 구강암세포 성장억제에 미치는 효과와 세포성장 억제기전을 분석하였다.

본 연구에서 quercetin과 rutin에 의한 구강암세포 성장억제와 그 기전을 조사하기 위해, 사람 구강암세포 KB에서 quercetin과 rutin을 처리한 후, MTT 분석, DAPI를

이용한 세포핵 염색, immunoblotting 및 caspase 활성분석 등을 시행하였으며, 그 결과는 다음과 같다.

1. 사람 구강암세포 KB 세포주에서 quercetin은 KB 세포의 성장을 시간과 농도에 의존적으로 억제하였으나, rutin은 KB 세포의 성장에 유의한 영향을 주지 않았다.
2. KB 세포에서 quercetin은 핵의 응집과 파쇄를 유도하였으나, rutin은 핵의 응집과 파쇄를 거의 유도하지 않았다.
3. KB 세포에서 quercetin은 procaspase-3의 proteolytic cleavage와 절단된 caspase-3의 증가를 촉진하였으나, rutin은 procaspase-3의 proteolytic cleavage를 촉진시키지 않았다.
4. KB 세포에 quercetin을 처리한 실험군에서 procaspase-7의 proteolytic cleavage 현상과 절단된 caspase-7의 증가를 확인할 수 있었으나, rutin을 처리한 실험군에서는 procaspase-7의 proteolytic cleavage 현상을 볼 수 없었다.
5. Quercetin은 procaspase-9의 proteolytic cleavage와 절단된 caspase-9의 증가를 촉진하였으나, rutin은 procaspase-9의 proteolytic cleavage를 촉진시키지 않았다.
6. KB 세포에 quercetin을 처리한 실험군에서 caspase-3/-7의 활성화를 확인할 수 있었으나, rutin은 caspase-3/-7의 활성화를 유도하지 않았다.

본 연구의 결과로 quercetin은 사람 구강암세포 KB의 apoptosis를 유도하여 구강암세포 성장을 억제시키는 것으로 사료된다. 또한 본 연구의 결과로, quercetin을 이용한 구강암세포의 성장억제에 관한 하나의 방향을 제시할 수 있을 것으로 사료된다.

중심어: Quercetin, Rutin, 세포사, Apoptosis, 항암치료제, 구강암

I. INTRODUCTION

Oral cancer is the sixth most common cancer globally (Notani, 2000). Despite the introduction of novel therapeutic modalities, there have only been modest improvements in the long-term survival rates (Todd et al., 1997). Better knowledge of the underlying mechanisms of oral cancer are necessary to improve the survival rates, which, despite the better early detection of oral cancer, have not improved over the past two decades and remain among the worst of all cancer sites (Todd et al., 1997). The current standard of clinical care in several cancers including oral cancer involves primary surgery followed by cytotoxic chemotherapy, but undesirable side effects and recurrence are significant problems (Tan et al., 2010).

In recent years, there has been a global trend toward the importance of naturally occurring phytochemicals in plants for the prevention and treatment of human diseases. Several of these phytochemicals have shown potential as cancer chemopreventive or therapeutic agents in the human body (Christou et al., 2001; Mukherjee et al., 2001; Pezutto, 1997; van Poppel and van den Berg, 1997). Most of these bioactive phytochemicals exert their cancer chemotherapeutic activity by blocking cell cycle progression and triggering apoptotic cell death (Christou et al., 2001; Mukherjee et al., 2001; Pezutto, 1997; van Poppel and van den Berg, 1997). Therefore, the induction of apoptosis in cancer cells is an important indicator of the cancer treatment response when employing a bioactive substance to reduce and control human mortality due to cancer (Paschka et al., 1998; Smets, 1994).

Quercetin (3,5,7,3′,4′-tetrahydroxyflavone), one of the active components of

flavonoids derived from the onions, fruits, buckwheat and Chinese herbs, has various beneficial biological activities including anti-oxidant, anti-inflammation, anti-atherosclerosis and anti-tumor properties (Naderi et al., 2003; Mamani-Matsuda, 2006; Lin et al., 2008; Lotito et al., 2006). Some studies have shown that quercetin has anti-tumor effects through inhibiting of melanoma and prostate cancers (Zhang, 2000; Zhang et al., 2004). In particular, quercetin is also considered pharmacologically safe as it is a naturally occurring compound (Naderi et al., 2003; Mamani-Matsuda, 2006; Lotito et al., 2006). Quercetin in diet bioflavonoids often presents with its glycoside derivatives such as rutin (quercetin-3-O-rutinoside) (Naderi et al., 2003; Mamani-Matsuda, 2006; Lin et al., 2008; Lotito et al., 2006). Rutin belongs to a class of plant secondary metabolites called flavonoids that are also known as rutoside, sophorin and quercetin-3-rutinoside (Jangaz et al., 2002; Joyeux et al., 1990; Martin-Aragón et al., 2001; Liu, 1995; Ramos et al., 2008). In fact, bioflavonoids, such as quercetin, are quite identical to the composition of rutin and, hence, several experts agree that rutin and quercetin actually work in tandem by harmonizing each other (Jangaz et al., 2002; Joyeux et al., 1990; Martin-Aragón et al., 2001; Liu, 1995; Ramos et al., 2008). Although it has been shown to characterize anti-tumor activities in some tumors, their action mechanisms remain to be determined. In addition, the effects and relationship of quercetin and rutin on oral cancer cells have not been analyzed.

In this study, therefore, the effects of quercetin and rutin on cell growth and the mechanisms of cell death elicited by quercetin and rutin were examined in KB human oral cancer cells.

II. MATERIALS AND METHODS

1. Materials

The KB human oral cancer cells were provided by American Type Culture Collection (ATCC, Rockville, MD, USA). Quercetin, rutin (Fig. 1) and 3-[4,5-Dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide (MTT) were supplied by Sigma (St Louis, MO, USA). Anti-cleaved caspase-3, anti-cleaved caspase-7 and anti-cleaved caspase-9 antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Cell-permeable fluorogenic substrate PhiPhiLux-G_1D_2 was purchased from OncoImmunin, Inc. (Gaithersburg, MD, USA). Other analytical reagents were purchased based on the analytical grade.

2. Cell cultures

The KB human oral cancer cells were grown in MEM containing NEAA (nonessential amino acids) at a ratio of 100:1, and the media was supplemented with 5% fetal bovine serum (FBS) (Kim et al., 2008). The KB cells were maintained as monolayers in plastic culture plates at 37℃ in the humidified atmosphere containing 5% CO_2.

3. Inhibition of cell growth (MTT assay)

The cell viability test was performed according to the previously described method with minor modifications (Hwang et al., 2007; Kim et al., 2010; Kwon et al., 2008). The cells were seeded at a concentration of 5 X 10^3 cells/well in 24-well plates. After 24 hours growth, the cells were treated with quercetin and rutin at various concentrations for 24 - 72 hours. The cell viability was assessed using MTT assay. Three separate experiments were performed for each concentration/exposure time combination.

4. Nuclear staining with DAPI

Nuclear staining with DAPI (40,60-diamidino-2-phenylindole) was performed to evaluate apoptosis. The KB cells were cultured in 24-well plates at a seeding density of 5 X 10^3 cells per well. After 24 hours growth, the cells were treated with quercetin and rutin (0 and 30 μM) for 24 - 72 hours. The treated KB cells were fixed with 1% paraformaldehyde for 30 min at room temperature and washed twice with PBS. Permeate the cells with ice-cold ethanol for 5 min at room temperature and washed twice with PBS. The fixed cells were stained with DAPI (300 nM) for 5 min at room temperature in dark, washed twice with PBS and examined by fluorescent inverted microscopy (IX71, Olympus, Japan).

5. Immunoblotting

The cells were treated with quercetin and rutin (0 and 30 μM) for 24 or 48 hours. Immunoblotting was performed according to the previously described method with minor modifications (Choi et al., 2010; Kim et al., 2010). The anti-cleaved caspase-3, anti-cleaved caspase-7 or anti-cleaved caspase-9 antibody (1:1000 dilution, Cell Signaling Technology, Inc., Danvers, MA, USA) was used as the primary antibody.

6. Determination of caspase activation

The activity of caspase-3/-7 was determined using the cell-permeable fluorogenic substrate PhiPhiLux-G_1D_2 (OncoImmunin, Inc. Gaithersburg, MD, USA), which was used according to the manufacturer's instructions. The cells were treated with quercetin and rutin (30 μM) for 24 or 48 hours and incubated with PhiPhiLux-G_1D_2. The activity of caspase-3/-7 was visualized by fluorescence microscopy (IX71, Olympus, Japan).

7. Data analysis

All experiments were performed at least in triplicate. The results are reported as the mean ± S.E.M. The statistical significance was analyzed using a Student's t-test for the two group comparison and one way analysis of variance for the multi-group comparisons. A p value <0.05 was considered significant.

Ⅲ. RESULTS

1. Cytotoxic effects of quercetin and rutin in KB cells

To analyze and compare the effect of quercetin and rutin on the viability of KB cells, the cells were treated with quercetin and rutin at various concentrations for 24 - 72 hours, and then the MTT assay was performed. As shown in Fig. 2A, treatment of quercetin 0.1 and 0.3 μM did not significantly affect the cell viability of KB cells, but quercetin from 1 to 300 μM reduced KB cell viability. When the KB cells were treated with quercetin for 24 - 72 hours, quercetin inhibited the growth of KB cells in the concentration- and time-dependent manners, suggesting that quercetin induces KB cell death (Fig. 2A). The IC_{50} values of quercetin on the cell viability after 24 - 72 hour treatments are shown in Table 1. Treatment of rutin from 0.1 to 300 μM for 24 - 72 hours did not significantly affect the cell viability of KB cells (Fig. 2B).

2. Changes in nuclear morphology by quercetin and rutin

The nuclear morphological changes by quercetin and rutin were assessed by DAPI staining. As shown in Fig. 3A, the control KB cell's nuclei had a normal regular and oval shape. Treatment with 30 μM quercetin for 24 - 72 hours resulted

in nuclear condensation and fragmentation, characteristics of apoptosis. Rutin 30 μM for 24 - 72 hours induced nuclear condensation and fragmentation scarcely (Fig. 3A).

As quantified in Fig. 3B, quercetin significantly increased the apoptotic rate of KB cells to 11.0 ± 0.89% (24 hours), 25.9 ± 2.80% (48 hours) and 46.7 ± 3.98% (72 hours), but rutin did not cause a statistically significant increase percentage of apoptotic rate (2.9 ± 0.68%, 24 hours; 4.3 ± 1.03%, 48 hours; 5.8 ± 0.97%, 72 hours).

Table 1. Antiproliferative effect of quercetin in KB cells

Time	IC_{50} (μM)
24 hours	102.5 ± 7.8
48 hours	22.4 ± 4.2
72 hours	10.2 ± 1.9

The IC_{50} values represent the mean ± SEM for three experiments.

3. Activation of caspases by quercetin and rutin

The levels of cleaved caspase-3, caspase-7 and caspase-9 were examined by immunoblotting and the activity of caspase-3/-7 was detected by fluorescence microscopy using a selective fluorogenic substrate since caspase-3, caspase-7 and caspase-9 are effector caspases of apoptotic cell death (Cohen, 1997; Datta et al., 1997; Liu et al., 1997). Treatment with 30 μM quercetin for 24 or 48 hours significantly promoted proteolytic cleavages of procaspase-3 in a time-dependent manner in the KB cells, with the increases in the amount of cleaved caspase-3, but treatment with 30 μM rutin did not significantly promote proteolytic cleavages of procaspase-3 (Fig. 4). Quercetin 30 μM for 24 or 48 hours also promoted proteolytic cleavages of procaspase-7 in a time-dependent manner, with the increases in the amount of cleaved caspase-7, but rutin did not (Fig. 5). Treatment with 30 μM quercetin significantly promoted proteolytic cleavages of procaspase-9 in a time-dependent manner, but treatment with 30 μM rutin did not significantly promote proteolytic cleavages of procaspase-9 (Fig. 6).

In addition, activation of caspase-3/-7 in quercetin- or rutin-treated KB cells was confirmed by fluorescence microscopy using fluorogenic substrate. As shown in Fig. 7, quercetin treatment for 24 or 48 hours led to activate the caspase-3/-7 in a time-dependent manner in the living KB cells. However, rutin 30 μM did not significantly lead to activate the caspase-3/-7 in the living KB cells (Fig. 7).

Ⅳ. DISCUSSION

Recent studies have shown that such chemicals derived from natural materials have been identified to elicit chemopreventive and therapeutic effects (Cheng et al., 2005; Park et al., 2005; Tan et al., 2005). It has been reported that this effect alters various factors associated with the cell cycle and thereby induce the apoptotic cell death (Cheng et al., 2005; Hoshino et al., 1991; Park et al., 2005; Tan et al., 2005; Tian et al., 2006a). Apoptosis, which is a major pathway of programmed cell death, plays an important role in regulating tissue development and homeostasis in eukaryotes (Green and Reed, 1998; Hengartner, 2000; Kaufmann and Hengartner, 2001). Apoptosis can occur via a death receptor-dependent extrinsic or a mitochondria-dependent intrinsic pathway, and is induced by a treatment with chemotherapeutic agents (Kaufmann and Earnshaw, 2000; Reed, 2001).

There is a need to find new anti-cancer agents that can kill cancerous cells with minimal toxicity. Quercetin, founded from the citrus fruit, buckwheat, onions and Chinese herbs, has been demonstrated significant anti-oxidant, anti-inflammation, anti-atherosclerosis and anti-tumor properties (Naderi et al., 2003; Mamani-Matsuda, 2006; Lin et al., 2008; Lotito et al., 2006). Several studies have suggested that quercetin has anti-tumor effects through inhibiting of melanoma and prostate cancers (Zhang, 2000; Zhang et al., 2004). Rutin, one of the glycoside derivatives of quercetin, belongs to a class of plant secondary metabolites that are

also known as rutoside, sophorin and quercetin-3-rutinoside (Jangaz et al., 2002; Joyeux et al., 1990; Martin-Aragón et al., 2001; Liu, 1995; Ramos et al., 2008). Although quercetin has been shown to characterize anti-tumor activities in some tumors, the mechanisms of action remain to be elucidated. In addition, the effects and relationship of quercetin and rutin on oral cancer cells are not clearly established. In this study, therefore, the cytotoxic effects of quercetin and rutin and the mechanism of cell death exhibited by quercetin and rutin were examined in KB human oral cancer cells.

In MTT assay, quercetin inhibited the growth of KB cells in the concentration- and time-dependent manners, but rutin did not significantly affect the cell viability of KB cells (Fig. 2). In this study, quercetin but not rutin showed anti-proliferative effects in KB cells. These are in agreement with previous reports that showed a generalized growth inhibitory effect of quercetin on several cancer cell lines (Alía et al., 2005; Nichenametla et al., 2006; Novotny et al., 2001; Tian et al., 2006b). Also, this corresponded with the results of rutin that has not anti-proliferative effects *in vitro* situation in several types of cancer cells (Lima et al., 2006; Ramos et al., 2008). These results speculated that quercetin has cytotoxicity for oral cancer cells. In addition, these results indicated that it has potential value for anti-cancer drug discovery.

Apoptosis is an important way to maintain cellular homeostasis between cell division and cell death (Green and Reed, 1998; Hengartner, 2000; Kaufmann and Hengartner, 2001). The induction of apoptosis in cancer cells is one of useful

strategies for anti-cancer drug development (Hu and Kavanagh, 2003). So, many studies were performed for screening of apoptosis from natural material-derived compounds (Christou et al., 2001; Mukherjee et al., 2001; Pezutto, 1997; van Poppel and van den Berg, 1997). In this study, treatment with quercetin induced nuclear condensation and fragmentation in KB cells and significantly increased the apoptotic rate of KB cells, suggesting apoptotic cell death (Fig. 3). In the case of rutin, although rutin induced nuclear condensation and fragmentation scarcely, rutin did not cause a statistically significant increase percentage of apoptotic rate (Fig. 3). These are in agreement with the MTT assay results of this study that showed the growth inhibitory effects of quercetin but not rutin on KB cells. These results indicated that quercetin inhibits the growth of KB cells by activating cell apoptosis.

The activation of a family of intracellular cysteine proteases, called caspases, is known to play an important role in the initiation and execution of apoptosis induced by various stimuli (Datta et al., 1997; Liu et al., 1997). Among the caspases identified in mammalian cells, caspase-3, caspase-7 and caspase-9 may serve as effector caspases of apoptotic cell death (Cohen, 1997; Datta et al., 1997; Liu et al., 1997). Caspase-3, caspase-7 and caspase-9 are synthesized as inactive proenzymes (of sizes 32 kDa, 35 kDa and 47 kDa, respectively), which require proteolytic activation to cleaved enzymes (of sizes 19 kDa, 20 kDa and 37 kDa, respectively) (Cohen, 1997; Datta et al., 1997; Liu et al., 1997). The results of this study show that low levels of cleaved capase-3, -7 and -9 were present in quercetin-untreated KB cells, and quercetin significantly promoted proteolytic

cleavages of procaspase-3, -7 and -9 in a time-dependent manner in the KB cells, with the increases in the amount of cleaved caspase-3, -7 and -9 (Fig. 4, 5 and 6). But rutin did not significantly promote proteolytic cleavages of procaspase-3. -7 and -9 (Fig. 4, 5 and 6). In addition, the activity of caspase-3/-7 was increased by quercetin treatment in living KB cells compared with DMSO treatment as a control, however rutin did not significantly lead to activate the caspase-3/-7 in the KB cells (Fig. 7). These corresponded with the MTT assay results and the nuclear morphological assay results of this study that showed the activating cell apoptosis by quercetin but not rutin in KB cells. These results suggested that quercetin induces apoptotic cell death through caspase-3-, caspase-7- and caspase-9-dependent processing in the KB cells.

In this study, quercetin induced apoptotic cell death of KB cells, while rutin did not affect the cell viability of KB cells and did not induce activating cell apoptosis. These results may be due to the lower hydrophobicity and bioavailability of rutin (Ramos et al., 2008). It has been reported that the estimated log *P* for quercetin is 1.48, whereas -2.02 is the value estimated for rutin in the estimates of a compound's hydrophobicity by using the KowWin (LogKow) software (Lima et al., 2006). Although rutin did not affect the cell viability of KB cells in an *in vitro* situation, dietary rutin can play a important role in the chemoprevention in an *in vivo* situation, since it is known that it can be deglycosylated to yield quercetin in the intestine by colon microflora (Kuo, 1996). However, the mechanisms of apoptosis induced by quercetin and rutin in KB cells are not yet completely

understood. Further studies will reveal the precise cellular and molecular mechanisms of apoptosis induced by quercetin and rutin.

In conclusion, these *in vitro* results indicate that the quercetin highly inhibits cell proliferation and induces apoptosis in KB human oral cancer cells, but not rutin. Moreover, these results suggest that the quercetin could be a model compound for further development of natural product-derived anti oral cancer agents. However, to elaborate this nascent possibility, further investigation of its activity including *in vivo* and purification of bioactive compounds is now in progress.

V. REFERENCES

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Ⅵ. FIGURE LEGENDS

Fig. 1. Chemical structures of quercetin (3,5,7,3′,4′-tetrahydroxyflavone) and rutin (quercetin-3-O-rutinoside).

Fig. 2. Time- and concentration-dependent effects of quercetin and rutin on the cell viability in KB human oral cancer cells. The KB cells were treated with various concentrations of quercetin (A) and rutin (B) for 24 (circle), 48 (square) and 72 (triangle) hours. The cell viabilities were determined by the MTT assays. The percentage of cell viability was calculated as a ratio of $A570_{nms}$ of quercetin or rutin treated cells and untreated control cells. Each data point represents the mean ± SEM of three experiments. $^{**}P<0.01$ vs. control and $^{***}P<0.001$ vs. control (the control cells measured in the absence of quercetin or rutin).

Fig. 3. Apoptotic nuclear changes induced by quercetin and rutin in KB cells. (A) Representative DAPI-stained fluorescence photomicrographs show the nuclei morphology of KB cells. Arrows indicate chromatin condensation, reduced nuclear size and nuclear fragmentation typically observed in apoptotic cells. Scale bar indicates 50 μm. (B) The percentage of apoptotic cells was calculated as the ratio of apoptotic cells to total cells. Results are express the mean ± SEM of three experiments. $^{***}P<0.001$ vs. control (the control cells measured in the absence of quercetin).

Fig. 4. Proteolytic cleavage of caspase-3 by treatments of quercetin and rutin in KB cells. (A) Activity of cleaved caspase-3 by quercetin and rutin in KB cells. The cells were treated with quercetin and rutin (30 μM) for 24 or 48 hours. The cell lysate was prepared and analyzed by immunoblotting as described in "MATERIALS AND METHODS". (B) Quantitative data for (A) were analyzed by using Imagegauge 3.12 software after β-actin normalization.

Fig. 5. Proteolytic cleavage of caspase-7 by treatments of quercetin and rutin in KB cells. (A) Activity of cleaved caspase-7 by quercetin and rutin was measured in KB cells. (B) Quantitative data for (A) were analyzed by using Imagegauge 3.12 software after β-actin normalization.

Fig. 6. Proteolytic cleavage of caspase-9 by treatments of quercetin and rutin in KB cells. (A) Activity of cleaved caspase-9 by quercetin and rutin was measured in KB cells. (B) Quantitative data for (A) were analyzed by using Imagegauge 3.12 software after β-actin normalization.

Fig. 7. Activation of caspase-3/-7 by treatments of quercetin and rutin in living KB cells. The cells were treated with quercetin and rutin (0 and 30 μM) for 24 or 48 hours and added specific cell-permeable substrate Phiphilux G_1D_2. Active of caspase-3/-7 was visualized by fluorescence microscopy.

Ⅶ. FIGURES (Fig. 1)

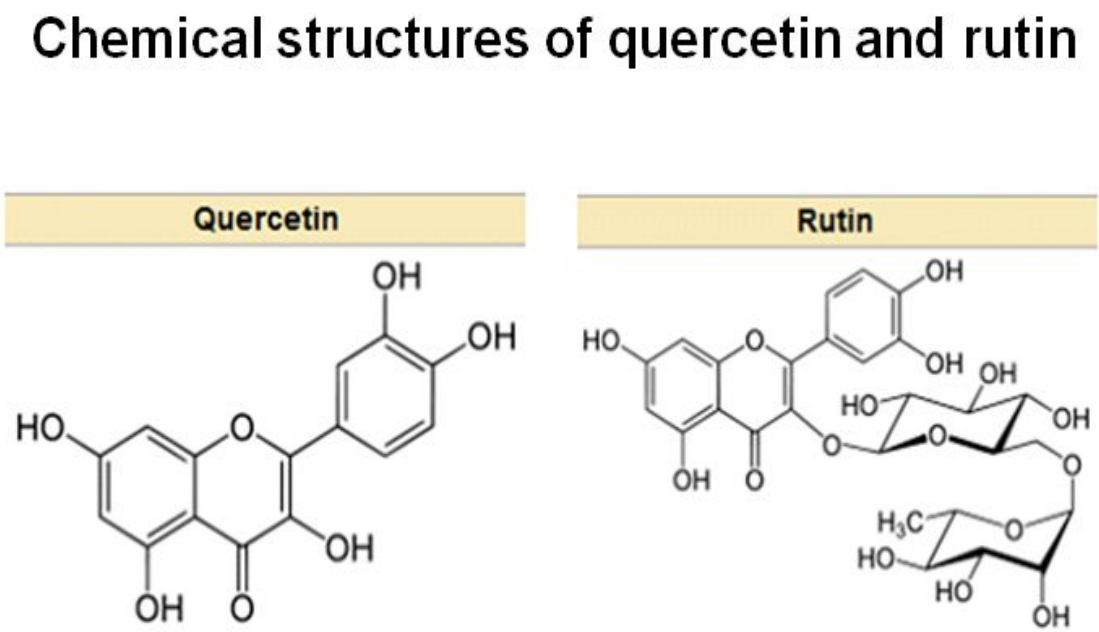

Fig. 1. Chemical structures of quercetin (3,5,7,3′,4′-tetrahydroxyflavone) and rutin (quercetin-3-O-rutinoside).

Ⅶ. FIGURES (Fig. 2)

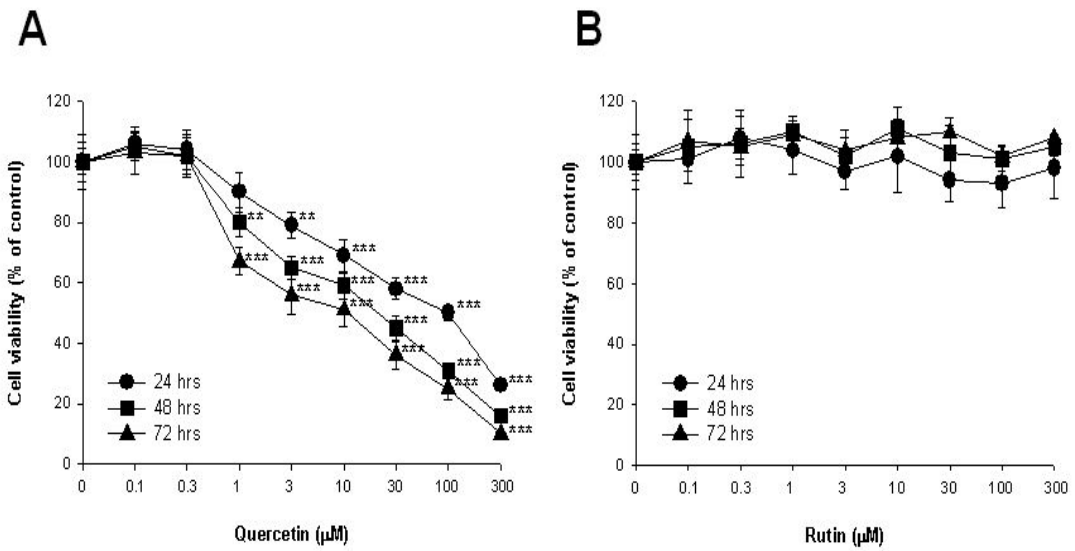

Fig. 2. Time- and concentration-dependent effects of quercetin and rutin on the cell viability in KB human oral cancer cells.

Ⅶ. FIGURES (Fig. 3A)

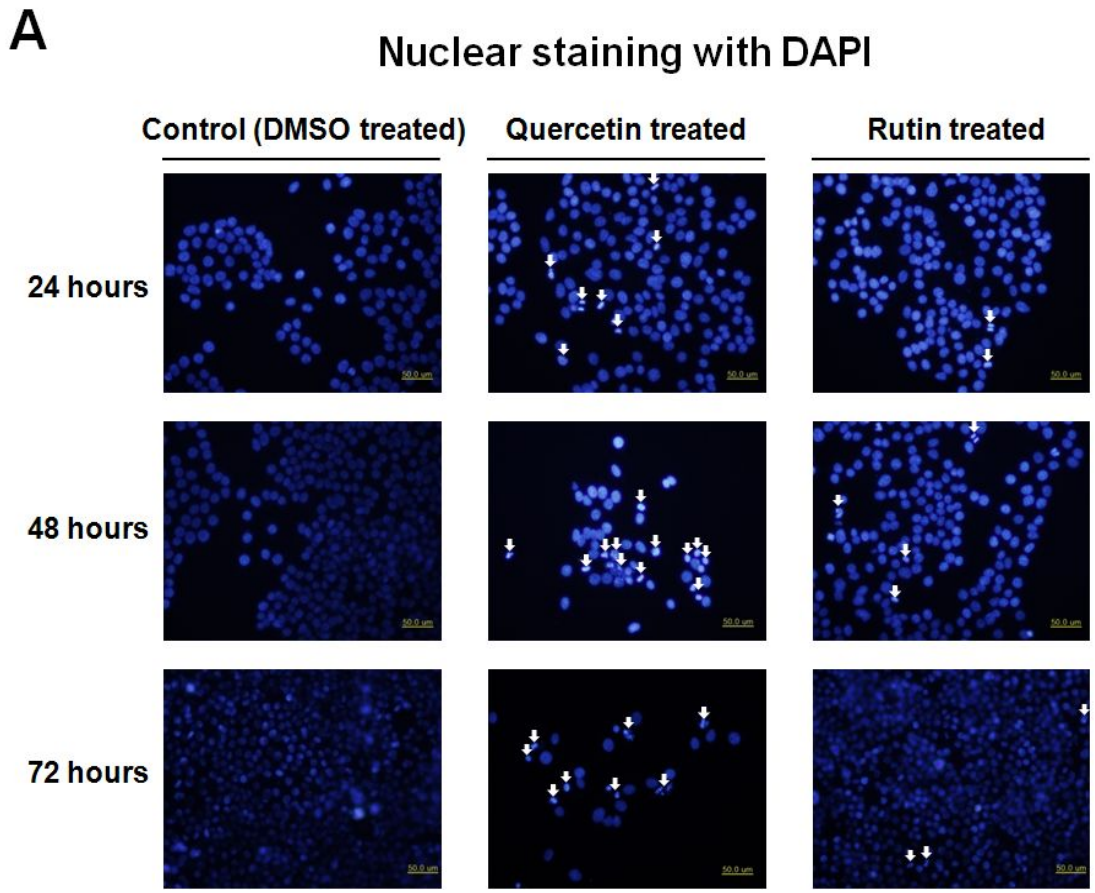

Fig. 3. Apoptotic nuclear changes induced by quercetin and rutin in KB cells.

(A) Representative DAPI-stained fluorescence photomicrographs show the nuclei morphology of KB cells.

Ⅶ. FIGURES (Fig. 3B)

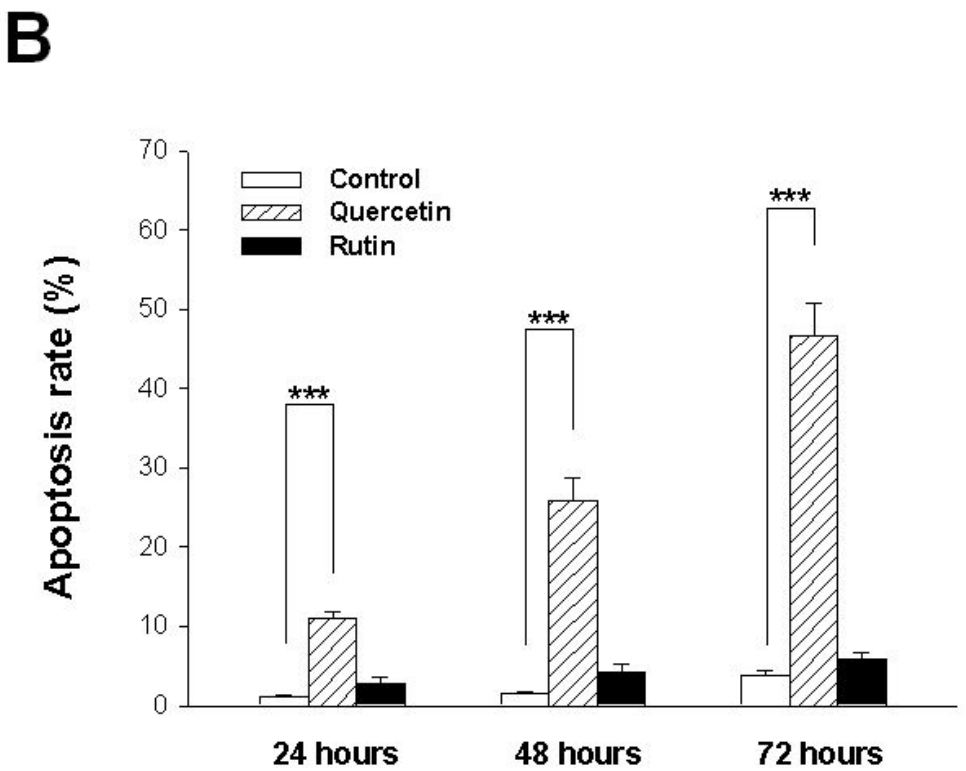

Fig. 3. Apoptotic nuclear changes induced by quercetin and rutin in KB cells.

(B) The percentage of apoptotic cells was calculated as the ratio of apoptotic cells to total cells.

Ⅶ. FIGURES (Fig. 4)

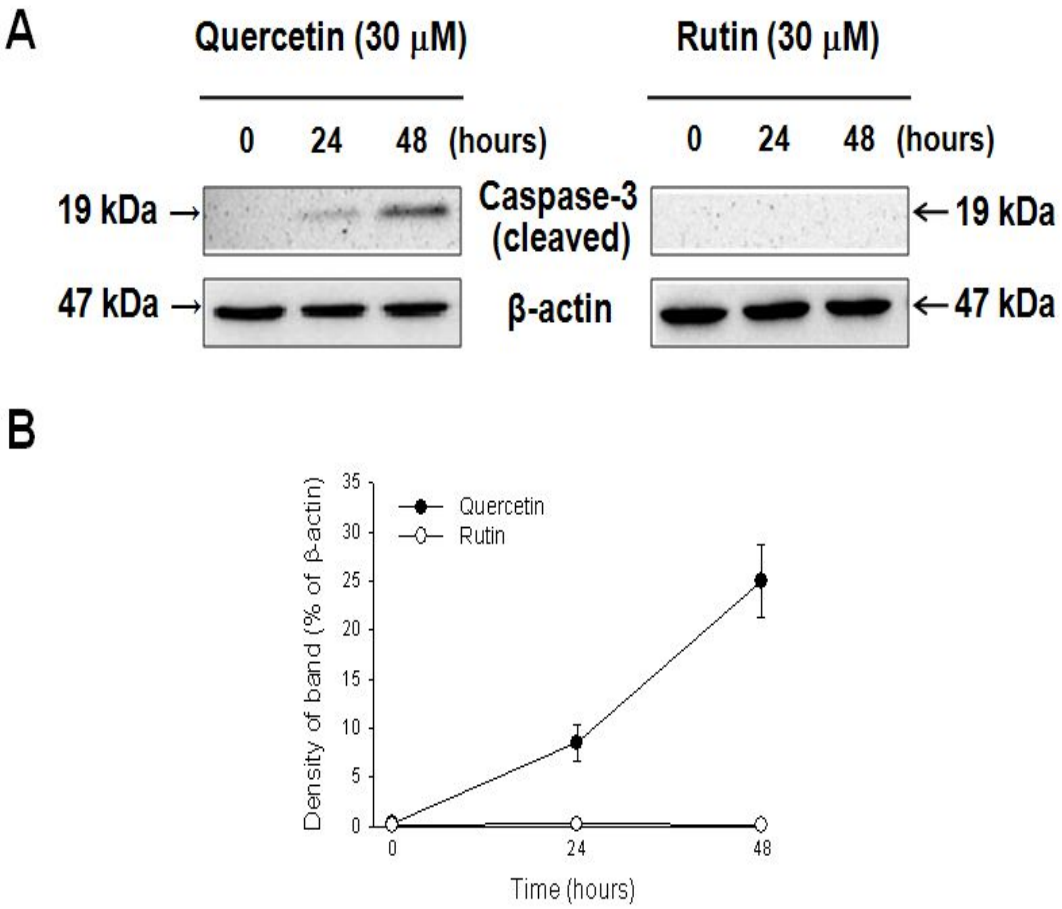

Fig. 4. Proteolytic cleavage of caspase-3 by treatments of quercetin and rutin in KB cells.

Ⅶ. FIGURES (Fig. 5)

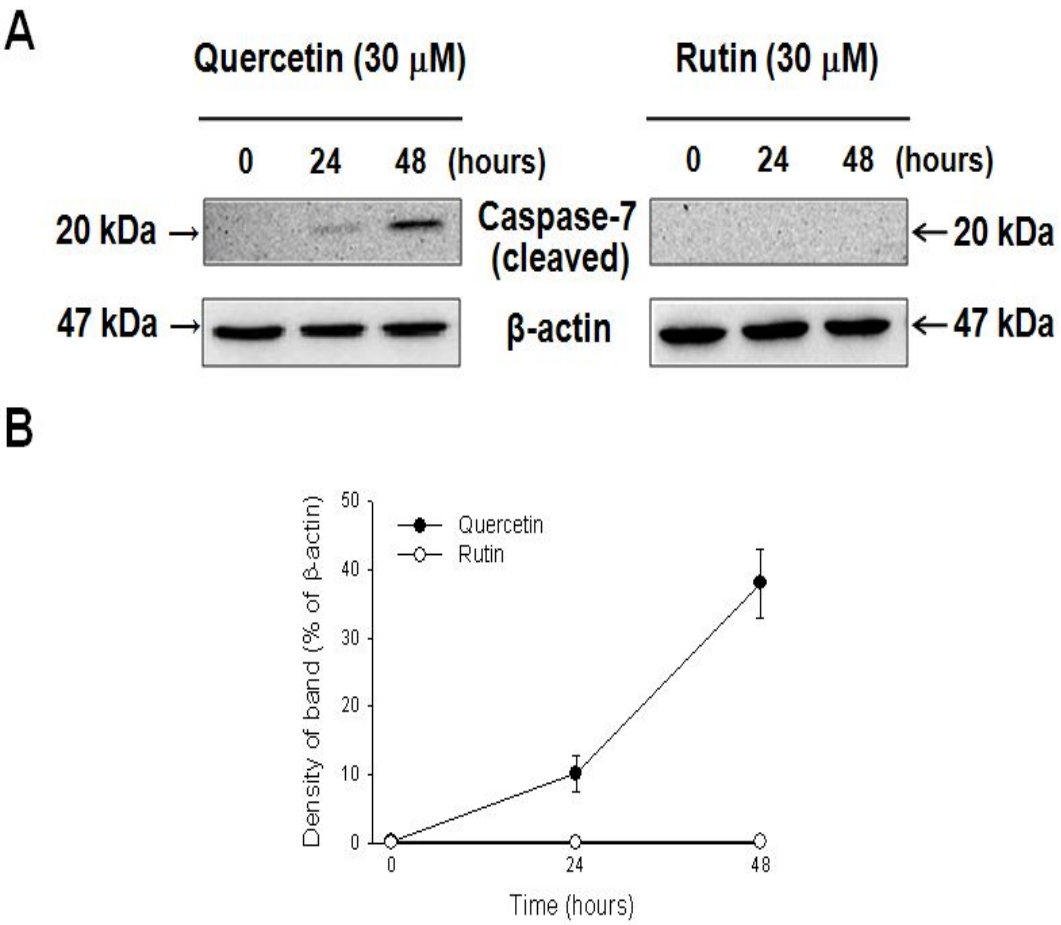

Fig. 5. Proteolytic cleavage of caspase-7 by treatments of quercetin and rutin in KB cells.

Ⅶ. FIGURES (Fig. 6)

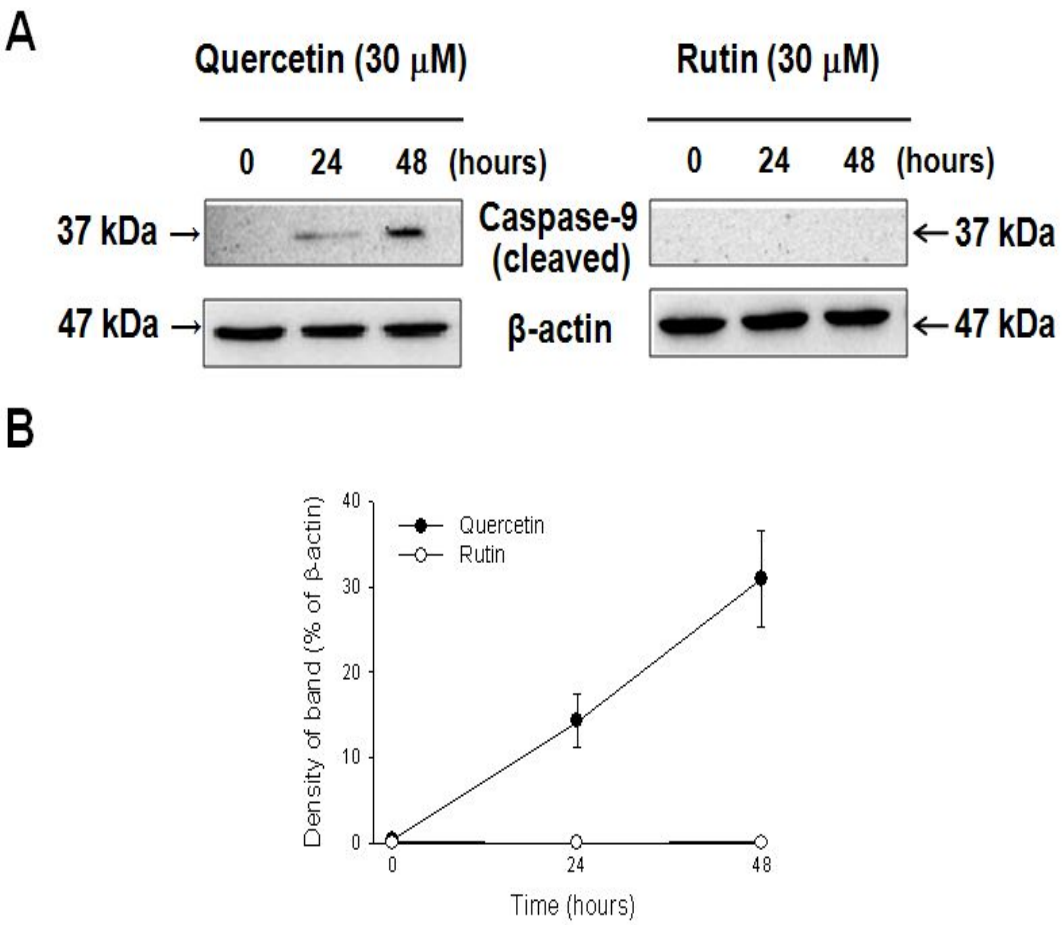

Fig. 6. Proteolytic cleavage of caspase-9 by treatments of quercetin and rutin in KB cells.

Ⅶ. FIGURES (Fig. 7)

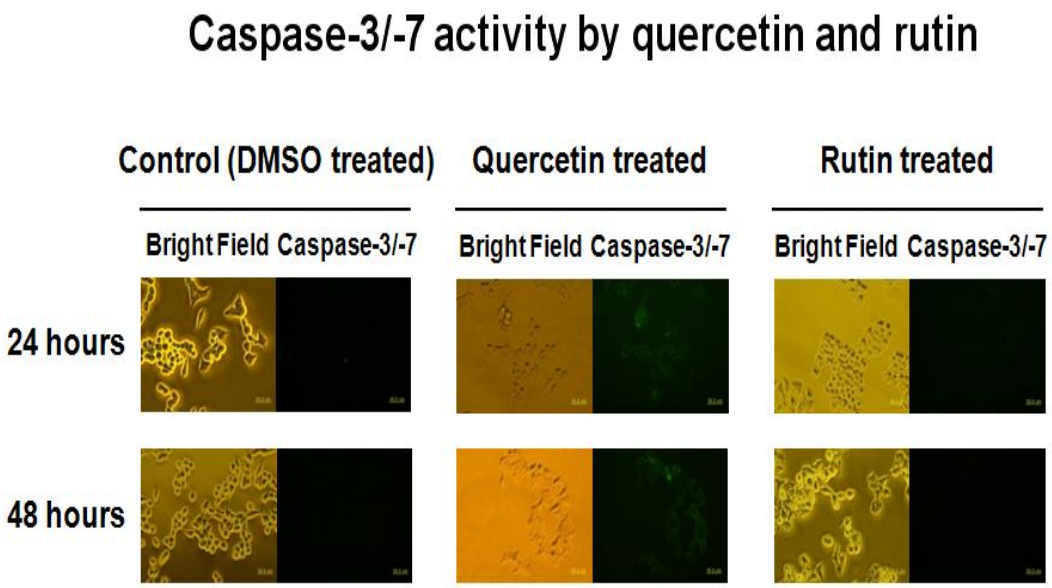

Fig. 7. Activation of caspase-3/-7 by treatments of quercetin and rutin in living KB cells.

ABSTRACT

Anticancer effects of quercetin and rutin on KB human oral cancer cells

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Quercetin (3,5,7,3′,4′-tetrahydroxyflavone), one of the active components of flavonoids derived from the onions, fruits, buckwheat and Chinese herbs, has various beneficial biological activities including anti-oxidant, anti-inflammation, anti-atherosclerosis and anti-tumor properties. Rutin (quercetin-3-O-rutinoside), one of the glycoside derivatives of quercetin, belongs to a class of plant secondary metabolites. The effects of quercetin and rutin on oral cancer cells are not clearly established. The main goal of this study was to investigate the effects of quercetin and rutin on cell growth and apoptosis induction in human oral cancer cells.

To determine the effects of quercetin and rutin on cell growth and apoptosis induction in human oral cancer cells, it was examined by inhibition of cell growth

(MTT assay), nuclear staining with DAPI, immunoblotting and determination of caspase activation in the KB human oral cancer cells. The results are as follows.

1. Quercetin inhibited the growth of KB cells in the concentration- and time-dependent manners, but rutin did not significantly affect the cell viability of KB cells.
2. Treatment of quercetin resulted in nuclear condensation and fragmentation in KB cells, but rutin induced nuclear condensation and fragmentation scarcely.
3. Quercetin promoted proteolytic cleavages of procaspase-3 in a time-dependent manner in the KB cells, with the increases in the amount of cleaved caspase-3, but rutin did not significantly promote proteolytic cleavages of procaspase-3.
4. The proteolytic processing of caspase-7 was increased by quercetin but not rutin in KB cells.
5. Quercetin promoted proteolytic cleavages of procaspase-9 with the increases in the amount of cleaved caspase-9, but rutin did not.
6. Activation of caspase-3 and -7 was detected in living KB cells treated quercetin by fluorescence microscopy, but rutin did not significantly lead to activate the caspase-3/-7 in the cells.

These results suggest that the quercetin highly inhibits cell proliferation and induces apoptosis in KB human oral cancer cells, and that it may have potential properties for anti oral cancer drug discovery.

KEY WORDS: Quercetin, Rutin, Cell death, Apoptosis, Anti-cancer therapy, Oral cancer