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Luteolin induced DNA damage leading to human lung squamous carcinoma CH27 cell apoptosis

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Abstract

Luteolin is an active compound from the *Lonicera japonica* (Caprifoliaceae). Luteolin (50 μM)-induced human lung carcinoma CH27 cell apoptosis is a typical apoptosis that was accompanied by a significant DNA condensation and apoptotic body formation. Luteolin-induced apoptosis is related to its ability to change the expression of apoptotic markers, such as caspase-3 (caspase-dependent) and apoptosis-inducing factor (caspase-independent) protein expression. The alkaline microgel electrophoresis technique (comet assay), which is the most sensitive, was used for estimation of the luteolin-induced DNA single strand breaks in this study. DNA-damaging effects of luteolin on DNA single strand breaks have been demonstrated in our study. In this study, luteolin induced S-phase cell cycle arrest and increased the mRNA of DNA repair enzymes such as human MutT homologue, 8-oxoguanine-glycosylase and apurinic endonuclease. Our data suggested that luteolin induces CH27 cell apoptosis by caspase-dependent and -independent pathway and the effect of luteolin on apoptosis of CH27 cells is associated with DNA damage and the expression of DNA repair enzymes.

Keywords: Luteolin; Human lung squamous carcinoma cell line CH27; Apoptosis; Cell cycle; Comet assay; DNA repair enzyme

1. Introduction

Luteolin is bioactive flavonoid of *Lonicera japonica* (Caprifoliaceae) and has been demonstrated its effect on various tumors such as, human leukemia cell line and pancreatic tumor cells (Ko et al., 2002; Lee et al., 2002). It exhibits a wide spectrum of pharmacological properties, but little is known about its biochemical targets. Several reports suggested that luteolin is an inhibitor of the topoisomerase I and II (Chowdhury et al., 2002; Webb and Ebeler, 2003; Yamashita and Kawanishi, 2000). Therefore, these results further support its therapeutic potential as a lead anti-cancer compound. Up to date, the effects of luteolin on human lung carcinoma cells have not been investigated. In this study, we examined the action of luteolin on human lung carcinoma cell CH27. Many reports also demonstrated that exposure to chemotherapy drug in cancer cells can lead to DNA damage (Han et al., 2002; Kowalska-Loth et al., 2002). The most abundant lesion produced is DNA single strand breakage, which can be sensitively detected by the alkaline microgel electrophoresis (comet) assay (Lee et al., 2004; Singh et al., 2002). The comet assay has also been previously used to detect DNA strand breaks in anti-tumor study (Cardile et al., 2003; Woods et al., 1997). One of the principal forms of oxidative DNA damage is 8-oxo-2-deoxyguanine (8-oxo dG), which as a result of mis-pairing to adenine during DNA-replication results in the formation of G to T transversions. The formation of 8-oxo dG may represent an important mechanism during DNA damage (Murata and Kawanishi, 2004; Murata et al., 2004). As a consequence

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of the formation of 8-oxo dG, there are four main repair systems that maintain the integrity of the human genome: prevention of incorporation (human MutT homologue, hMTH1), mismatch repair (human MutY homologue, hMYH), base excision repair (human homologue of the 8-oxoguanine-glycosylase enzyme, hOGG1) and nucleotide excision repair (Michaels et al., 1992; Parsons et al., 2004). In the current study we investigated mRNA expression of DNA repair enzymes in human lung carcinoma cells exposed to luteolin to determine whether luteolin is able to modulate expression of DNA repair enzymes.

Cells can respond to DNA damage either by undergoing cell cycle arrest, to facilitate DNA repair, or by undergoing cell suicide. Apoptosis is a major form of cell death and is associated with characteristic morphological changes including the formation of membrane blebs and apoptotic bodies, chromatin and nuclear condensation and DNA fragmentation. It is widely accepted that there are two principal pathways of apoptosis, namely, caspase-dependent and -independent. Caspases, a family of cysteine proteases, play a critical role during apoptosis. There are at least two major mechanisms by which a caspase cascade resulting in the activation of effector caspase-3 may be initiated by the most apical caspase, one involving caspase-8 and the other involving caspase-9 (Srinivasula et al., 1998; Zou et al., 1997). Therefore, the activation of caspase-3 is required of apoptosis whether caspase-8 or caspase-9 pathway. Apoptosis-inducing factor (AIF) is a 57 kDa protein that resides mainly within the space between the inner and outer mitochondrial membrane. Upon loss of mitochondrial membrane integrity, AIF is released from the mitochondria to induce nuclear condensation and large-scale DNA fragmentation (Liou et al., 2003). This leads to cell death without the participation of caspases; hence, AIF is a key player in eliciting caspase-independent apoptosis in the cells. Luteolin-mediated DNA damage might be a possible mechanism by which luteolin exert its cytotoxicity potential. This study examined whether luteolin induced changes of the expression of the important members of apoptosis on CH27 cell apoptosis.

2. Materials and methods

2.1. Materials

Luteolin (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one), antipain, aprotinin, dithiothreitol, ethylenediaminetetraacetic acid (EDTA), EGTA, leupeptin, pepstatin, phenylmethylsulfonyl fluoride and Tris were purchased from Sigma Chemical (St. Louis, MO); anti-mouse and anti-rabbit IgG peroxidase-conjugated secondary antibody were purchased from Amersham (Buckinghamshire). Antibodies to various proteins were obtained from the following sources: caspase-3 was obtained from BD Biosciences (San Diego, CA); apoptosis-inducing factor was from Sigma Chemical. Enhanced chemiluminescent (Renaissance) detection reagents were obtained from NEN Life Science Products (Boston, MA).

2.2. Cell culture

CH27 cells were grown in monolayer culture in Dulbecco's modified Eagle's medium (Life Technologies, Rockville, MD) containing 5% fetal bovine serum (Hyclone, Logan, UT), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco BRL, Rockville, MD) and 2 mM glutamine (Merck, Darmstadt, Germany) at 37 °C in a humidified atmosphere comprised of 95% air and 5% CO_2. When CH27 cells were treated with luteolin, the culture medium containing 1% fetal bovine serum was used. All data presented in this report are from at least three independent experiments showing the same pattern of expression.

2.3. Single-cell gel electrophoresis

To examine the DNA damage in these single-cell suspensions, we performed an alkaline single-cell gel electrophoresis (comet assay). The comet assay carried out was based on the method of Singh (2000) with some modification. Fully frosted microscope slide were covered with 85 μl of 1% normal melting agarose in PBS (phosphate-buffered saline), immediately coverslipped and kept at room temperature for 30 min to allow the agarose to solidify. The removal of the cover glass from the agar layer was followed by the addition of a second layer of 75 μl of 1% normal melting agarose containing approximately 10^5 cells at 37 °C. Cover glasses were immediately placed and the slides were placed at room temperature for 30 min. After the solidification of the normal melting agarose, the cover glasses were removed and the slides were immersed in the cold lysis solution (pH 11) containing 2.5 M NaCl, 100 mM Na_2-EDTA, 10 mM Tris–HCl and 1% Triton X-100 for 1 h at room temperature. Gels are then incubated in an alkaline buffer (0.3 M NaOH, 1 mM Na_2-EDTA) for 20 min at 4 °C. The slides were removed from the alkaline buffer and placed on a horizontal electrophoresis tank. The electrophoresis was carried out for 1 h at 25 V. After electrophoresis, the gels were washed gently twice with water and Tris–HCl (0.5 M, pH 7). The slides were immersed in 100% methanol for 30 min at room temperature. The methanol was then removed and the gels were stained with propidium iodide (40 μg/ml). The gels were washed twice with water and examined by fluorescence microscopy (Olympus IX 70).

2.4. Extraction of total RNA

Total RNAs were isolated from control or luteolin-treated CH27 cells with RNeasy Mini kit (QIAGEN, USA)

according to the manufacturer's descriptions. RNA concentration was quantified using a spectrophotometer at a wavelength of 260 nm.

2.5. Reverse transcriptase-polymerase chain reaction (RT-PCR)

cDNA was prepared by reverse transcription of 1.5 μg of total RNA. hMTH1, hOGG1 and apurinic endonuclease transcripts were determined by reverse transcriptase-polymerase chain reaction (RT-PCR) with RNA PCR kit (Invitrogen life technologies, USA). The primers of hMTH1 are 5′-GAGCGGCGGTGCAGAACCCAG-3′ and 5′-AGAAGACATGCACGTCCATGAG-3′ (Sato et al., 2003), hOGG1 are 5′-ATCTGTTCCTCCAACAACAA-3′ and 5′-GCCAGCATAAGGTCCCCACAG-3′ (Hodges and Chipman, 2002), apurinic endonuclease are 5′-ATAGGCGATGAGGATCATGA-3′ and 5′-CAACATTCTTGGATCGAGCA-3′ (Hodges and Chipman, 2002). The primers of β-actin (GenBank accession no. G15871) are 5′-ACAAAACCTAACTTGCGCAG-3′ and 5′-TCCTGTAACAACGCATCTCA-3′, which amplify a 241 bp product. The amplification of hMTH1 (285 bp), hOGG1 (495 bp) and apurinic endonuclease (419 bp) was performed with one denaturing cycle at 95 °C for 5 min, then 30 cycles at 95 °C for 1 min, at 55 °C for 1 min, at 72 °C for 1 min, and one final extension at 72 °C for 10 min. RT-PCR products were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining.

2.6. Flow cytometry analysis

The percentage of hypodiploid cells was determined as described previously (Lee, 2001). Briefly, 2×10^6 cells were trypsinized, washed twice with PBS and fixed in 80% ethanol. Fixed cells were washed with PBS, incubated with 100 μg/ml RNase for 30 min at 37 °C, stained with propidium iodide (50 μg/ml) and analyzed on a FACScan flow cytometer (Becton Dickinson Instruments). The average of the percentages of each phase in the cell cycle was representative of three independent experiments.

2.7. Cell viability assay

Cells were seeded at a density of 5×10^4 cells/well onto 12-well plate (Falcon, Franklin Lakes, NJ) 48 h before drugs treated. Drugs were added to medium, at various indicated times and concentrations. The control cultures were treated with 0.1% DMSO (dimethylsulfoxide; Merck). After incubation, cells were washed with PBS (phosphate-buffered saline). The number of viable cells was determined by staining cell population with Trypan blue (Sigma Chemical). One part of 0.2% Trypan blue dissolved in PBS was added to one part of the cell suspension, and the number of unstained (viable) cells was counted.

2.8. 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI) staining

Cells were seeded at a density of 5×10^4 cells/well onto 12-well plate 48 h before drugs treated. CH27 cells were cultured for 16 h in 1% serum medium with vehicle alone (0.1% DMSO), 30, 50 or 80 μM luteolin. After treatment, cells were fixed with 3.7% formaldehyde for 15 min, permeabilized with 0.1% Triton X-100 and stained with 1 μg/ml DAPI for 5 min at 37 °C. The cells were then washed with PBS and examined by fluorescence microscopy (Olympus IX 70).

2.9. Protein preparation

Protein was extracted as previously described (Lee, 2001). Adherent and floating cells were collected at the indicated times and washed twice in ice-cold PBS. Cell pellets were resuspended in modified RIPA buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.25% sodium deoxycholate, 1 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 1 mM sodium fluoride, 5 μg/ml aprotinin, 5 μg/ml leupeptin and 5 μg/ml antipain) for 30 min at 4 °C. Lysates were clarified by centrifugation at 13,000×*g* for 30 min at 4 °C and the resulting supernatant was collected, aliquoted (50 μg/tube) and stored at −80 °C until assay. The protein concentrations were estimated with the Bradford method (Bradford, 1976).

2.10. Western blot analysis

Samples were separated by various appropriate concentrations (11 and 13%) of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE; Bio-Rad Life Science Products, Hercules, CA). The SDS-separated proteins were equilibrated in transfer buffer [50 mM Tris, pH 9.0–9.4, 40 mM glycine (Bio-Rad Life Science Products), 0.375% SDS (Bio-Rad Life Science Products), 20% methanol (Merck)] and electrotransferred to Immobilon-P Transfer Membranes (Millipore, Bedford, MA). The blot was blocked with a solution containing 5% nonfat dry milk in Tris-buffered saline [10 mM Tris, 150 mM NaCl (Sigma Chemical)] with 0.05% Tween 20 (TBST; Merck) for 1 h, washed and incubated with antibodies to β-actin (1:5000; Sigma Chemical, the detection of β-actin was used as an internal control in all of the data of Western blotting analysis), AIF (1:1000, Sigma Chemical) and caspase-3 (1:1000, PharMingen). Secondary antibody consisted of a 1:20,000 dilution of horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG. The enhanced chemiluminescent (NEN Life Science Products, Boston, MA) detection system was used for immunoblot protein detection.

2.11. Data analysis and statistics

Values are presented as percentage±S.D. of control. Statistically significant difference from the control group

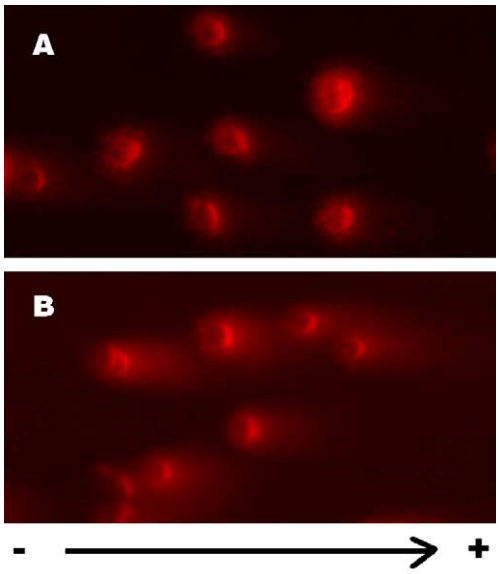

Fig. 1. Luteolin-induced DNA single strand breaks in CH27 cells. Cells were incubated with (B) or without (A) 50 μM luteolin in the presence of 1% serum for 16 h. Single-cell gel electrophoresis was determined as described in Materials and methods. The symbols of + and − indicated the anode and cathode, respectively, during electrophoresis of negatively charged DNA. Results are representative of three independent experiments.

was identified by Student's *t* test for paired data. A *P* value less than 0.05 was considered significant for all tests.

3. Results

3.1. The effect of luteolin on DNA damage in CH27 cells

To study the DNA damage in the luteolin treated cells, we performed an alkaline single-cell gel electrophoresis (comet assay). Alkaline comet assay is a sensitive method by which DNA single strand breaks at a single-cell level can be monitored. Fig. 1 is a photomicrograph of DNA migration patterns in human lung squamous carcinoma CH27 cells, processed using comet assay. When cells were treated with 50 μM luteolin for 16 h, DNA damage, as indicated by increased tail migration, is significantly observed in the majority of the luteolin-treated cells compared to those of control cells. The comet assay results in CH27 cells showed that luteolin induced DNA damage in this study.

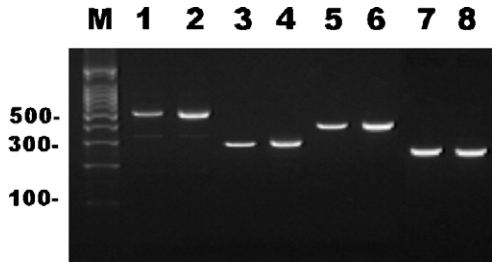

Fig. 2. Effects of luteolin on the mRNA expression of hMHT1, hOGG1 and apurinic endonuclease in CH27 cells. Luteolin-induced gene expression of hMHT1, hOGG1 and apurinic endonuclease was detected by RT-PCR. CH27 cells were incubated with 0.1% DMSO or 50 μM luteolin in the presence of 1% serum for 24 h. RNA samples were prepared from control or luteolin-treated cells. PCR products run on a 2% agarose gel. Lane M, molecular weight marker; lane 1 (hOGG1), control cells; lane 2 (hOGG1), luteolin-treated cells; lane 3 (hMHT1), control cells; lane 4 (hMHT1), luteolin-treated cells; lane 5 (apurinic endonuclease), control cells; lane 6 (apurinic endonuclease), luteolin-treated cells; lane 7 (β-actin), control cells; lane 8 (β-actin), luteolin-treated cells. Results are representative of three independent experiments.

Table 1
Luteolin-induced S-phase arrest in flow cytometry assay

Luteolin (μM)	G0/G1	S	G2/M
0	59.7±2	29.3±3	11.0±2
30	57.2±3	35.3±3	7.5±1
50	55.0±3	38.1±1	6.9±1
80	55.0±1	39.1±2	5.9±1

Cells were treated with vehicle alone, 30, 50 or 80 μM luteolin in the presence of 1% serum for 24 h. After treatment, cells were harvested and subjected to cytometric analysis. The percentages of each phase in the cell cycle were representative of three independent experiments.

3.2. The effects of luteolin on the gene expression of DNA repair enzymes in CH27 cells

To elucidate whether luteolin affects the gene expression of hMHT1, hOGG1 and apurinic endonuclease, RT-PCR techniques were used in this study. The detection of β-actin was used as an internal control in the data of PCR. After CH27 cells were treated with 50 μM luteolin for 24 h, there was a significant increase in the gene expression of hMHT1, hOGG1 and apurinic endonuclease in CH27 cells (Fig. 2).

3.3. Luteolin induced CH27 cells S-phase arrest

Due to luteolin-induced DNA damage, we investigated the effect of luteolin on cell cycle in CH27 cells. Flow

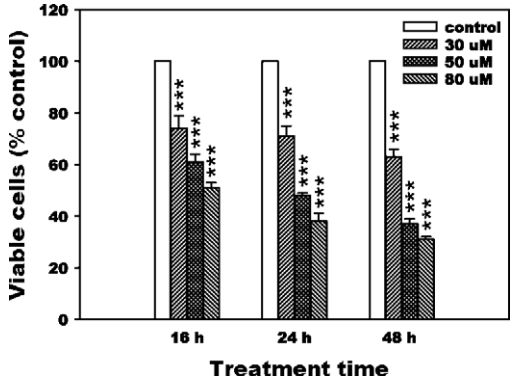

Fig. 3. Luteolin induces CH27 cell death. Cells were cultured for 48 h before drug treatment in 12-well plates. Cells were treated without (0.1% DMSO) or with luteolin (30, 50 and 80 μM) in the presence of 1% serum at 37 °C for different times (16, 24 and 48 h) and cells were washed and counted for Trypan blue exclusion with a hemocytometer. All results are expressed as the mean percentage of control±S.D. of triplicate determinations from three independent experiments. Asterisks indicate values significantly different from control values (***$P<0.001$).

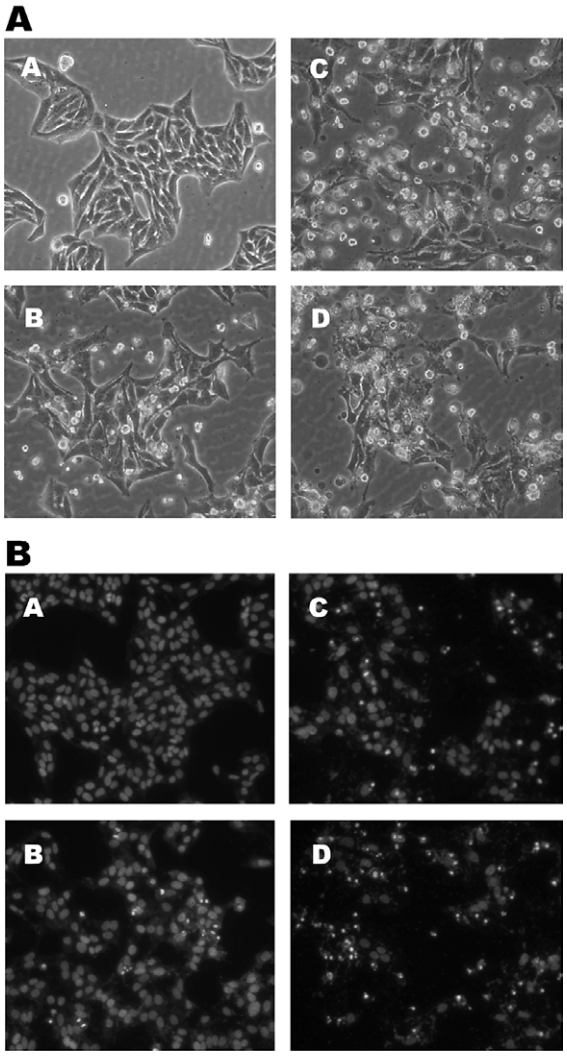

Fig. 4. Luteolin induces CH27 cell apoptosis. (A) Changes in CH27 cell morphology during luteolin-induced apoptotic cell death. Shown are phase-contrast views of CH27 cells cultured for 24 h under control conditions (A) or in the presence of 30 (B), 50 (C) and 80 (D) μM luteolin. CH27 cells formed apoptotic bodies, and then floated after treatment with luteolin. (B) Luteolin induced phenotypic changes in cell nucleus. CH27 cells were cultured for 16 h in 1% serum medium with vehicle alone (A), 30 (B), 50 (C) or 80 (D) μM luteolin. After treatment, cells were fixed with 3.7% formaldehyde for 15 min, permeabilized with 0.1% Triton X-100 and stained with 1 μg/ml DAPI for 5 min at 37 °C. Cells were then washed with PBS and examined by fluorescence microscopy. Results are representative of three independent experiments.

cytometric analysis was performed on cells treated with 30, 50 or 80 μM luteolin for 24 h. Cell cycle analysis revealed a concentration-dependent S-phase arrest after treatment with luteolin. Significant S-phase arrest was indicated by decreased proportion of cells in G2/M-phase (Table 1).

3.4. Luteolin induced CH27 cell death in a dose- and time-dependent manners

The present study served to evaluate the effects of luteolin on cell death of lung carcinoma cell line CH27. We determined the effect of luteolin on cell viability by Trypan blue dye exclusion. The data are presented as proportional viability (%) by comparing the treated group with the untreated cells, the viability of which was assumed to be 100%. As shown in Fig. 3, 48 h of continuous exposure to various concentrations of luteolin (30, 50 and 80 μM) on CH27 cells resulted in time- and dose-dependent decreases in cell number relative to control cultures. The concentration of luteolin-induced cell death was significant at 30 μM.

3.5. Luteolin induces apoptosis in CH27 cells

Based on the above data, CH27 cells failing to progress to mitosis may be destined to apoptosis by luteolin. The phenotypic characteristics of luteolin-treated CH27 cells were evaluated by microscopic inspection of overall morphology. CH27 cells formed apoptotic bodies, and then floated after treatment with 30, 50 or 80 μM luteolin for 24 h (Fig. 4A). To further investigate whether the induction of S-phase arrest by luteolin could be linked to apoptosis in CH27 cells, nuclear morphological changes were performed. Treatment of CH27 with luteolin resulted in changes in nuclear morphology, evidenced by the DAPI staining, a DNA binding dye. There was a gradual dose-dependent increase in the number of nuclear condensation after treatment with luteolin (30, 50 and 80 μM) for 16 h in CH27 cells (Fig. 4B). In this study, the luteolin-induced CH27 cells nuclear morphological change, DNA condensation and apoptosis were observed. Luteolin-induced CH27 cell death was indicative of a typical apoptosis.

3.6. The effects of luteolin on apoptosis-inducing factor and caspase-3 expression on CH27 cells

To obtain further support for the induction of DNA damage and apoptosis by luteolin in CH27 cells, the expression of apoptosis-inducing factor and caspase-3 proteins, which are the key indicators of intracellular signaling of apoptosis-dependent and -independent, were performed. In this study, the expression of proform of caspase-3 was significantly decreased after treatment with

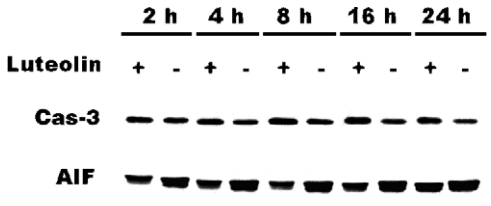

Fig. 5. Effects of luteolin on the expression of apoptosis-inducing factor and caspase-3 proteins. The effect of luteolin (50 μM) on apoptosis-inducing factor (AIF) and caspase-3 (Cas-3) proteins was detected by Western blot analysis in CH27 cells. Cells were incubated with or without 50 μM luteolin in the presence of 1% serum for 2, 4, 8, 16 and 24 h. Cell lysates were analyzed by 11% (AIF) and 13% (caspase-3) SDS–PAGE and probed with primary antibodies as described in Materials and methods. Results are representative of three independent experiments.

luteolin for 2 h (Fig. 5). However, AIF protein levels increases after 2 h treatment with luteolin (Fig. 5). Based on the above results, luteolin-induced cell death provides apoptotic characteristics and triggers changes in the proteins, which are intimately associated with apoptosis.

4. Discussion

Luteolin exhibits a wide spectrum of pharmacological properties, but little is known about its anti-cancer mechanisms. The previous study has demonstrated that luteolin induced HL-60 cell apoptosis via topoisomerase II-mediated DNA damage (Yamashita and Kawanishi, 2000). Chowdhury et al. (2002) have suggested that luteolin is similar to camptothecin, a class I inhibitor, with respect to its ability to form the topoisomerase I-mediated 'cleavable complex'. Luteolin has also been examined with regard to the inhibition of proliferation and induction of apoptosis in several tumor cell lines (Ko et al., 2002; Lee et al., 2002). Based on the above suggestions, luteolin is an inhibitor of the topoisomerases and poisons topoisomerases that resulted in DNA damage. Therefore, luteolin may be considered as a potential chemotherapeutic agent. Our results, in CH27 exposed to luteolin, support earlier reports on the inducing of DNA damage. DNA-damaging effects of luteolin on DNA single strand breaks or alkali labile sites have been demonstrated in our study. The alkaline microgel electrophoresis technique (comet), which is the most sensitive, was used for estimation of DNA single strand breaks in this study. In order to prevent DNA from chemical-mediated damage, there are many specific DNA repair enzymes, which help in the safeguarding and repairing of DNA. During DNA damage, up-regulation the mRNA expression of DNA repair enzyme human MutT homologue (hMTH1) has been demonstrated. Human MTH1 prevents the cell from incorporating 8-oxo-deoxyguanosine (8-oxo dG) into DNA by hydrolyzing 8-oxo-dGTP to 8-oxo-dGMP. 8-Oxo dG, which is one of the major forms of oxidative DNA damage, is produced in cells by exposure to a wide range of chemical agents. 8-Oxo dG paired to cytosine in DNA is recognized and repaired by DNA-glycosylase or endonuclease. The mRNA expression of hMTH1 was significantly increased by exposure to luteolin in CH27 cells. It seems to suggest that luteolin-induced increase in DNA damage results in enhancing the ability of hMHT1 in the hydrolysis of 8-oxo-dGTP to 8-oxo-dGMP in this study. Dybdahl et al. (2003) have demonstrated that diesel exhaust particle-induced DNA strand breaks were accompanied by a significant increase in the level of hOGG1 mRNA, encoding an enzyme involved in repair of 8-oxo dG. Human OGG1 cleaves the glycosidic bond of 8-oxo dG preferentially at 8-oxo dG:C base pairs during DNA repair process. Our study has demonstrated that the up-regulation of hOGG1 gene expression appeared to play a role in luteolin-induced CH27 cells apoptosis. The increase of the mRNA expression of apurinic endonuclease that can cleave the phosphodiester bond at the apurinic-site in the process of base excision repair was also observed during luteolin-induced CH27 cells apoptosis. Based on the above data, it seems to suggest that luteolin induces the increase in the amount of mRNA of DNA base excision repair enzymes, such as hOGG1 and apurinic endonuclease, yet those enzymes could not repair completely the severe DNA damage.

Based on the above findings, we found that the DNA damage was an important factor in luteolin-induced CH27 cell apoptosis. We focused the attention on the proteins associated with DNA fragmentation, such as caspase-3 (caspase-dependent apoptosis pathway) and apoptosis-inducing factor (caspase-independent apoptosis pathway). Caspases are involved in various programmed cell death pathways reported in many investigations. They are a family of cysteine proteases, and many of them are implicated as important initiators or effectors of the apoptosis process. To date, at least 14 members of this family have been identified. Among them, the caspase-3 is required for many of the nuclear changes associated with apoptosis, including DNA fragmentation and chromatin condensation. Apoptosis-inducing factor is released from the mitochondria to induce nuclear condensation and large-scale DNA fragmentation, resulting in cell death. In this study, luteolin has been shown to be inducing apoptosis due to its capability to damage DNA, directly or indirectly, via activation of caspase-3 or apoptosis-inducing factor. Generally, cells with damaged DNA must arrest to allow repair of damage before replication. Due to luteolin-induced DNA damage, we also investigated the effect of luteolin on cell cycle in CH27 cells. Cell cycle analysis revealed an increase in the cell population in S-phase following treatment with luteolin for 24 h. Based on our above results, it indicated that luteolin induced cell cycle arrest and increased the mRNA expression of DNA repair enzymes in order to allow DNA repair.

In summary, the results of our study provide experimental evidence that luteolin-induced CH27 cell apoptosis is a typical apoptosis that was accompanied by a significant DNA condensation and apoptotic body formation. Luteolin-induced apoptosis is probably related to its ability to change the expression of apoptotic markers, such as caspase-3 and apoptosis-inducing factor protein expression in CH27 cells. In this study, luteolin induced S-phase cell cycle arrest and increased in mRNA of DNA repair enzymes. However, the severe DNA damage is beyond the capacity of the DNA repair enzymes. In order to eliminate the damaged cells, the apoptosis was observed after treatment with luteolin in CH27 cells.

Acknowledgements

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