Emodin, Aloe-emodin and Rhein Induced DNA Damage and Inhibited DNA Repair Gene Expression in SCC-4 Human Tongue Cancer Cells

YA-YIN CHEN[1,2], SU-YIN CHIANG[1], JAUNG-GENG LIN[1], JAI-SING YANG[3], YI-SHIH MA[1], CHING-LUNG LIAO[1], TUNG-YUAN LAI[4,5], NOU-YING TANG[1] and JING-GUNG CHUNG[6]

Schools of [1]Chinese Medicine, [4]Post-baccalaureate Chinese Medicine, Departments of [3]Pharmacology, [6]Biological Science and Technology, China Medical University, Taichung 404, Taiwan, R.O.C.; Departments of [2]Chinese Medicine and [5]Chinese Internal Medicine, China Medical University Hospital, Taichung 404, Taiwan, R.O.C.

Abstract. *In our primary studies, we have shown that emodin, aloe-emodin and rhein induced cytotoxic effects, including cell cycle arrest and apoptosis in SCC-4 human tongue cancer cells. However, details regarding their effects on DNA damage and repair gene expression in SCC-4 cells are not clear. We investigated whether or not emodin, aloe-emodin and rhein induced DNA damage and inhibited DNA repair gene expression in SCC-4 cells. Comet assay (single cell electrophoresis) indicated that incubation of SCC-4 cells with 0, 20, 30 and 40 μM of emodin, 0, 25, 50 and 100 μM of aloe-emodin or rhein led to a longer DNA migration smear (comet tail). This means that all examined agents induced DNA damage in SCC-4 cells and these effects are dose-dependent but emodin is stronger than that of aloe-emodin or rhein. The results from real-time PCR assay demonstrated that 30 μM of emodin or aloe-emodin used for 24 and 48 h treatment in SCC-4 cells significantly inhibited expression of genes associated with DNA damage and repair [ataxia telangiectasia mutated (ATM); ataxia-telangiectasia and Rad3-related (ATR); 14-3-3sigma (14-3-3σ); breast cancer 1, early onset (BRCA1); and DNA-dependent serine/threonine protein kinase (DNA-PK)]; only rhein suppressed the expression of O^6-methylguanine-DNA methyltransferase (MGMT) mRNA with 48 h treatment, but had no effect on ATM expression. On 24 h treatment, only aloe-emodin significantly affected ATM expression. These effects may be the vital factors for emodin, aloe-emodin and rhein induction of DNA damage in vitro. In conclusion, these agents induced DNA damage followed by the inhibition of DNA repair-associated gene expressions, including ATM, ATR, 14-3-3σ, BRCA1, DNA-PK and MGMT in SCC-4 human tongue cancer cells.*

The maintenance of the genome relies upon the repair of damaged DNA before cell replication; cell cycle arrest allows cells to repair such damage before the start of DNA synthesis. It is well-known that cells lacking p53 fail to arrest in response to a wide variety of DNA-damaging agents (1-3). The p53-dependent transactivation of 14-3-3σ plays a role in the inhibition of G_2/M phase progression (4), whereas G_1/S phase arrest after DNA damage is controlled, at least in part, by up-regulation of *p21* (5-6). DNA repair for eliminating spontaneous and carcinogen-induced DNA damage is an important cellular defense mechanism against mutagenesis and carcinogenesis (7-8).

Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is one of the active constituents of the herb of *Rheum palmatum* L. (9-10) and possesses anticancer, antibacterial, diuretic, and vasorelaxant effects (11-13). It was reported that emodin can inhibit the formation of 1-nitropyrene-induced DNA adducts in *Salmonella typhimurium* (TA98) (14). Emodin induced apoptosis in LNCaP human prostate cancer cells (15), lung adenocarcinoma cells (16), HepG2 hepatocellular carcinoma cells (17), and BCap-37 breast cancer cells (18). Recently, our studies have shown that emodin mediated DNA damage based on reactive oxygen species (ROS) production and endoplasmic reticulum (ER) stress based on the levels of growth arrest and DNA damage inducible gene 153 (*GADD153*) and glucose regulated protein 78 (*GRP78*) that

Correspondence to: Jing-Gung Chung, Department of Biological Science and Technology, China Medical University, No 91, Hsueh-Shih Road, Taichung 404, Taiwan. Tel: +886 4220533662161, Fax: +886 422053764, e-mail: jgchung@mail.cmu.edu.tw Jaung-Geng Lin, Graduate Institute of Chinese Medical Science, China Medical University, No 91, Hsueh-Shih Road, Taichung 404, Taiwan, R.O.C. Tel: +886 422053366/3311, Fax: +886 422035192, e-mail: jglin@mail.cmu.edu.tw

Key Words: ATM, BRCA1, RT-PCR, Comet assay.

acts as an early and upstream change in the cell death cascade to caspase- and mitochondria-dependent signaling pathways, triggered mitochondrial dysfunction from Bcl-2 and Bax modulation, mitochondrial cytochrome *c* release and caspase activation, consequently leading to apoptosis in SCC-4 human tongue cancer cells (19).

Aloe-emodin (1,8-dihydroxy-3-(hydroxymethyl)-anthraquinone) is an active component contained in the root and rhizome of *Rheum palmatum* L. (Polygonaceae) (20). Pecere *et al*. reported that aloe-emodin has a specific anti-neuroectodermal tumor activity (21). From *in vitro* studies, it was demonstrated the genotoxicity of aloe-emodin (22) and its ability to promote malignant transformation of cells (23), and showed that it is not mutagenic *in vivo* (22). It was reported that aloe-emodin has selective activity against neuroectodermal tumors (22, 24) and it has shown antiproliferative activity in human hepatoma (25) and lung carcinoma cell lines (26). Aloe-emodin was also reported to induce apoptosis in human gastric carcinoma cells (27), H460 non-small cell lung carcinoma cells (28) and hepatoma cells (29). Recently in our laboratory, we found that aloe-emodin induced apoptosis in SCC-4 human tongue cancer cells through the death-receptor, mitochondria and caspase cascade-dependant pathways (30).

Rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid) is a compound isolated from the root of rhubarb (*Rheum palmatum* L.), and suppresses phorbol ester-induced tumor promotion in JB6 mouse epidermal cell line (31). It was reported that rhein suppresses the growth of tumor cells in rat liver (32), human glioma (33), and Ehrlich ascites tumor (34) *in vivo*. Rhein induced apoptosis in human colonic adenocarcinoma monolayer cells (35) and HL-60 leukemia cells (36). Recently in our laboratory, we found that rhein induced apoptosis in Ca Ski human cervical cancer cells (37), nasopharyngeal carcinoma cells (38) and SCC-4 human tongue cancer cells *via* caspase, ROS and mitochondrial death pathways (39).

Despite much evidence suggesting that emodin, aloe-emodin and rhein induced apoptosis in many cancer cell lines, there is not enough information to show that these compounds induced DNA damage and inhibited DNA repair gene expression. Therefore, in this study, we investigated the effects of emodin, aloe-emodin and rhein on DNA damage and DNA repair genes in SCC-4 cells.

Materials and Methods

Chemicals and reagents. Emodin, aloe-emodin, rhein, dimethyl sulfoxide (DMSO), propidium iodide (PI), Tris-HCl, triton X-100 and trypan blue were obtained from Sigma Chemical Co. (St. Louis, MO, USA). RPMI-1640 medium, fetal bovine serum (FBS), L-glutamine, penicillin-streptomycin and trypsin-EDTA were obtained from Invitrogen/Gibco BRL (Grand Island, NY, USA). High Capacity cDNA Reverse Transcription Kit and 2X SYBR Green PCR Master Mix were obtained from Applied Biosystems (Carlsbad, CA, USA).

Human tongue cancer cells. Human tongue cancer cell line (SCC-4) was obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan, ROC) and were cultured at 37˚C under a humidified 5% CO_2 and 95% air at one atmosphere with RPMI-1640 medium supplemented with 10% FBS, 100 Units/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine. The medium was changed every 2 days (19, 39).

Assessment of viability of SCC-4 cells after exposure to emodin, aloe-emodin and rhein. SCC-4 cells (2×10^5 cells/well) were placed in 12-well plates and incubated at 37˚C for 24 h before each well was treated with 0, 20, 30 or 40 μM emodin; or 0, 25, 50 or 100 μM aloe-emodin; or 0, 25, 50 or 100 μM rhein for 24 h. DMSO (solvent) was used for the control regimen. The cells were stained with PI (5 μg/ml) and analyzed by flow cytometry (Becton-Dickinson, San Jose, CA, USA) as previously described (19, 38-39).

Comet assay for examining DNA damage in SCC-4 cells. Approximately 2×10^5 cells/well of SCC-4 cells in 12-well plates were incubated with emodin at final concentrations of 0, 20, 30 or 40 μM, 1 μl DMSO (vehicle) and 5 μM of H_2O_2 (positive control), and exposed to aloe-emodin or rhein at final concentrations of 0, 25, 50 and 100 μM, 1 μl DMSO and 5 μM of H_2O_2 in RPMI-1640 medium grown at 37˚C in 5% CO_2 and 95% air. The cells were harvested for the examination of DNA damage using the comet assay as described previously (19, 30, 40).

Real-time PCR of ATM, ATR, 14-3-3σ, BRCA1, DNA-PK and MGMT in SCC-4 cells. Total RNA isolation, cDNA synthesis, and real-time PCR were carried out as described previously (41). Briefly, SCC-4 cells (1×10^6 cells/well) in 6-well plates were maintained in RPMI-1640 medium with or without emodin (30 μM), aloe-emodin (50 μM) or rhein (50 μM), respectively were incubated for 24 and 48 h. The total RNA from each sample was extracted by using the Qiagen RNeasy Mini Kit (Qiagen, inc, Valencia, CA, USA) as described previously (41). RNA samples were reverse-transcribed for 30 min at 42˚C with High Capacity cDNA Reverse Transcription Kit according to the standard protocol of the supplier (Applied Biosystems, Carlsbad, CA, USA). The quantitative PCR was performed under the following the conditions: 2 min at 50˚C, 10 min at 95˚C, and 40 cycles of 15 s at 95˚C, 1 min at 60˚C using 1 μl of the cDNA reverse-transcribed as described above, 2X SYBR Green PCR Master Mix (Applied Biosystems) and 200 nM of forward and reverse primers as shown in Table I (42). Finally, each assay was run on an Applied Biosystems 7300 Real-Time PCR System in triplicates and expression fold-changes were derived using the comparative C_T method (41).

Statistical analysis. Student's *t*-test was used to analyze differences between groups treated with emodin, aloe-emodin and rhein and the untreated (control) group.

Results

Emodin, aloe-emodin and rhein reduced the viability of human tongue cancer (SCC-4) cells. The cells were exposed to different concentrations of emodin, aloe-emodin and rhein for 24 h, and cells were collected for PI staining for viability analysis. The results are presented in Figure 1 and there were fewer viable cells as concentration increased when compared

Table I. *Sequences used in real-time PCR analysis. The DNA sequence was evaluated using the Primer Express software.*

Primer name	Primer sequence
Human *ATM*F	TTTACCTAACTGTGAGCTGTCTCCAT
Human *ATM*R	ACTTCCGTAAGGCATCGTAACAC
Human *ATR*F	GGGAATCACGACTCGCTGAA
Human *ATR*R	CTAGTAGCATAGCTCGACCATGGA
Human *14-3-3σ*F	GCCATGGACATCAGCAAGAA
Human *14-3-3σ*R	GGCTGTTGGCGATCTCGTA
Human *BRCA1*F	CCAGGGAGTTGGTCTGAGTGA
Human *BRCA1*R	ACTTCCGTAAGGCATCGTAACAC
Human *DNA-PK*F	CCAGCTCTCACGCTCTGATATG
Human *DNA-PK*R	CAAACGCATGCCCAAAGTC
Human *MGMT*F	CCTGGCTGAATGCCTATTTCC
Human *MGMT*R	TGTCTGGTGAACGACTCTTGCT
Human *GAPDH*F	ACACCCACTCCTCCACCTTT
Human *GAPDH*R	TAGCCAAATTCGTTGTCATACC

Each assay was conducted at least twice to ensure reproducibility. F, Forward; R, reverse.

to control groups. These effects were dose dependent and the results demonstrated that emodin, aloe-emodin and rhein, respectively displayed a remarkable cytotoxic effect with concentrations causing the death of 50% of cells (LC_{50}) of 29.13±1.09, 48.53±1.12 and 56.87±3.59 μM in SCC-4 cells at 24h.

Emodin, aloe-emodin and rhein induced DNA damage in human tongue cancer (SCC-4) cells as demonstrated by comet assay. Previous studies have shown that emodin, aloe-emodin and rhein induce cytotoxic effects including apoptosis on SCC-4 cells. In the present study, we investigated whether or not emodin, aloe-emodin and rhein induced DNA damage in SCC-4 cells. The results from the comet assay are presented in Figure 2. From each treatment, the high concentration of emodin, aloe-emodin and rhein led to a longer comet tail (Figure 2A, B and C, respectively). Quantification of each sample tail length also confirmed this (Figure 2A, B and C), indicating that DNA was damaged in the cells in a dose-dependent manner. These results also showed DNA damage in SCC-4 cells was induced with an order of emodin> aloe-emodin> rhein.

Emodin, aloe-emodin and rhein inhibited DNA damage repair gene expressions in SCC-4 cells as shown by real-time PCR. SCC-4 cells were treated individually with 30 μM of each agent for 24 and 48 h. The results of analysis of gene expression by real-time PCR are shown in Figure 2A, B and C, respectively. Expression of *ATM, ATR, 14-3-3σ, BRCA1, DNA-PK* and *MGMT* mRNA was significantly inhibited by aloe-emodin with the exception of *MGMT* under 48 h treatment. Emodin also had no effect on *MGMT* at all, while

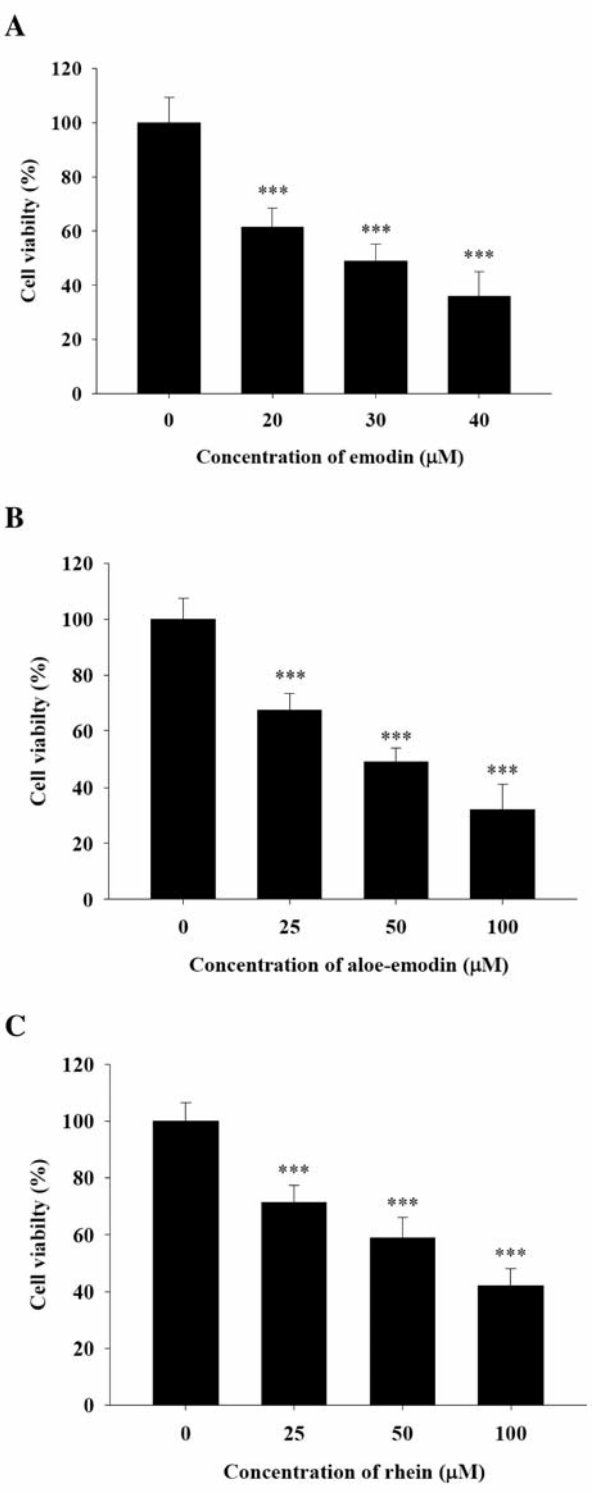


Figure 1. *Emodin, aloe-emodin and rhein affected the viability of SCC-4 cells. The SCC-4 cells (2×10^5 cells/well) were placed in 12-well plates and incubated at 37˚C for 24 h then treated with different doses of emodin, aloe-emodin or rhein for 24 h. DMSO (solvent) was used for the control. The cells were stained with PI and were analyzed by flow cytometry as described in Materials and Methods. Each point is the mean±S.D. of three experiment; ***p<0.001.*

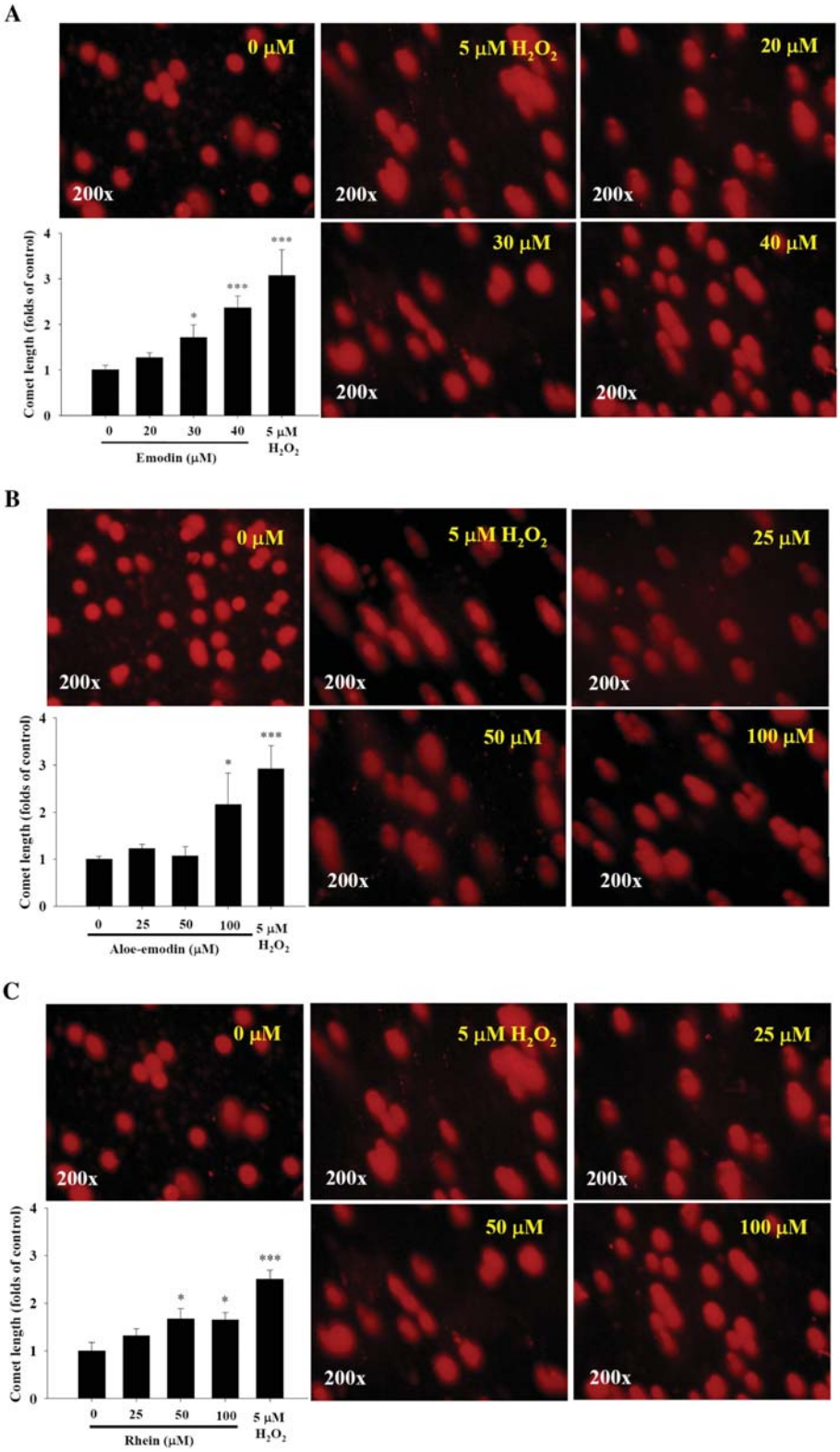
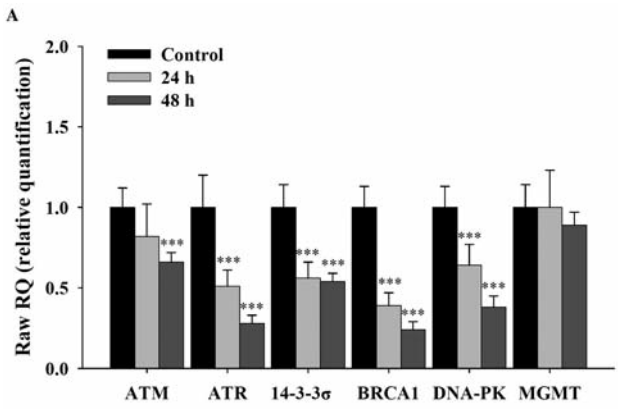
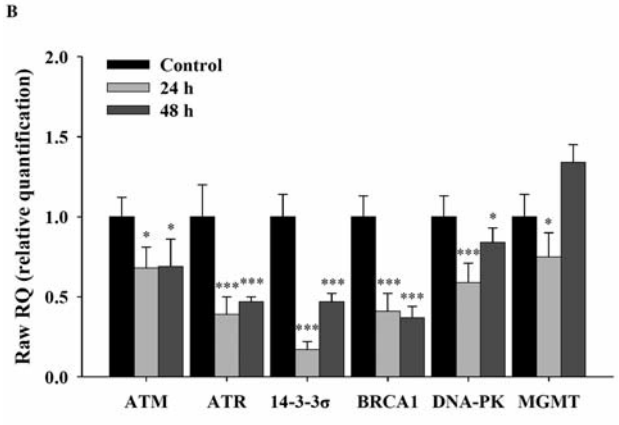


Figure 2. *DNA damage induced by emodin (A), aloe-emodin (B) and rhein (C) in SCC-4 cells was determined by comet assay. The SCC-4 cells (2×10^5 cells/well; 12-well plates) were incubated with different concentrations of emodin, aloe-emodin or rhein for 24 h and DNA damage was determined by comet assay as described in Materials and Methods. Representative images of comet assay and comet length are shown. Each point is the mean±S.D. of three experiments; *p<0.05, ***p<0.001.*





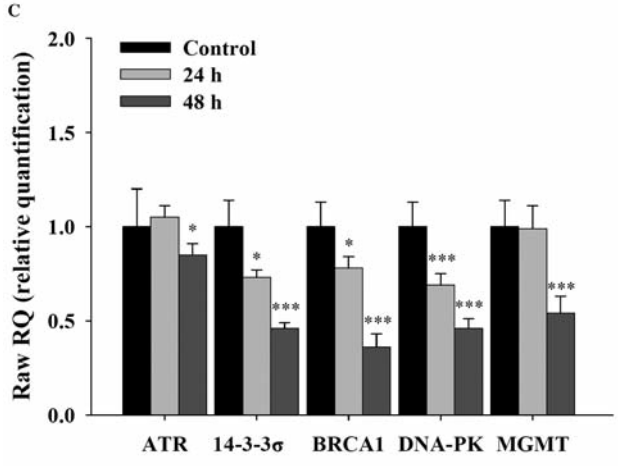


Figure 3. *Emodin-(A), aloe-emodin-(B) and rhein-(C)inhibited DNA damage repair gene expression in SCC-4 cells were determined by real-time PCR. The total RNA was extracted from the SCC-4 cells after treatment of 30 μM emodin, aloe-emodin or rhein for 24 and 48 h, and RNA samples were reverse-transcribed for cDNA then for real-time PCR as described in Materials and Methods. The ratios of ATM, ATR, 14-3-3σ, BRCA1, DNA-PK and MGMT mRNA to that of GAPDH (relative quantification) are presented. Data represent the mean±S.D. of three experiment; *p<0.05, ***p<0.001.*

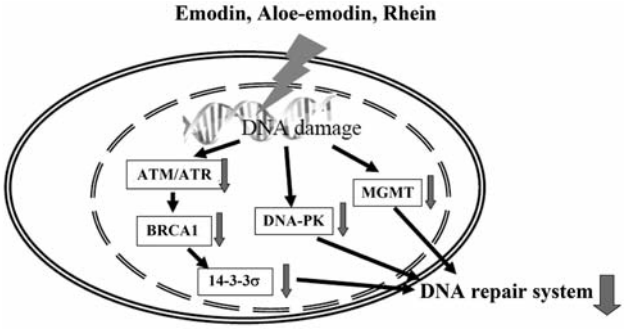


Figure 4. *A possible flow chart for DNA repair gene inhibition by emodin, aloe-emodin and rhein in SCC-4 cells.*

rhien reduced its expression only after 48 h incubation. Moreover, rhein induced similar changes in *ATR* and had no effect on *ATM* expression.

Discussion

In the present study, we investigated emodin, aloe-emodin and rhein induced DNA damage, using the comet assay. We found that a significant dose-dependent increase in DNA damage (longer comet tail; Figure 2) was observed in SCC-4 human tongue cancer cells, which was associated with a loss of cell viability (Figure 1) ($p<0.01$). The comet assay has been used for examining DNA damage in single cells after exposure to agents (43, 44). It was reported that the comet assay was used for measuring the strand-break formation during the process of excision repair of DNA which may also cause DNA migration (42, 45).

In cells, DNA damage can be reduced *via* DNA repair through eliminating DNA lesions. Therefore, the analysis of the finer mechanisms of enzymatic repair of DNA damage in the mammalian genome has attracted more attention and has also been the subject of intensive research in recent years (46-48). Figure 3 data from real-time PCR examination indicated that emodin, aloe-emodin and rhein inhibited DNA repair gene expression including of *ATM, ATR, 14-3-3σ, BRCA1, DNA-PK* and *MGMT* in the examined SCC-4 cells. Our previous studies have shown that emodin, aloe-emodin and rhein induced cell cycle arrest and apoptosis in SCC-4 cells (19, 30, 39). It is well-known that DNA damage checkpoints play a role in signal transduction pathways that are involved in the cell cycle and cellular responses to DNA damage for maintaining genomic integrity.

It was reported that ATM and ATR are two master checkpoint kinases activated by double-stranded DNA breaks (DSBs) (49) and ATR kinase is responsible for initiating the DNA damage checkpoint (50). BRCA1 (tumor suppressor) plays critical roles in DNA repair, cell cycle checkpoint

control and maintenance of genomic stability in breast and ovarian cancer (51). 14-3-3σ overpression may be used as an effective therapeutic target in breast cancer patients (52). It was reported that DNA-dependent protein kinase (DNA-PK) also plays a critical role in DNA damage repair (53). In cells, O^6-methylguanine DNA methyltransferase (MGMT) can reduce the cytotoxicity of therapeutic and environmental alkylating agents (54).

Our previous studies have shown that emodin, aloe-emodin and rhein promoted the production of ROS in SCC-4 cells (19, 30, 39). In the present study, emodin, aloe-emodin and rhein induced DNA damage in SCC-4 cells and these effects occur in a dose-dependent manner.

In conclusion, emodin, aloe-emodin and rhein induce DNA damage in SCC-4 cells followed by the inhibition of DNA repair-associated gene expressions including *ATM, ATR, 14-3-3σ, BRCA1, DNA-PK* and *MGMT* (Figure 4).

Acknowledgements

This work was supported by Grants CMU96-086 and CMU96-087 from China Medical University, Taichung, Taiwan, R.O.C.

References

1 Morgan SE and Kastan MB: p53 and ATM: cell cycle, cell death, and cancer. Adv Cancer Res *71*: 1-25, 1997.

2 Levine AJ: p53, the cellular gatekeeper for growth and division. Cell *88*: 323-331, 1997.

3 Cox LS and Lane DP: Tumour suppressors, kinases and clamps: how p53 regulates the cell cycle in response to DNA damage. Bioessays *17*: 501-508, 1995.

4 Hermeking H, Lengauer C, Polyak K, He TC, Zhang L, Thiagalingam S, Kinzler KW and Vogelstein B: 14-3-3 sigma is a p53-regulated inhibitor of G_2/M progression. Mol Cell *1*: 3-11, 1997.

5 Waldman T, Lengauer C, Kinzler KW and Vogelstein B: Uncoupling of S phase and mitosis induced by anticancer agents in cells lacking p21. Nature *381*: 713-716, 1996.

6 Brugarolas J, Chandrasekaran C, Gordon JI, Beach D, Jacks T and Hannon GJ: Radiation-induced cell cycle arrest compromised by p21 deficiency. Nature *377*: 552-557, 1995.

7 Gensler HL and Bernstein H: DNA damage as the primary cause of aging. Q Rev Biol *56*: 279-303, 1981.

8 Wang Z, Wu X and Friedberg EC: The detection and measurement of base and nucleotide excision repair in cell-free extracts of the yeast *Saccharomyces cerevisiae*. Methods *7*: 177-186 1995.

9 Tsai TH and Chen CF: Ultraviolet spectrum identification of emodin in rabbit plasma by HPLC and its pharmacokinetics application. Asia Pac J Pharmacol *7*: 53-56, 1992.

10 Liang JW, Hsiu SL, Huang HC and Lee-Chao PD: HPLC analysis of emodin in serum, herbs and Chinese herbal prescriptions. J Food Drug Anal *1*: 251-257, 1993.

11 Huang HC, Chu SH and Chao PD: Vasorelaxants from Chinese herbs, emodin and scoparone, possess immunosuppressive properties. Eur J Pharmacol *198*: 211-213, 1991.

12 Zhou XM and Chen QH: Biochemical study of Chinese rhubarb. XXII. Inhibitory effect of anthraquinone derivatives on Na^+-K^+-ATPase of the rabbit renal medulla and their diuretic action. Acta Pharmaceutica Sinica *23*: 17-20, 1988.

13 Koyama M, Kelly TR and Watanabe KA: Novel type of potential anticancer agents derived from chrysophanol and emodin. Some structure activity relationship studies. J Med Chem *31*: 283-284, 1988.

14 Su HY, Cherng SH, Chen CC and Lee H: Emodin inhibits the mutagenicity and DNA adducts induced by 1-nitropyrene. Mutat Res *329*: 205-212, 1995.

15 Yu CX, Zhang XQ, Kang LD, Zhang PJ, Chen WW, Liu WW, Liu QW and Zhang JY: Emodin induces apoptosis in human prostate cancer cell LNCaP. Asian J Androl *10*: 625-634, 2008.

16 Su YT, Chang HL, Shyue SK and Hsu SL: Emodin induces apoptosis in human lung adenocarcinoma cells through a reactive oxygen species-dependent mitochondrial signaling pathway. Biochem Pharmacol *70*: 229-241, 2005.

17 Wang XD, Gu LQ and Wu JY: Apoptosis-inducing activity of new pyrazole emodin derivatives in human hepatocellular carcinoma HepG2 cells. Biol Pharm Bull *30*: 1113-1116, 2007.

18 Huang Z, Chen G and Shi P: Emodin-induced apoptosis in human breast cancer BCap-37 cells through the mitochondrial signaling pathway. Arch Pharm Res *31*: 742-748, 2008.

19 Lin SY, Lai WW, Ho CC, Yu FS, Chen GW, Yang JS, Liu KC, Lin ML, Wu PP, Fan MJ and Chung JG: Emodin induces apoptosis of human tongue squamous cancer SCC-4 cells through reactive oxygen species and mitochondria-dependent pathways. Anticancer Res *29*: 327-335, 2009.

20 Yang F, Zhang T, Tian G, Cao H, Liu Q and Ito Y: Preparative isolation and purification of hydroxyanthraquinones from *Rheum officinale* Baill by high-speed counter-current chromatography using pH-modulated stepwise elution. J Chromatogr A *858*: 103-107, 1999.

21 Pecere T, Gazzola MV, Mucignat C, Parolin C, Vecchia FD, Cavaggioni A, Basso G, Diaspro A, Salvato B, Carli M and Palu G: Aloe-emodin is a new type of anticancer agent with selective activity against neuroectodermal tumors. Cancer Res *60*: 2800-2804, 2000.

22 Heidemann A, Volkner W and Mengs U: Genotoxicity of aloeemodin *in vitro* and *in vivo*. Mutat Res *367*: 123-133, 1996.

23 Wolfle D, Schmutte C, Westendorf J and Marquardt H: Hydroxyanthraquinones as tumor promoters: enhancement of malignant transformation of C3H mouse fibroblasts and growth stimulation of primary rat hepatocytes. Cancer Res *50*: 6540-6544, 1990.

24 Pecere T, Sarinella F, Salata C, Gatto B, Bet A, Dalla Vecchia F, Diaspro A, Carli M, Palumbo M and Palu G: Involvement of p53 in specific anti-neuroectodermal tumor activity of aloe-emodin. Int J Cancer *106*: 836-847, 2003.

25 Kuo PL, Lin TC and Lin CC: The antiproliferative activity of aloe-emodin is through p53-dependent and p21-dependent apoptotic pathway in human hepatoma cell lines. Life Sci *71*: 1879-1892, 2002.

26 Lee HZ: Protein kinase C involvement in aloe-emodin- and emodin-induced apoptosis in lung carcinoma cell. Br J Pharmacol *134*: 1093-1103, 2001.

27 Chen SH, Lin KY, Chang CC, Fang CL and Lin CP: Aloe-emodin-induced apoptosis in human gastric carcinoma cells. Food Chem Toxicol *45*: 2296-2303, 2007.

28 Lai MY, Hour MJ, Wing-Cheung Leung H, Yang WH and Lee HZ: Chaperones are the target in aloe-emodin-induced human lung nonsmall carcinoma H460 cell apoptosis. Eur J Pharmacol *573*: 1-10, 2007.
29 Lu GD, Shen HM, Chung MC and Ong CN: Critical role of oxidative stress and sustained JNK activation in aloe-emodin-mediated apoptotic cell death in human hepatoma cells. Carcinogenesis *28*: 1937-1945, 2007.
30 Chiu TH, Lai WW, Hsia TC, Yang JS, Lai TY, Wu PP, Ma CY, Yeh CC, Ho CC, Lu HF, Wood WG and Chung JG: Aloe-emodin induces cell death through S-phase arrest and caspase-dependent pathways in human tongue squamous cancer SCC-4 cells. Anticancer Res *29*: 4503-4511, 2009.
31 Lin S, Li JJ, Fujii M and Hou DX: Rhein inhibits TPA-induced activator protein-1 activation and cell transformation by blocking the JNK-dependent pathway. Int J Oncol *22*: 829-833, 2003.
32 Miccadei S, Pulselli R and Floridi A: Effect of lonidamine and rhein on the phosphorylation potential generated by respiring rat liver mitochondria. Anticancer Res *13*: 1507-1510, 1993.
33 Delpino A, Paggi MG, Gentile PF, Castiglione S, Bruno T, Benass M and Floridi A: Protein synthetic activity and adenylate energy charge in Rhein-treated cultured human glioma cells. Cancer Biochem Biophys *12*: 241-252, 1992.
34 Castiglione S, Fanciulli M, Bruno T, Evangelista M, Del Carlo C, Paggi MG, Chersi A and Floridi A: Rhein inhibits glucose uptake in Ehrlich ascites tumor cells by alteration of membrane-associated functions. Anticancer Drugs *4*: 407-414, 1993.
35 Raimondi F, Santoro P, Maiuri L, Londei M, Annunziata S, Ciccimarra F and Rubino A: Reactive nitrogen species modulate the effects of rhein, an active component of senna laxatives, on human epithelium *in vitro*. J Pediatr Gastroenterol Nutr *34*: 529-534, 2002.
36 Lin S, Fujii M and Hou DX: Rhein induces apoptosis in HL-60 cells *via* reactive oxygen species-independent mitochondrial death pathway. Arch Biochem Biophys *418*: 99-107, 2003.
37 Ip SW, Weng YS, Lin SY, Mei D, Tang NY, Su CC and Chung JG: The role of Ca^{2+} on rhein-induced apoptosis in human cervical cancer Ca Ski cells. Anticancer Res *27*: 379-389, 2007.
38 Lin ML, Chen SS, Lu YC, Liang RY, Ho YT, Yang CY and Chung JG: Rhein induces apoptosis through induction of endoplasmic reticulum stress and Ca^{2+}-dependent mitochondrial death pathway in human nasopharyngeal carcinoma cells. Anticancer Res *27*: 3313-3322, 2007.
39 Lai WW, Yang JS, Lai KC, Kuo CL, Hsu CK, Wang CK, Chang CY, Lin JJ, Tang NY, Chen PY, Huang WW and Chung JG: Rhein induced apoptosis through endoplasmic reticulum stress, caspase- and mitochondria-dependent pathways in SCC-4 human tongue squamous cancer cells. In Vivo *23*: 309-316, 2009.
40 Hsia TC, Yang JS, Chen GW, Chiu TH, Lu HF, Yang MD, Yu FS, Liu KC, Lai KC, Lin CC and Chung JG: The roles of endoplasmic reticulum stress and Ca^{2+} on rhein-induced apoptosis in A-549 human lung cancer cells. Anticancer Res *29*: 309-318, 2009.
41 Lu HF, Yang JS, Lai KC, Hsu SC, Hsueh SC, Chen YL, Chiang JH, Lu CC, Lo C, Yang MD and Chung JG: Curcumin-induced DNA damage and inhibited DNA repair genes expressions in mouse-rat hybrid retina ganglion cells (N18). Neurochem Res *34*: 1491-1497, 2009.
42 Olive PL, Banath JP and Durand RE: Detection of etoposide resistance by measuring DNA damage in individual Chinese hamster cells. J Natl Cancer Inst *82*: 779-783, 1990.
43 Ashby J, Tinwell H, Lefevre PA and Browne MA: The single cell gel electrophoresis assay for induced DNA damage (comet assay): measurement of tail length and moment. Mutagenesis *10*: 85-90, 1995.
44 Pool-Zobel BL, Lotzmann N, Knoll M, Kuchenmeister F, Lambertz R, Leucht U, Schroder HG and Schmezer P: Detection of genotoxic effects in human gastric and nasal mucosa cells isolated from biopsy samples. Environ Mol Mutagen *24*: 23-45, 1994.
45 Tice RR, Andrews PW and Singh NP: The single cell gel assay: a sensitive technique for evaluating intercellular differences in DNA damage and repair. Basic Life Sci *53*: 291-301, 1990.
46 Terleth C, van de Putte P and Brouwer J: New insights in DNA repair: preferential repair of transcriptionally active DNA. Mutagenesis *6*: 103-111, 1991.
47 Hanawalt PC: Heterogeneity of DNA repair at the gene level. Mutat Res *247*: 203-211, 1991.
48 Hanawalt PC: DNA repair comes of age. Mutat Res *336*: 101-113, 1995.
49 Shiotani B and Zou L: Single-stranded DNA orchestrates an ATM-to-ATR switch at DNA breaks. Mol Cell *33*: 547-558, 2009.
50 Choi JH, Sancar A and Lindsey-Boltz LA: The human ATR-mediated DNA damage checkpoint in a reconstituted system. Methods *48*: 3-7, 2009.
51 Venkitaraman AR: Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell *108*: 171-182, 2002.
52 Neal CL, Yao J, Yang W, Zhou X, Nguyen NT, Lu J, Danes CG, Guo H, Lan KH, Ensor J, Hittelman W, Hung MC and Yu D: 14-3-3zeta overexpression defines high risk for breast cancer recurrence and promotes cancer cell survival. Cancer Res *69*: 3425-3432, 2009.
53 Mi J, Dziegielewski J, Bolesta E, Brautigan DL and Larner JM: Activation of DNA-PK by ionizing radiation is mediated by protein phosphatase 6. PLoS One *4*: e4395, 2009.
54 Jesien-Lewandowicz E, Jesionek-Kupnicka D, Zawlik I, Szybka M, Kulczycka-Wojdala D, Rieske P, Sieruta M, Jaskolski D, Och W, Skowronski W, Sikorska B, Potemski P, Papierz W, Liberski PP and Kordek R: High incidence of MGMT promoter methylation in primary glioblastomas without correlation with *TP53* gene mutations. Cancer Genet Cytogenet *188*: 77-82, 2009.

Received July 28, 2009
Revised February 4, 2010
Accepted February 24, 2010