Biochemical Pharmacology, Vol. 58, pp. 49–57, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(99)00075-1

A Novel Function of Emodin

ENHANCEMENT OF THE NUCLEOTIDE EXCISION REPAIR OF UV- AND CISPLATIN-INDUCED DNA DAMAGE IN HUMAN CELLS

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ABSTRACT. Nucleotide excision repair (NER) is the main pathway by which mammalian cells remove carcinogenic DNA lesions caused by UV light and many other common mutagens. To explore the effect of emodin on NER, its influence on the repair of UV- and cisplatin-induced DNA damage in human fibroblast cells (WI38) was evaluated. Emodin increased unscheduled DNA synthesis (UDS) of UV-treated cells and reduced cisplatin-induced DNA adducts in WI38 in a concentration-dependent manner, indicating that emodin might promote NER capability in cells. The resultant NER complex is a cooperative assembly of XPF, ERCC1, XPA, RPA, and XPG subunits. The gene regulations of the subunits after emodin treatment were determined by reverse transcription-polymerase chain reaction (RT-PCR) using specific primers. Among the subunits, the expression of ERCC1 in WI38 cells was up-regulated significantly after emodin treatment. All other expressions remained essentially unchanged. In addition, calcium influx in WI38 was increased in proportion to the concentration of emodin. Since UV-induced NER is Ca^{2+} dependent, elevation of calcium influx may be another mechanism by which emodin facilitates DNA repair. In conclusion, emodin can increase the repair of UV- and cisplatin-induced DNA damage in human cells, and elevated ERCC1 gene expression and Ca^{2+}-mediated DNA repair processes may be involved in the repair mechanism of emodin. BIOCHEM PHARMACOL **58**;1:49–57, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. emodin; nucleotide excision repair; UV; cisplatin; DNA adducts; calcium influx

Carcinogenesis generally is considered to be a multistep process that may involve cumulative mutations in the genome. Most mutations in cancer-associated genes are acquired and secondary to environmental carcinogens such as cigarette smoking and/or exposure to chemicals and radiation; only a small minority are inherited in the germline [1]. Damage to numerous regulatory genes ultimately results in the development of invasive and metastatic cancers [2]. Despite the immense efforts made to improve treatment of cancers, the overall mortality rates for most cancers have not declined significantly in the past 25 years [3]. Moreover, the investigations of chemical agents used in fighting cancer generally focus more on "killing" cells than on the prevention of cancer formation. However, these chemical agents usually injure not only cancer cells but normal cells as well, especially the hematopoietic cells. This is a common problem in the chemotherapeutic treatment of cancers, and the severe side-effects of these chemical agents may force patients to withdraw from therapeutic cycles.

Genetic analysis now has the potential to identify large numbers of people who are at increased risk for the development of invasive cancers. Thus, strategies for preventing carcinogenesis are becoming increasingly important. DNA repair that eliminates spontaneous and carcinogen-induced DNA damage is an important cellular defense mechanism against mutagenesis and carcinogenesis [4, 5]. In mammalian cells, DNA lesions are eliminated by two basic mechanisms: BER‖ and NER [6]. BER cleaves mismatched and damaged bases off the deoxyribose by DNA glycosylase. Then the one-nucleotide gap is filled and ligated [7]. NER is the main DNA repair pathway for UV- and carcinogen-induced DNA damage in mammalian cells, and is highly conserved in nature, with analogous repair systems described in *Escherichia coli* and yeast [8–10]. The mechanism of NER is diagrammed in Fig. 1. In general, the XPA, RPA, XPG, ERCC1, and XPF subunits and a basal transcription factor, TFIIH, are involved in NER function [11–14]. Recently, with the cloning of DNA repair genes in *Saccharomyces cerevisiae* and human cells, there has been a veritable explosion of information about DNA repair in

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Received 19 August 1998; accepted 16 November 1998.

‖ *Abbreviations*: BER, base excision repair; NER, nucleotide excision repair; UDS, unscheduled DNA synthesis; fluo 3-AM, 3-acetoxymethyl ester; RT-PCR, reverse transcription-polymerase chain reaction; and MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium salt.

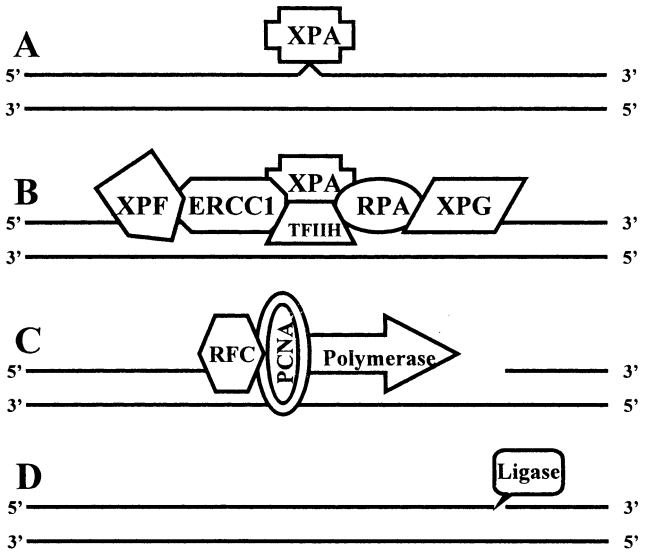

FIG. 1. Diagram of the mechanism of NER. (A) DNA damage is recognized by XPA. (B) RPA binds to XPA, which increases the binding affinity of XPA to the damaged DNA. Another part of the XPA protein can also bind to the basal transcription factor TFIIH. The ERCC1-XPF and XPG nucleases cut the DNA on either side of the damage. The result is the removal of a piece of DNA about 29 nucleotides in length. (C) DNA polymerase, replication factor C (RFC), and proliferating-cell nuclear antigen (PCNA) fill in the resulting gap, and (D) the new DNA fragment is joined to the old by DNA ligase.

eukaryotes. Associated with this complex control of NER in cells, a considerable variation in the expression of DNA repair gene may be related to cancer susceptibility [15].

Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is an active constituent of *Rheum palmatum* herb [16, 17]. Pharmacological studies have demonstrated that emodin possesses anticancer, antibacterial, diuretic, and vasorelaxant effects [18–20]. Moreover, emodin can inhibit the formation of 1-nitropyrene-induced DNA adducts in *Salmonella typhimurium* (TA98) [21]. To explore the effect of emodin on NER in mammalian cells, the modulation of NER by emodin was evaluated with UV- and cisplatin-induced DNA adducts in WI38 cells. UV and cisplatin are utilized widely for the evaluation of NER capability in cells [22, 23]. The repair of UV-induced cyclobutane pyrimidine dimers [24] and cisplatin-induced intrastrand cross-linked DNA adducts [25, 26] was determined by UDS and cell viability assay [23, 27, 28]. It has been shown that emodin increased UDS for UV-induced damage and reduced the cytotoxicity of cisplatin to WI38 cells in a concentration-dependent manner, suggesting that emodin may promote NER activity. In addition, the strategy of primer design on open regions of mRNA secondary structures offers an efficient primer for RT-PCR [29]. The specific primer sets for NER subunits were determined by this strategy. Gene regulation of the NER subunits after emodin treatment was determined by these primers. Since NER of UV-induced DNA repair in human lymphocytes is Ca^{2+} dependent [30], the cellular influx of Ca^{2+} after emodin treatment was characterized by a laser cytometer. This paper reports a novel function of emodin in the modulation of NER in human cells, and the mechanism of the action of emodin on NER is investigated.

MATERIALS AND METHODS

Cell Lines and Cell Cultures

Human lung diploid fibroblast cells (WI38) were cultured in minimum essential medium (MEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 1.0 mM sodium pyruvate, 100 μg/mL of streptomycin, and 100 U/mL of penicillin at 37° in a humidified atmosphere of 95% air–5% CO_2. The doubling time of WI38 cells was 22 hr [31].

MTS and [^{3}H]Thymidine Incorporation Assay

Cell viability was measured by CellTiter 96^{TM} AQ_{ueous} non-radioactive MTS assay (Promega). WI38 cells were seeded in 96-well plates and incubated at 37° for at least 24 hr prior to use. Serial amounts of emodin (Sigma) were added to the wells. Emodin is a reddish color after dissolution. Therefore, to avoid the color-interference of emodin at $A_{490\ nm}$, the medium was replaced with fresh medium at the end of emodin treatment. Twenty microliters of MTS reagent was added to each well. The conversion of MTS into aqueous soluble formazan was measured at $A_{490\ nm}$. Absorbance is proportional to the number of living cells in the culture. For the evaluation of cell viability caused by UV, the cells were irradiated immediately after removal of the medium. Then fresh medium was added to each well, and incubated continuously at 37° for 4 hr. All the other procedures were the same as described above. The determination of cell replication by the [^{3}H]thymidine incorporation method was described in a previous report [32]. In short, WI38 cells were seeded in 1 mL each of 24-well multi-dishes, the cells were UV irradiated immediately after removal of the medium, and fresh medium containing [^{3}H]thymidine (2 μCi) was added to the well and incubated continuously at 37° for 4 hr. The [^{3}H]DNA was measured with a Packard model 2100TR scintillation counter. For determination of the cytotoxicity of cisplatin by [^{3}H]thymidine incorporation, the cells were incubated with cisplatin for 20 hr. All the other procedures were the same as described above.

UDS

The UDS was carried out in accordance with established procedures [27]. UV (254 nm) emitted from a Bio-Link model BLX (Vilber Lourmat) was used as the source of UV light. WI38 cells (5×10^4) in 1 mL of MEM–10% fetal bovine serum medium were inoculated in each of 24-well multi-dishes for at least 24 hr prior to use. The cells were preincubated with emodin (0, 10, 20, and 30 μg/mL) for 4 hr prior to UV irradiation. Then the medium was removed, and the cells were washed with 1x PBS. After irradiation

TABLE 1. Primer sequences for the determination of NER subunits

Subunit	Oligonucleotide (5′→ 3′)	Sense/Antisense	Size (bp)
XPF	TGACCCTAGCAAGCCTTTCTCTCT	Sense	130
	GGGGCACCAGAGAATCCGTAGTC	Antisense	
ERCC1	GGAGCTGGCTAAGATGTGTATCCTG	Sense	143
	AGTCCTGCTCTAGCTTCTCCATCAG	Antisense	
XPA	GAGTTTCAACCCAGGTCTTCTCATTTT	Sense	128
	AATGGTAACAGTGAGTAGTAGTAGTGC	Antisense	
RPA	GGAAGAGTAACCGCCAAGGCCACC	Sense	121
	GAGGGAAGTCATGGATAATTTTCACAG	Antisense	
XPG	CATCAAGAGCTACTTTGGCCGTAAG	Sense	156
	ATGCGAATGGTGCGGTAGAACATGC	Antisense	

with 0, 10, 20, 30, 40, and 50 J/m^2, the cells were incubated continuously at 37° for 4 hr with medium containing the previous amount of emodin and [^{3}H]thymidine (2 μCi). The incorporation of [^{3}H]thymidine was terminated by rinsing the cells with 1 mL of PBS containing unlabeled thymidine (100 μg/mL). The measurement of [^{3}H]DNA was performed as described in a previous report [32].

RNA Preparation

WI38 cells were incubated with emodin (30 μg/mL) for 0, 2, 4, 6, and 8 hr. Then the RNA was extracted from the cells using an RNA isolation kit (Stratagene) according to the manufacturer's protocol.

RT-PCR

cDNA was synthesized from 0.5 μg of fresh preparation of RNA using an Advantage RT-for-PCR kit (Clontech). Reverse transcription was performed with 1 μL oligo $(dT)_{18}$ primer in a final volume of 20 μL containing 10 mM dNTP, recombinant RNase inhibitor, and MMLV reverse transcriptase. The mixture was incubated at 42° for 1 hr, and then was heated to 94° for 5 min. The cDNA amplification was carried out with *Taq* DNA polymerase (Promega) according to the supplier's instructions. The primer sequences of NER subunits are indicated in Table 1. The cDNA of human β-actin was amplified as a control gene using the specific primers 5′-CGGAACCGCTCATTGCC-3′ and 5′-ACCCACACTGTGCCCATCTA-3′. The PCR conditions were: 98° for 3 min and 94°/60°/75° for 1 min each for a total of 25 cycles.

Southern Hybridization

The cDNA products were separated on a 2% agarose gel and transferred onto a nylon membrane (Bio-Rad). After prehybridization, the blot was hybridized with the cDNA probes of NER subunits labeled with [^{32}P]dATP using a randomly primed labeling procedure (Promega).

Cytosolic Calcium Concentration

Intracellular free calcium was measured with fluo 3-AM agent [33]. Fluo 3-AM was dissolved in DMSO for a concentration of 5 mM. The fluo 3-AM was stored at −20° in a desiccator and diluted immediately prior to use. To measure the intracellular free [Ca^{2+}] by laser cytometer, WI38 cells were grown on 35-mm plastic petri dishes. The cells were washed twice with buffer containing 140 mM NaCl, 2 mM $CaCl_2$, 4.6 mM KCl, 1.0 mM $MgCl_2$, 10 mM glucose, and 10 mM HEPES, pH 7.4. The cells were incubated in the same buffer supplemented with 5 mM fluo 3-AM at room temperature for 40 min in the dark. The final concentration of DMSO did not exceed 0.1% (v/v). The dish was washed several times with buffer to remove the excess fluo 3-AM. Fluorescence was recorded using an interactive Laser Cytometer (Meridian ACAS 570) equipped with an argon-ion laser source. The fluo 3-AM was excited at 488 nm, and the emitted fluorescence was recorded at 520 nm. Emodin (30 μg/mL) then was added to the dish. Image acquisition was performed by scanning an image area of 200 × 200 pixels at the highest possible magnification. Cells with detectable motion artifacts were excluded from the study.

For the concentration–response experiment, equal volumes of the serial concentrations of emodin (0.3, 3, 10, and 30 μg/mL) were added cumulatively to the well. A single line selected from the confocal image was scanned repeatedly (up to a frequency of 250 Hz), and the acquired lines were arranged successively in a top-down order to build up the line-scan image.

RESULTS

Determination of the Optimum Concentrations of Emodin for WI38 Cells

The cytotoxicity of emodin was determined by MTS assay to select feasible concentrations of emodin for WI38 cells. Constant amounts of WI38 cells were treated with serial concentrations of emodin for 20 hr. As shown in Fig. 2,

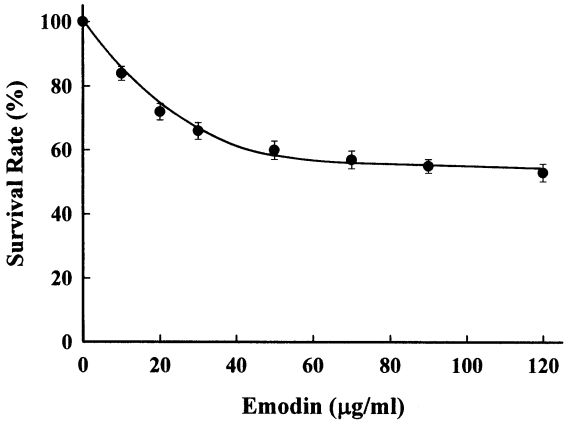

FIG. 2. Cytotoxicity of emodin to WI38 cells. The optimum concentration of emodin for the cells was measured by the MTS assay as described in Materials and Methods. Data represent means ± SD of the survival rate (%) from quadruplicate determinations.

emodin was not cytotoxic to WI38 cells. The cell survival rates after emodin treatment were 84, 72, and 66% for 10, 20, and 30 μg/mL, respectively, and there was no significant decrease of survival rates after 40 μg/mL. Thus, the concentrations at 10, 20, and 30 μg/mL were the optimal concentrations in these experiments for determining the effects of emodin.

Production of DNA Damage in WI38 Cells by UV Irradiation

WI38 cells were irradiated with serial energies of UV to induce different degrees of DNA damage. As shown in Fig. 3, the DNA synthesis in WI38, as indicated by [^{3}H]thymidine incorporation, was blocked by the serial energies of UV irradiation. However, cell viability as measured by MTS assay was not changed significantly under the same

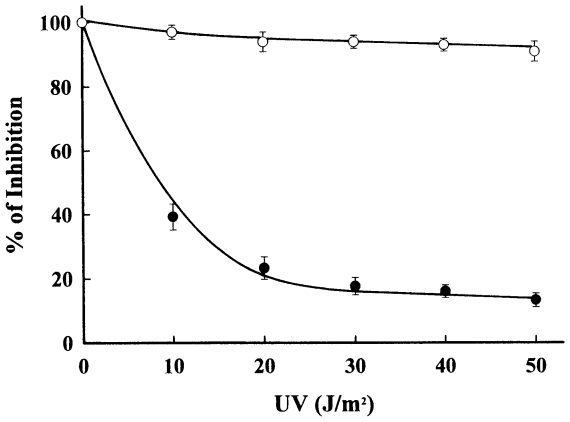

FIG. 3. Effect of UV irradiation on WI38 cells. UV irradiation was applied to the cells immediately after removal of medium from the wells. Then fresh medium containing either MTS reagent or [^{3}H]thymidine was added to the wells. After incubation for 4 hr, cell viability (○) and replication (●) of WI38 cells were determined by MTS assay and [^{3}H]thymidine incorporation. Data represent means ± SD from triplicate determinations.

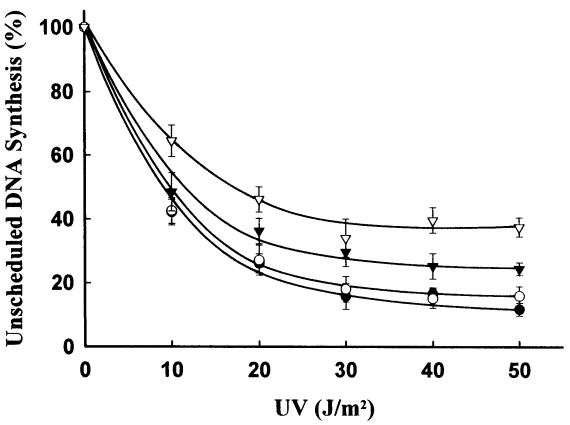

FIG. 4. Modulation of the UDS of UV-induced DNA damage in WI38 cells by emodin. A constant amount of cells were preincubated with serial concentrations of emodin (●, 0; ○, 10; ▼, 20; ▽, 30 μg/mL) for 4 hr. UV irradiation was applied onto the cells immediately after removal of medium from the wells. Fresh medium containing the previous amount of emodin and [^{3}H]thymidine (2 μCi) was added and incubated continuously at 37° for 4 hr. Data represent means ± SD of [^{3}H]thymidine incorporation calculated from triplicate determinations.

conditions. These results indicate that UV (10–50 J/m^2) produces DNA damage and simultaneously arrests DNA replication in WI38 cells without affecting cell viability. Thus, 10–50 J/m^2 of UV irradiation were the optimum dosages for the production of DNA damage in WI38 cells.

Modulation of NER of UV- and Cisplatin-Induced DNA Damage in WI38 Cells by Emodin

UV- and cisplatin-induced DNA damage is eliminated by NER in mammalian cells. UV irradiation causes an early depression of scheduled DNA synthesis and, at the same time, induces UDS to replace those sections that were excised in the course of repairing DNA damage. In this experiment, WI38 cells were preincubated with a constant amount of emodin (0, 10, 20, and 30 μg/mL) for 4 hr. Increasing doses of UV irradiation then were applied immediately after removal of the medium. Fresh medium containing [^{3}H]thymidine (2 μCi) and the previous amount of emodin was added to the wells and incubated continuously at 37° for 4 hr. The control cells were treated with 1x PBS instead of emodin under the same conditions. As shown in Fig. 4, the emodin-treated cells displayed a higher UDS than the control cells in proportion to the concentrations of emodin. A difference of approximately 25% in [^{3}H]thymidine levels could be detected between the control and 30 μg/mL of emodin-treated cells. Because [^{3}H]thymidine in UDS was incorporated for repairing damaged DNA domains, it is logical that the differing levels of radioactivity in areas being repaired should not be as high as that in the replication of genomes.

On the other hand, cisplatin produces DNA cross-links, which result in cytotoxicity to cells. WI38 cells were treated with constant amounts of emodin (0, 10, 20, and 30

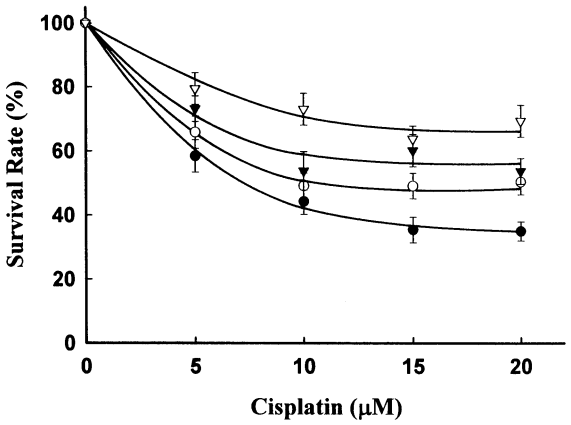

FIG. 5. Reduction of the cytotoxicity of cisplatin to WI38 cells by emodin. WI38 cells were incubated with increasing concentrations of cisplatin and serial amounts of emodin (●, 0; ○, 10; ▼, 20; and ▽, 30 μg/mL) for 20 hr. The cytotoxicity of cisplatin was determined by the [^{3}H]thymidine incorporation method. Data represent means ± SD calculated from triplicate determinations.

μg/mL) and increasing concentrations of cisplatin for 20 hr. The cytotoxicity of cisplatin to WI38 cells was determined by the [^{3}H]thymidine incorporation method. As shown in Fig. 5, the cytotoxicity of cisplatin to WI38 was reduced by emodin in a concentration-dependent manner. These results imply that emodin may promote the capacity of NER to repair UV- and cisplatin-induced DNA damage in WI38 cells.

Regulation of Gene Expression of NER Subunits by Emodin

To explore the mechanism of action of emodin on NER, regulation of the gene expressions of XPF, ERCC1, XPA, RPA, and XPG subunits by emodin was examined by RT-PCR. The primer sequences, as designed from open regions of mRNA secondary structures of NER subunits, are indicated in Table 1. All the primer sets amplified a single band of the expected size. As shown in Fig. 6, constant amounts of WI38 cells were treated with emodin (30 μg/mL) for 0, 2, 4, 6, and 8 hr. At the time-intervals stated, the cellular RNAs were extracted immediately from the cells. Triplicate RNA extractions from each group of emodin-treated cells were employed for the experiments. The quantity of cDNA and the number of PCR cycles were chosen based on our preliminary test for each primer set to ensure that the amounts of PCR products fell within a linear range. Human β-actin was utilized as a quantity control gene in each experiment. The RT-PCR products were separated on a 2% agarose gel and transferred onto a nylon membrane. After hybridization with the ^{32}P-labeled cDNA probes of NER subunits, the intensities of the subunits were integrated by a densitometer. As shown in Fig. 7, the ERCC1 gene was the most up-regulated gene after 2, 4, and 6 hr of emodin treatment, increasing to approximately five times higher than the control. The

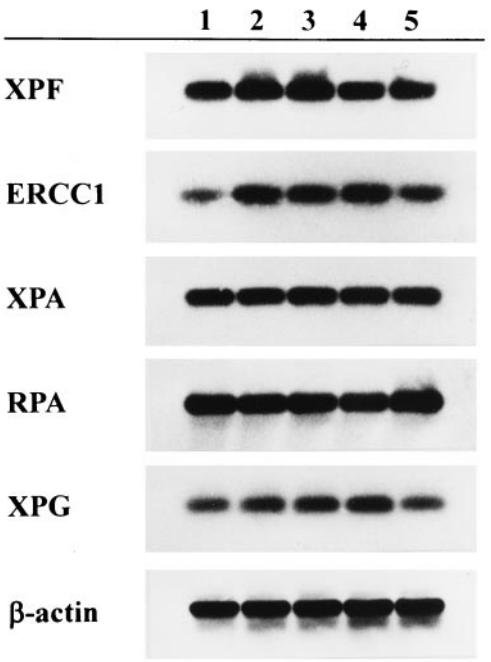

FIG. 6. Regulation of the gene expression of NER subunits by emodin. Constant amounts of cells were treated with emodin (30 μg/mL) for different time-intervals (1, control; 2, 2 hr; 3, 4 hr; 4, 6 hr; and 5, 8 hr). At these intervals, the RNA was extracted from the cells. The gene expression of NER subunits was determined by RT-PCR using the specific primers. The expressed cDNAs were verified by Southern hybridization using ^{32}P-labeled cDNA probes. Human β-actin gene was used as a control under the same conditions.

expression of XPG was also enhanced after emodin treatment. Both overexpressed genes, ERCC1 and XPG, gradually decayed after 8 hr of emodin treatment. All other expressions remained essentially unchanged.

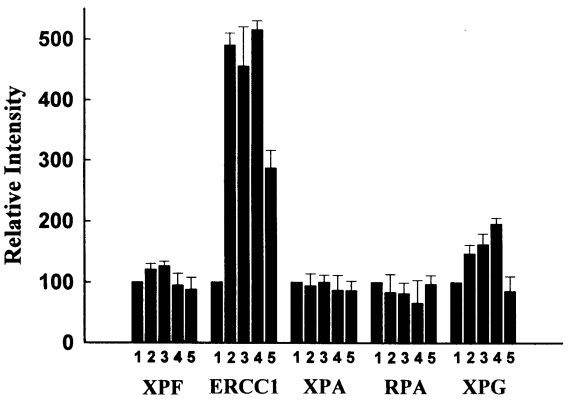

FIG. 7. Relative intensity of the gene expression of NER subunits after emodin treatment. The relative intensity of the NER subunits was measured by a densitometer. The expression of NER subunits was calculated by the ratio of the presence and absence of emodin in each determination from three individual experiments. Key: 1, control; 2, 2 hr; 3, 4 hr; 4, 6 hr; and 5, 8 hr of emodin treatment. Data represent means ± SD calculated from triplicate determinations.

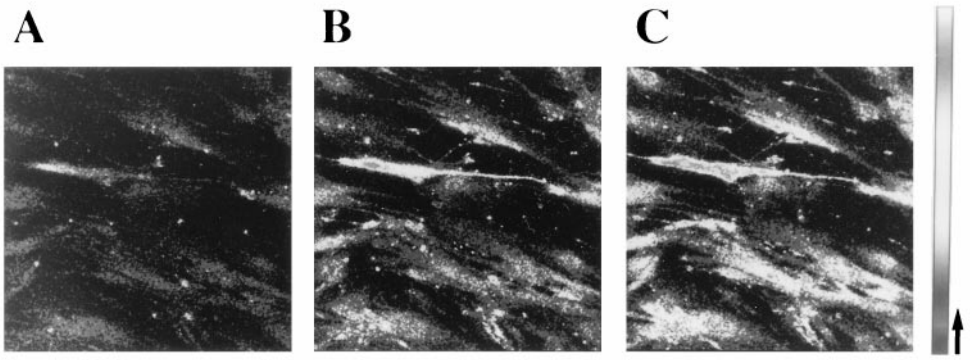

FIG. 8. Analysis of the calcium influx in WI38 cells after emodin treatment. The fluorescence images of cells after emodin treatment were analyzed by a laser cytometer. The cells were treated with emodin (30 μg/mL) for (A) control; (B) 10 sec; and (C) 1 min. The intensity bar indicates the relative amount of Ca^{2+} fluorescence in WI38 cells. The control was treated with the same volume of buffer without emodin addition.

Effect of Emodin on the Ca^{2+} Influx in WI38 Cells

The NER process for UV-induced DNA damage is Ca^{2+} dependent [30]. As shown in Fig. 8, the image scan indicated that the elevation of calcium fluorescence in WI38 cells was parallel to the time of incubation with a constant concentration of emodin (30 μg/mL). To further verify this elevation of calcium influx by emodin, a concentration–response curve for emodin treatment was determined in WI38 cells. As shown in Fig. 9, the control cells exhibited a uniform relative intensity of Ca^{2+} fluorescence. Cell fluorescence increased after cumulative concentrations of emodin treatment (0.3, 3, 10, and 30 μg/mL). These results reveal that emodin can trigger cellular uptake of Ca^{2+} in WI38 cells. The threshold of Ca^{2+} elevation in cells seems to be at 10 μg/mL of emodin, because the elevation of Ca^{2+} fluorescence at that point was higher than at other levels of emodin.

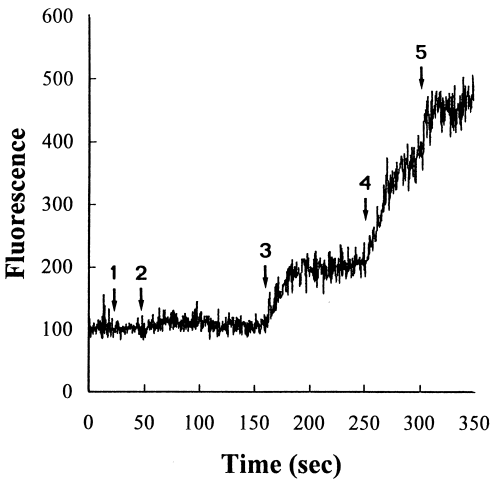

FIG. 9. Concentration–response effect of emodin on the calcium influx of WI38 cells. The line scan analysis of the calcium influx of WI38 cells in response to the emodin treatment was recorded by a laser cytometer. The line was positioned perpendicularly to the longitudinal axis of the WI38 cells. A serial concentration of emodin was added cumulatively to the well: 1, 0; 2, 0.3; 3, 3; 4, 10; and 5, 30 μg/mL of emodin.

DISCUSSION

Chinese herbs are composed of active components that exhibit the ability to regulate cell activities [34, 35]. Previous treatments of diseases with herbs were empirical more than theoretical. Therefore, clarification of the mechanisms of action of components in herbs may be important in developing their applications. Emodin, a constituent of *R. palmatum* herb, is traditionally applied in cancer therapy in Chinese medicine. This is the first study on the modulation of NER capability and gene regulation of NER subunits by emodin. It has been shown that emodin can increase UDS of UV-treated WI38 cells and reduce the cytotoxicity of cisplatin on cells in a concentration-dependent manner.

Cells arrest proliferation in response to DNA damage and allow the process of DNA repair [36]. Incorporation of [^{3}H]thymidine during resynthesis of the damaged DNA domains allows the detection of the repair process by UDS [27]. The proliferation of WI38 cells ceased after UV irradiation (Fig. 2). However, the elevation of UDS of UV-treated cells was correlated to the increasing concentrations of emodin under the same conditions (Fig. 3). Thus, it indicates that the increase of [^{3}H]thymidine incorporation of UDS in cells results from the resynthesis of DNA fragments that were utilized to replace the damaged DNA domains.

Cisplatin is used extensively in studying NER. Cells resistant to cisplatin exhibit enhanced DNA repair [26]. The cytotoxicity of cisplatin results from its ability to cross-link DNA, leading to unwinding, kinking, and the inhibition of polymerase involved in replication and transcription [37]. In addition, enhanced expression of the *HER-2/neu* proto-oncogene, which encodes the tyrosine kinase receptor p185^{neu}, has been observed frequently in many human cancers [38]. *HER-2/neu* overexpression in non-small-cell lung cancer has been reported to induce resistance to chemotherapeutic drugs such as cisplatin, doxorubicin, and etoposide [39, 40]. Emodin, a tyrosine kinase inhibitor, sensitizes lung cancer cells that overex-

press the *HER-2/neu* gene to cisplatin, whereas no such synergistic antiproliferation activity has been observed in cells that express low levels of *HER-2/neu* [38]. Since WI38 is a normal lung fibroblast, the expression of *HER-2/neu* proto-oncogene in the cells may be low. Thus, the result that emodin enhanced NER and consequently reduced the cytotoxicity of cisplatin was rational.

Impaired NER activity has been investigated extensively in cells taken from xeroderma pigmentosum patients. Xeroderma pigmentosum is an autosomal recessive human genetic disorder manifested as extreme sensitivity to sunlight (UV), resulting in a very high incidence of skin cancer [41]. The primer sequences for the determination of NER subunits have not been well established. To realize the regulation of NER subunits by emodin, gene expression of the subunits after emodin treatment was determined by RT-PCR. Since the open-region strategy of primer design yields an efficient primer for *in vitro* amplification of cDNA by RT-PCR [29], the mRNA secondary structures of human NER subunits were folded with a DNA and protein sequence analysis software system (DNASIS Pro^{TM}) (Hitachi), in accordance with the minimization of free energy rule (data not shown). The specific primers for NER subunits were synthesized on the open regions of mRNA secondary structures. The cDNA sequences of the subunits were retrieved from the GenBank database, and the accession numbers for XPF, ERCC1, XPA, RPA, and XPG were L77890, AF001925, U16815, L07493, and X71341, respectively. It has been demonstrated that all the primer sets amplified a single band by RT-PCR. Hence, these specific primer sets for RT-PCR of NER subunits may have provided a tool for monitoring the gene regulation of NER subunits by diverse agents.

Not all the enzymes in a series of biochemical reactions can be regulated. However, the XPA and ERCC1 gene products, which recognize and excise DNA damage, might play a role in the rate-limiting step in NER [42], and, therefore, at least one of the rate-limiting enzymes may control the NER reaction process. The ERCC1 gene product is involved in the incision step of excision repair of bulky platinum (Pt)-DNA adducts. The resistance to cisplatin-induced cytotoxicity in Chinese hamster ovary cells is dependent upon ERCC1 expression [42]. In the present paper, elevation of ERCC1 and XPG expression in WI38 cells could result in a significant promotion of NER capability. Therefore, it seems that ERCC1 and XPG may play a crucial role in the modulation of NER for UV- and cisplatin-induced DNA adducts by emodin. Alternately, TFIIH is a multiprotein basal transcription factor and is essential for both RNA polymerase II and NER [43]. The change of gene expression of TFIIH in cells may not actually specifically reflect the fact of NER. Thus, the gene expression of TFIIH was not determined in this paper. Moreover, expression of wild-type p53 is required for efficient global genomic NER in UV-irradiated human fibroblasts [44]. The activation of p53 following irradiation and other forms of DNA damage is proposed to be part of a checkpoint function, allowing damaged cells to arrest progress through the cell cycle and carry out DNA repair. In the present study, WI38 contains a wild-type p53 gene. The halt of cell proliferation after UV irradiation (Fig. 2) implied that p53 might be involved in the reaction. On the other hand, p21 can modulate the NER process to facilitate the repair of UV-type DNA damage in the absence of wild-type p53 [45]. Emodin could enhance NER in WI38 cells. However, the correlation of NER with emodin in mutant p53 cancer cells remains to be clarified.

The real mechanism of the involvement of calcium in DNA repair is largely unknown [30]. Cells stimulated with UV irradiation had a simultaneous elevation in their $[Ca^{2+}]$, suggesting that NER might be calcium dependent [46]. The confocal laser cytometer has made the study of Ca^{2+} patterns in single cells possible because it provides a high spatial resolution and the ability to measure the fluorescent signal from a specific optical section [47]. By time-course and concentration-dependence experiments, the elevation of $[Ca^{2+}]$ influx in WI38 cells by emodin was observed. It is assumed that elevation of $[Ca^{2+}]$ in cells is one of the effects of emodin. However, whether the effect of $[Ca^{2+}]$ elevation in cells is solely capable of facilitating DNA repair remains to be clarified.

In conclusion, carcinogenesis usually is preventable. NER is the main pathway to repair DNA damage in mammalian cells caused by UV irradiation and many other common carcinogens. It is of increasing importance to identify agents that are able to promote NER activity, especially for those people living in areas at high risk of environmental mutagens. In this paper, emodin purified from Chinese herb promotes NER for UV- and cisplatin-induced DNA damage. The gene regulation of the NER subunit by emodin is characterized, and it has been demonstrated that the elevation of the expression of ERCC1 and XPG genes and cytosolic $[Ca^{2+}]$ influx may be involved in the mechanism of action of emodin. This is the first paper reporting the effects of emodin on NER activity.

The authors wish to thank Dr. J. C. Batimus for reading this manuscript. This project was supported by a grant from the Committee on Chinese Medicine and Pharmacy, Department of Health, Executive Yuan, Taiwan, R.O.C. (CCMP 86-RD-020).

References

1. Hodgson SV and Maher ER, General principles of cancer genetics. In: *A Practical Guide to Human Cancer Genetics* (Eds. Hodgson SV and Maher ER), pp. 1–22. Cambridge University, London, 1993.
2. Bohr VA, Evans MK and Fornace AJ Jr, DNA repair and its pathogenetic implications. *Lab Invest* **61:** 143–161, 1989.
3. Hong WK and Sporn MB, Recent advances in chemoprevention of cancer. *Science* **278:** 1073–1077, 1997.
4. Gensler HL and Bernstein H, DNA damage as the primary cause of aging. *Q Rev Biol* **56:** 279–303, 1981.
5. 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.
6. Sancar A, DNA repair in humans. *Annu Rev Genet* **29:** 69–105, 1995.
7. Seeberg E, Eide L and Bjoras M, The base excision repair pathway. *Trends Biochem Sci* **20:** 391–397, 1995.
8. Hoeijmakers JHJ, How relevant is the *Escherichia coli* UvrABC model for excision repair in eukaryotes? *J Cell Sci* **100:** 687–691, 1991.
9. Wang Z, Wu X and Friedberg EC, Nucleotide-excision repair of DNA in cell-free extracts of the yeast *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **90:** 4907–4911, 1993.
10. Reardon JT, Thompson LH and Sancar A, Rodent UV-sensitive mutant cell lines in complementation groups 6–10 have normal general excision repair activity. *Nucleic Acids Res* **25:** 1015–1021, 1997.
11. Bardwell AJ, Bardwell L, Tomkinson AE and Friedberg EC, Specific cleavage of model recombination and repair intermediates by the yeast Rad 1–Rad 10 DNA endonuclease. *Science* **265:** 2082–2085, 1994.
12. O'Donovan A, Davies AA, Moggs JG, West SC and Wood RD, XPG endonuclease makes the 3′ incision in human DNA nucleotide excision repair. *Nature* **371:** 432–435, 1994.
13. He Z, Henricksen LA, World MS and Ingle CJ, RPA involvement in the damage-recognition and incision steps of nucleotide excision repair. *Nature* **374:** 566–569, 1995.
14. Park C-H, Mu D, Reardon JT and Sancar A, The general transcription-repair factor TFIIH is recruited to the excision repair complex by the XPA protein independent of the TFIIE transcription factor. *J Biol Chem* **270:** 4896–4902, 1995.
15. Cleaver JE, Charles WC, McDowell ML, Sadinski WJ and Mitchell DL, Overexpression of the *XPA* repair gene increases resistance to ultraviolet radiation in human cells by selective repair of DNA damage. *Cancer Res* **55:** 6152–6160, 1995.
16. 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.
17. 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.
18. Koyama M, Kelly TR and Watanabe KA, Novel type of potential anticancer agents derived from chrysophanol and emodin. *J Med Chem* **31:** 283–284, 1988.
19. Zhou XM and Chen QH, Biochemical study of Chinese rhubarb XXII. Inhibitory effect of anthraquinone derivatives on sodium-potassium-ATPase of a rabbit renal medulla and their diuretic action. *Acta Pharm Sin* **23:** 17–20, 1988.
20. Huang HC, Chu SH and Chao-Lee PD, Vasorelaxants from Chinese herbs, emodin and scoparone, possess immunosuppressive properties. *Eur J Pharmacol* **198:** 211–213, 1991.
21. Su HY, Cheng SH, Chen CC and Lee H, Emodin inhibits the mutagenicity and DNA adducts induced by 1-nitropyrene. *Mutat Res* **329:** 205–212, 1995.
22. Smith ML, Kontny HU, Zhan Q, Sreenath A, O'Connor PM and Fornace AJ Jr, Antisense *GADD45* expression results in decreased DNA repair and sensitizes cells to u.v.-irradiation or cisplatin. *Oncogene* **13:** 2255–2263, 1996.
23. Yen L, Zeng-Rong N, You X-L, Richard S, Langton-Webster BC and Alaoui-Jamali MA, Regulation of cellular response to cisplatin-induced DNA damage and DNA repair in cells overexpression $p185^{erbB-2}$ is dependent on the ras signaling pathway. *Oncogene* **14:** 1827–1835, 1997.
24. Trosko JE, Krause D and Isoun M, Sunlight-induced pyrimidine dimers in human cells *in vitro*. *Nature* **228:** 358–359, 1970.
25. Bohr VA, Gene specific DNA repair. *Carcinogenesis* **12:** 1983–1992, 1991.
26. Zamble DB and Lippard SJ, Cisplatin and DNA repair in cancer chemotherapy. *Trends Biochem Sci* **20:** 435–439, 1995.
27. Nishigori C, Zghal M, Yagi T, Imamura S, Komoun MR and Takebe H, High prevalence of the point mutation in exon 6 of the xeroderma pigmentosum group A-complementing (XPAC) gene in xeroderma pigmentosum group A patients in Tunisia. *Am J Hum Genet* **53:** 1001–1006, 1993.
28. Korr H, Philippi V, Helg C, Schiefer J, Graeber MB and Kreutzberg GW, Unscheduled DNA synthesis and mitochondrial DNA synthetic rate following injury of the facial nerve. *Acta Neuropathol (Berl)* **94:** 557–566, 1997.
29. Kuo KW, Leung MFKL and Leung WC, Intrinsic secondary structure of human TNFR-1 mRNA influences the determination of gene expression by RT-PCR. *Mol Cell Biochem* **177:** 1–6, 1997.
30. Gafter U, Malachi T, Ori Y and Breitbart H, The role of calcium in human lymphocyte DNA repair ability. *J Lab Clin Med* **130:** 33–41, 1997.
31. Miwa N, Mizuno S and Okamoto S, Differential cytotoxicity to human lung normal diploid, virus-transformed and carcinoma cells by the antitumor antibiotics, auromomycin and macromomycin, and their non-protein chromophores. *J Antibiot (Tokyo)* **36:** 715–720, 1983.
32. Kuo KW, Yeh HW, Chu DJ and Yeh YC, Separation and microanalysis of growth factors by Phast system gel electrophoresis and by DNA synthesis in cell culture. *J Chromatogr* **543:** 463–470, 1991.
33. Burnier M, Centeno G, Burki E and Brunner HR, Confocal microscopy to analyze cytosolic and nuclear calcium in cultured vascular cells. *Am J Physiol* **266:** 1118–1127, 1994.
34. Kaku H, Tanaka Y, Tazaki K, Minami E, Mizuno H and Shibuya N, Sialylated oligosaccharide-specific plant lectin from Japanese elderberry (*Sambucus sieboldiana*) bark tissue has a homologous structure to type II ribosome-inactivating proteins, ricin and abrin. cDNA cloning and molecular modeling study. *J Biol Chem* **271:** 1480–1485, 1996.
35. Zhang L, Chang CJ, Bacus SS and Hung M-C, Suppressed transformation and induced differentiation of *HER-2/neu*-overexpressing breast cancer cells by emodin. *Cancer Res* **55:** 3890–3896, 1995.
36. Alberts B, Bray D, Lewis J, Raff M, Roberts K and Watson JD, The cell-division cycle. In: *Molecular Biology of the Cell* (Ed. Robertson M), pp. 863–910. Garland Publishing, New York, 1994.
37. Chu G, Cellular response to cisplatin: The roles of DNA-binding proteins and DNA repair. *J Biol Chem* **269:** 787–790, 1994.
38. Zhang L and Hung M-C, Sensitization of *HER-2/neu*-overexpression non-small cell lung cancer cells to chemotherapeutic drugs by tyrosine kinase inhibitor emodin. *Oncogene* **12:** 571–576, 1996.
39. Tsai C-M, Chang K-T, Perng R-P, Mitsudomi T, Chen M-H, Kadoyama C and Gazdar AF, Correlation of intrinsic chemoresistance of non-small-cell lung cancer cell lines with HER-2/neu gene expression but not with ras gene mutations. *J Natl Cancer Inst* **85:** 897–901, 1993.
40. Tsai C-M, Yu D, Chang K-T, Wu L-H, Perng R-P, Ibrahim NK and Hung M-C, Enhanced chemoresistance by elevation of $p185^{neu}$ levels in HER-2/neu-transfected human lung cancer cells. *J Natl Cancer Inst* **87:** 682–684, 1995.
41. Satokata I, Tanaka K, Miura N, Miyamoto I, Satoh Y, Kondo S and Okada Y, Characterization of a splicing mutation in group A xeroderma pigmentosum. *Proc Natl Acad Sci USA* **87:** 9908–9912, 1990.
42. Dabholkar M, Vionnet J, Bostick-Bruton F, Yu JJ and Reed E, Messenger RNA levels of XPAC and ERCC1 in ovarian cancer tissue correlate with response to platinum-based chemotherapy. *J Clin Invest* **94:** 703–708, 1994.

43. Satoh MS and Hanawalt PC, TFIIH-mediated nucleotide excision repair and initiation of mRNA transcription in an optimized cell-free DNA repair and RNA transcription assay. *Nucleic Acids Res* **24:** 3576–3582, 1996.
44. Ford JM and Hanawalt PC, Expression of wild-type p53 is required for efficient global genomic NER in UV-irradiated human fibroblasts. *J Biol Chem* **272:** 28073–28080, 1997.
45. Sheikh MS, Chen YQ, Smith ML and Fornace AJ Jr, Role of p21 Waf1/Cip1/Sdi1 in cell death and DNA repair as studied using a tetracycline-inducible system in p53-deficient cells. *Oncogene* **14:** 1875–1882, 1997.
46. Schieven GL, Kiribara JM, Gilliland LK, Uckun FM and Ledbetter JA, Ultraviolet radiation rapidly induces tyrosine phosphorylation and calcium signaling in lymphocytes. *Mol Biol Cell* **4:** 523–530, 1993.
47. Lopez JR, Ghanbari RA and Terzic A, A K_{ATP} channel opener protects cardiomyocytes from Ca^{2+} waves: A laser confocal microscopy study. *Am J Physiol* **270:** H1384–H1389, 1996