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Differential Apoptosis by Indomethacin in Gastric Epithelial Cells through the Constitutive Expression of Wild-Type p53 and/or Up-regulation of c-myc

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ABSTRACT. Nonsteroidal anti-inflammatory drug (NSAID)-induced apoptosis is considered to be an important mechanism in the antineoplastic effects and damage produced by the drugs in the gastrointestinal tract. In this study, two different gastric cancer cell lines, MKN28 (mutant-type p53) and AGS (wild-type p53), were compared as to growth inhibition, apoptosis, and cell cycle and apoptosis-related gene expression in response to indomethacin treatment. Cell growth was measured by MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide) assay. Apoptosis was characterized by acridine orange staining and DNA fragmentation, and cell cycle kinetics by flow cytometry. The mRNA and protein levels of p53, $p21^{waf1/cip1}$, and c-myc were determined by Northern and Western blotting. The results showed that indomethacin initiated growth inhibition and apoptosis in both cell lines without cell cycle shifting. AGS cells were more sensitive to growth inhibitory activity and apoptosis of indomethacin than MKN28 cells. In MKN28 cells, the levels of p53, $p21^{waf1/cip1}$, and c-myc mRNA remained unchanged over the 24-hr treatment with indomethacin, but the p53 protein level was elevated after 4 hr. There was no change in the $p21^{waf1/cip1}$ and c-myc protein levels in the MKN28 cells. In AGS cells, a progressive increase in c-myc mRNA and protein levels was noted, while p53 and $p21^{waf1/cip1}$ remained unchanged. It can be concluded that wild-type p53 and/or up-regulation of c-myc is associated with indomethacin-mediated differential apoptosis in gastric epithelial cells. BIOCHEM PHARMACOL **58**;1:193–200, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. apoptosis; stomach; oncogene; NSAIDs; cell cycle; chemoprevention

Apoptosis (programmed cell death) is a highly regulated form of cell death defined by distinct morphological and biological features [1]. It plays a crucial role in the maintenance of gastrointestinal mucosal integrity. Abnormal regulation of apoptosis is associated with atrophic gastritis, peptic ulcer, and tumorigenesis of the stomach [2].

The tumor suppressor gene *p53* mediates either cell cycle arrest or apoptosis in response to DNA damage, thus acting as a molecular "guardian of the genome" [3]. p53-mediated cell cycle arrest was shown to depend on its ability to act as a sequence-specific transcriptional activator [4]. The most important target gene of p53 in the regulation of growth arrest is $p21^{waf1/cip1}$, which acts as a potent inhibitor of cyclin–Cdk complexes and thereby arrests cell cycle progression at G_0/G_1 stage [5, 6]. However, cell cycle checkpoint can also be regulated by a pathway other than $p53/p21^{waf1/cip1}$ [7, 8]. It has been demonstrated that the role of p53 in apoptosis is distinct from its function in cell cycle arrest [9]. The mechanism by which wild-type p53 activates apoptosis has not yet been clarified. As one of the most common tumors worldwide, 60% of gastric carcinoma involves *p53* gene alteration [10, 11]. Since apoptosis usually is triggered off in response to DNA damage, it is thought to prevent harmful genetic damage from being carried over to the next cellular generation [3]. Loss of normal p53-mediated apoptosis contributes to tumor development *in vivo* and transformation *in vitro* [12, 13]. In addition, the resistance of cancers to chemotherapy may be due in part to the high frequency of *p53* mutations, which impairs p53-dependent apoptosis [14, 15].

Activation of c-myc is thought to be involved in the development of gastric cancer since it is commonly amplified and/or overexpressed in human gastric carcinoma [16, 17]. Inappropriate overexpression of c-myc promotes apoptosis in some cell systems [18–20]. Unlike p53, c-myc protein is a positive regulator of cell progression and sufficient for the induction of cell cycle re-entry. Recent results indicated that c-myc and p53 may be interdependent in apoptosis induction. Activation of c-myc in fibroblasts only induces apoptosis if functional p53 is present [21]. It is still unclear whether wild-type p53 is required for the c-myc-mediated apoptosis in human gastric epithelial cells.

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There is substantial experimental, clinical, and epidemiological evidence that regular use of aspirin and NSAIDs* is associated with a reduced risk of colon cancer as well as gastric carcinoma [22–25]. Recent studies in colon cancer cells demonstrated that NSAIDs could induce apoptosis, which was considered to be relevant to their antitumor effects *in vivo* [26–28]. However, until now, there has not yet been a study on apoptosis by NSAIDs in gastric epithelial cells. An understanding of their underlying mechanisms is important for the development of effective chemopreventive strategies for gastrointestinal tumors as well as for the effective prevention of NSAID-related gastritis and peptic ulcer.

To identify the role of p53 in indomethacin-induced apoptosis, we used two different human gastric cancer cell lines. MKN28 (mutant-type p53) and AGS (wild-type p53) [29], and compared their growth inhibition and apoptotic response to indomethacin treatment. We further examined the biological role of $p21^{waf1/cip1}$ and c-myc in these cancer cell lines.

MATERIALS AND METHODS

Cell Lines and Culture

AGS, a gastric adenocarcinoma cell line bearing wild-type p53, was purchased from the American Type Culture Collection. MKN28, as gastric tubular adenocarcinoma cell line, does not contain wild-type p53, but has missense mutation (codon 251, isoleucine to leucine) [29]. This was kindly donated by Professor Xiao SD (Shanghai Second Medical University). All experiments involved cells that were passaged no more than 10 times. They were grown in RPMI 1640 medium containing 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin (GIBCO BRL, Life Technologies). Cells were kept in 25-cm^2 culture flasks (Corning) as monolayer in a 95% air, 5% CO_2 humidified atmosphere at 37°.

Chemicals and Drug Treatment

Indomethacin (Sigma) was freshly prepared in absolute ethanol before use. Vehicle control of absolute ethanol (less than 0.1%) was included in the studies. Cells were incubated for 24 hr with various concentrations of indomethacin. After treatment, adherent cells were removed by trypsinization and combined with the floating cells in the medium [26]. Cells were collected by centrifugation for further analysis.

MTT Assay

Cell growth was measured by a modified MTT assay [30], based on the viability of live cells to utilize thiazolyl blue and its subsequent conversion into a dark blue formazan produced by these cells. The assay detects living but not dead cells, and the signal generated directly correlates to the number of metabolically active cells in the culture. About 3,000 cells/well were plated in 96-well microtiter plates and incubated overnight in 100 μL of culture media. Cells were then treated with various doses of indomethacin (0–400 μM) for three days as described previously [31]. Ten μL stock MTT (2.5 mg/mL) was then added to each well, and the cells were further incubated at 37° for 4 hr. The supernatant was removed and 100 μL 0.04 M HCl in isopropanol was added to each well to solublize the formazan production. The absorbance at a wavelength of 595 nm was measured by a microELISA reader (BioRad). The negative control well, into which no cells had been seeded and only the medium had been added, was used for zeroing the absorbance. Each assay was performed three times in triplicate.

Morphological Measurement of Apoptosis

The morphological change of apoptosis was assayed by fluorescence microscope following staining with AO, as described previously [32]. Single cell suspension was fixed in 1% formalin/PBS, stained with 5 μg/mL AO (Sigma), then visualized under UV fluorescence microscope. Apoptotic cells were defined as cells showing cytoplasmic and nuclear shrinkage and chromatin condensation or fragmentation. Necrotic cells were identified as cells with poorly staining "hollow". At least 300 cells were counted and the percentage of apoptotic cells was determined.

Cell Cycle Phase Distribution

The proportion of cells in G_0/G_1, S, and G_2/M phases of cell cycle was determined by flow cytometric analysis of DNA content. Cells were treated with various concentrations of indomethacin for 24 hr. DNA content was then determined after labeling cells with propidium iodide as described previously [33]. Cell suspension was prepared as before, fixed in ice-cold 70% ethanol in PBS, and stored at −20°. Prior to analysis, the cells were washed and resuspended in PBS and incubated with 0.1 mg/mL RNase I and 40 μg/mL propidium iodide (Sigma) at 37° for 30 min. The analysis of samples was performed by a flow cytometer (Coulter EPICS XL). The resulting histogram was analyzed using Multicycle AV software (Phoenix Flow System). The apoptotic cells can be observed on a DNA histogram as a subdiploid or 'pre-G_1' peak.

DNA Fragmentation Analysis

DNA fragmentation was analyzed by a previously described method [34] with some modifications. Briefly, following indomethacin treatment, cells were harvested and rinsed twice in ice-cold PBS. The final pellet was lysed in 0.3 mL 10 mM Tris–HCl (pH 7.4) buffer containing 25 mM

* *Abbreviations:* AO, acridine orange; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MTT, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide; and NSAIDs, nonsteroidal anti-inflammatory drugs.

EDTA, 0.5% SDS, and 0.1 mg/mL proteinase K (Sigma) at 50° for 12 hr. DNA was extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) and precipitated with 2 volumes of ice-cold absolute ethanol and 1/10 volume 3 M sodium acetate. DNA was collected, washed once with 70% ethanol, and dissolved in TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0). Then, the isolated DNA samples were incubated with 10 μg/mL RNase I for 1 hr at 37°. An equal amount (10 μg/well) of DNA was electrophoresed in 1.8% agarose gels impregnated with ethidium bromide (0.1 μg/mL) for 2 hr at 60 V. DNA markers (50–1000 bp, FMC BioProducts) were run at the same time. DNA fragments were visualized by ultraviolet transillumination.

RNA Preparation and Northern Blotting Analysis of p53, p21$^{waf1/cip1}$, and c-myc

Total cellular RNA was isolated following the methods described previously [35] with some modifications. Briefly, cells were washed with ice-cold PBS, lysed with 4 M LiCl/8M urea/6 mM EDTA, and then precipitated overnight at 4°. After centrifugation for 15 min at 12,000 *g* at 4°, RNA pellet was dissolved in dicarbonic acid diethylester-treated H_2O, extracted with phenol/chloroform/isoamyl alcohol (25:24:1), and then precipitated with ethanol and sodium acetate. Northern blot analysis was performed according to the method described by Ausubel *et al.* [36]. Twenty μg of total RNA per lane was electrophoresed on 1.0% denatured formaldehyde agarose gel and transferred onto nylon membrane. The cDNA probe of p53, p21$^{waf1/cip1}$, and c-myc (Oncogene Research Products) was radiolabeled with [α-^{32}P]dCTP (Amersham) by the Megaprime random prime labeling system (Amersham Life Science). After hybridization with the probe overnight at 42°, the membrane was washed with 2.0 × SSPE/0.1% SDS, 1.0 × SSPE/0.1% SDS, and 0.2 × SSPE/0.1% SDS at 42°, then exposed to Kodak XAR film at −70°. The membrane was reprobed for use in another hybridization in which GAPDH mRNA served as an internal control. GAPDH oligonucleotide probe (Oncogene Research Products) was radiolabeled with [γ-^{32}P] ATP by the 5′-end labeling kit (Amersham Life Science).

Western Blot Analysis of p53, p21$^{waf1/cip1}$, and c-myc

After the drug treatment, the whole cell lysates were extracted with lysis buffer (1% Triton-100, 50 mM NaCl, 50 mM NaF, 20 mM Tris pH 7.4, 1 mM EDTA, 1 mM EGTA, 1 mM sodium vanadate, 0.2 mM phenylmethylsulfonyl fluoride, and 0.5% Nonidet P-40). Western blotting was carried out as described previously [37]. An equal amount of total cell lysates (60 μg) was solubilized in sample buffer by boiling for 5 min and subjected to 10% SDS-PAGE followed by electrotransfer onto a nitrocellulose filter (Sigma). The filter was incubated first with an appropriate primary antibody and then with peroxidase-conjugated anti-mouse immunoglobulin G (IgG) in the second reaction. Proteins were detected using the ECL Western blot detection system (Transduction Laboratories). Mouse monoclonal antibodies against p21$^{waf1/cip1}$ (Ab-1), p53 (Ab-6), c-myc (Ab-1), and peroxidase-conjugated anti-mouse IgG were purchased from Oncogene Research Products.

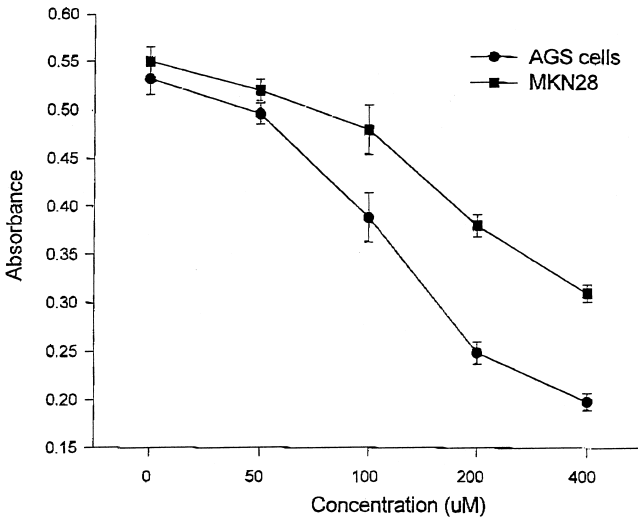

FIG. 1. Dose–response of indomethacin on growth inhibition of both MKN28 and AGS cells. The cells were treated with various concentrations of indomethacin for three days. The antiproliferative effects were measured by the MTT assay as described in Materials and Methods. The values were expressed as a means ± SEM from three independent experiments.

Statistical Analysis

The data shown were mean values of at least three different experiments and expressed as mean ± SEM. Student's *t*-test was used to compare data. A P value of less than 0.05 was considered as statistically significant.

RESULTS

Cell Growth Inhibition of Indomethacin on MKN28 and AGS Cells

As shown in Fig. 1, indomethacin could inhibit the cell growth of both the MKN28 and AGS cell lines in a dose-dependent manner in the MTT assay. AGS cells were more sensitive to growth inhibition than MKN28 cells.

Induction of Apoptosis by Indomethacin on MKN28 and AGS Cells

Apoptosis of cells was evaluated in three different ways as described in Materials and Methods: 1) measurement of DNA content of cells by propidium iodide staining and flow cytometry analysis to detect the pre-G_1 peak; 2) agarose gel electrophoresis of genomic DNA to detect DNA fragmentation; and 3) AO staining to detect typical

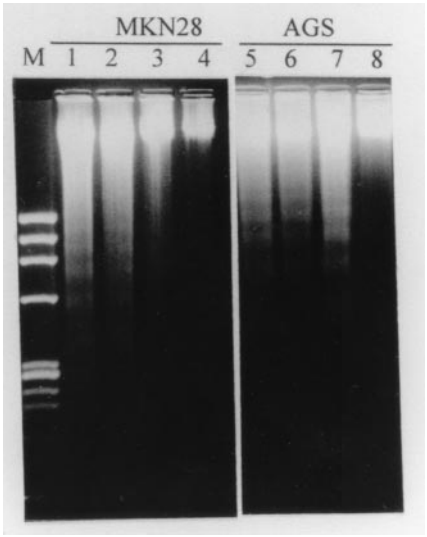

FIG. 2. DNA ladder pattern formation of MKN28 and AGS. Both cell lines were treated with different concentrations of indomethacin for 24 hr and the formation of oligonucleosomal fragments was determined by 1.8% agarose gel electrophoresis. M, DNA markers; lanes 1–4, MKN28 treated with 400, 200, 100, and 0 μM of indomethacin; lanes 5–8, AGS cells treated with 400, 200, 100, and 0 μM of indomethacin as with MKN28.

morphological changes under fluorescence microscopy. The flow cytometry analysis showed that both MKN28 and AGS cells had a typical subdiploid peak on the DNA histogram. Analysis of DNA from MKN28 and AGS cells demonstrated that indomethacin caused the generation of nucleosomal-sized ladders of DNA fragments in treated cells (≥100 μM for AGS cells and ≥200 μM for MKN28 cells). (Fig. 2). The AO staining showed that indomethacin could induce apoptosis in MKN28 and AGS cells morphologically, characterized by both cytoplasmic and nuclear shrinkage and chromatin condensation and fragmentation (Fig. 3). Apoptosis induction in both cell lines followed a dose-dependent pattern as quantified by AO staining and flow cytometry (Fig. 4). A more profound apoptotic effect was observed in AGS cells than in MKN28 cells.

Cell Cycle Phase Distribution of Gastric Cancer Cells by Indomethacin

To investigate the mechanisms involved in differential growth inhibition in indomethacin-treated cell lines, we studied the effect of indomethacin on the cell cycle kinetics of MKN28 and AGS cells. The analysis of flow cytometry after indomethacin treatment for 24 hr showed that there was no cell cycle alteration of either cell line compared with controls (Table 1).

Differential Expression of p53, $p21^{waf1/cip1}$, and c-myc mRNA in Indomethacin-treated Cells

Several gene products are known to be important in controlling the apoptotic process. To determine if indomethacin has any effect on the expression of these genes, a time-course experiment was performed to analyze the mRNA level by Northern blotting. As shown in Fig. 5, the level of p53, $p21^{waf1/cip1}$, and c-myc mRNA remained unchanged in 200 μM indomethacin-treated MKN28 cells throughout the 24-hr period. In contrast, there was a progressive increase in c-myc mRNA in AGS cells which was noted as early as 2 hr and peaked at 12 hr, while p53 and $p21^{waf1/cip1}$ were unchanged.

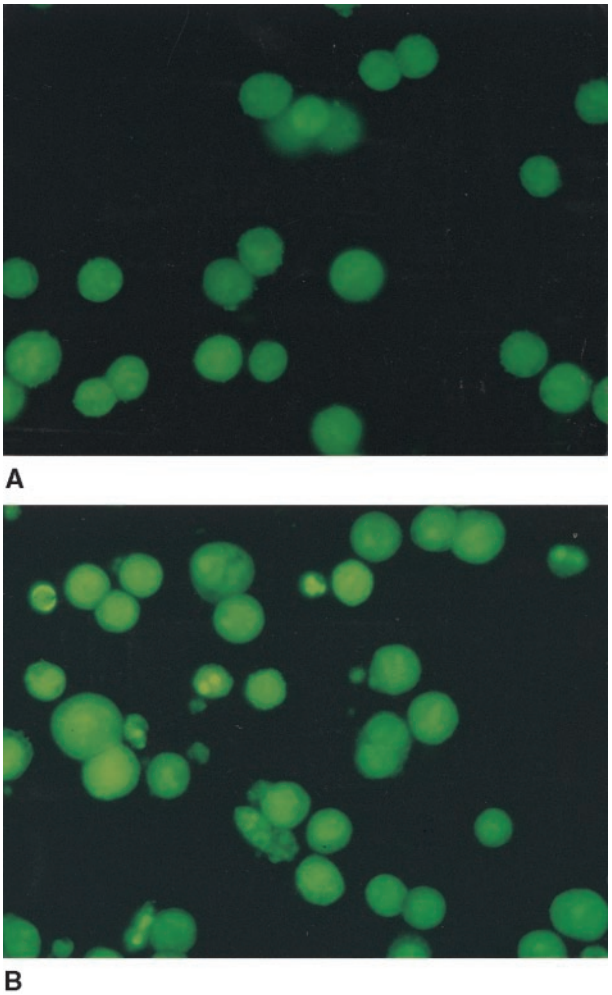

FIG. 3. Fluorescence photography of MKN28. Cells were stained with AO and examined under fluorescence microscope. A, control; B, treatment with 200 μM indomethacin. Original magnification was 400×.

Differential Expression of p53, $p21^{waf1/cip1}$, and c-myc Protein in Indomethacin-treated Cells

Time-course experiments were carried out to detect the protein levels of apoptosis-related genes. As shown in Fig. 6, there was a sustained elevation of the p53 protein level in 200 μM indomethacin-treated MKN28 cells, starting at 4 hr and continuing until 24 hr. However there was no change in $p21^{waf1/cip1}$ or c-myc protein during the whole period of treatment. In AGS cells undergoing the same treatment, the protein expression of the three genes exhib-

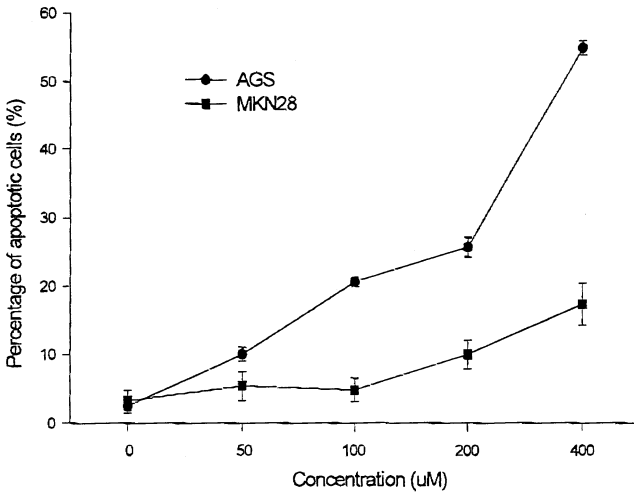

FIG. 4. Dose–response of indomethacin on apoptosis of both MKN28 and AGS cells. The cells were treated with various concentrations of indomethacin for 24 hr. The percentage of apoptosis was quantified by AO staining and flow cytometry. The values were expressed as means ± SEM from three different experiments.

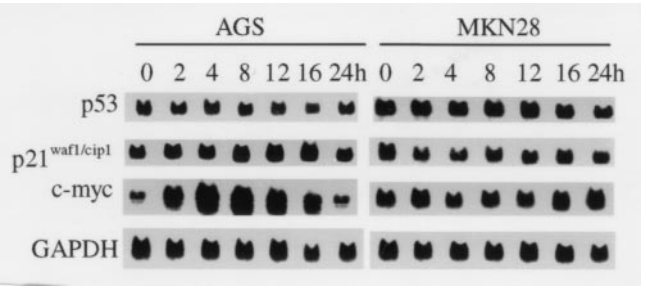

FIG. 5. Analysis of $p21^{waf1/cip1}$, p53, and c-myc mRNA levels in indomethacin-treated cells. The Northern blot was performed on total RNA (20 μg per lane) extracted from cells exposed to 200 μM indomethacin for specified times. Blots were probed sequentially with ^{32}P-labeled probes coding for $p21^{waf1/cip1}$, p53, and c-myc. The housekeeping GAPDH was used as an internal control. The figure is representative of three different experiments.

ited a similar change to the expression of their mRNA, i.e. c-myc protein was induced while p53 and p21 were invariable.

DISCUSSION

Our results indicated that AGS cells bearing the wild-type *p53* gene were more sensitive to the growth inhibitory effect of indomethacin than MKN28 cells with mutant-type p53. Similarly, indomethacin-induced apoptosis occurred more readily in AGS than in MKN28 cells. In addition, the increase in apoptosis paralleled the growth inhibition of indomethacin. However, neither cell line exhibited cell cycle phase alteration after indomethacin treatment. These data suggested that the level of apoptosis induction by indomethacin was the major factor contributing to differential cell growth inhibition in both cell lines. Studies carried out in colon cancer cell lines confirm that sulindac and sulindac sulfide could arrest the cell at G_0/G_1 phase [27]. The discrepancy between our results and previous findings may be due to different tissue origin. An *in vivo* study in human gastric intestinal-type adenocarcinoma indicated that expression of a mutated *p53* gene could lead to attenuation of gastric cancer cell apoptosis [38]. Our *in vitro* results further confirmed the previous observation that the degree of apoptosis in gastric epithelial cells was p53-dependent. p53-Dependent apoptosis has also been demonstrated in recent studies to modulate the genotoxic and hormone ablation therapies [17, 15, 39], probably due to the disruption of the apoptotic pathway. Our present study led us to speculate that NSAIDs achieved their growth inhibition effect by the same mechanism. The p53 protein level was increased in MKN28 cells in response to indomethacin treatment, while the mRNA level was unchanged despite prolonged incubation. This indicated that the elevation of the mutant-type p53 protein after indomethacin treatment was due to increased translation or stabilization of the p53 protein rather than increased transcription. The mechanisms by which NSAIDs effect their post-transcriptional regulation in the mutant-type but not the wild-type p53 has not yet been explored. In HT-29 colon carcinoma cells which harbor a G to A mutation at codon 273 in the *p53* gene, the level

TABLE 1. Effect of indomethacin on cell cycle kinetics of MKN28 and AGS cells

Treatment	% G_0/G_1	% S	% G_2/M
AGS cells			
Vehicle	73.6 ± 3.9	12.2 ± 3.4	14.2 ± 0.4
Indomethacin (100 μM)	75.8 ± 0.9	11.0 ± 0.8	13.0 ± 0.5
Indomethacin (200 μM)	75.1 ± 1.4	12.3 ± 2.2	12.3 ± 1.3
Indomethacin (400 μM)	74.8 ± 2.1	13.6 ± 0.9	11.5 ± 2.1
MKN28			
Vehicle	66.3 ± 2.5	15.0 ± 0.4	18.5 ± 3.0
Indomethacin (100 μM)	66.9 ± 2.2	15.9 ± 3.7	17.3 ± 2.4
Indomethacin (200 μM)	66.9 ± 2.2	15.9 ± 3.7	17.3 ± 2.3
Indomethacin (400 μM)	64.6 ± 0.8	18.5 ± 3.0	16.9 ± 3.4

The cells were treated with various concentrations of indomethacin for 24 hr. The cell cycle distribution was determined by flow cytometry analysis. The data were expressed as means ± SEM from three independent experiments. There is no statistical difference in cell cycle distribution of AGS and MKN28 with or without indomethacin treatment.

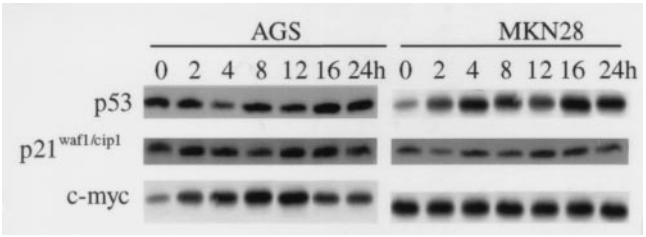

FIG. 6. Analysis of p21$^{waf1/cip1}$, p53, and c-Myc protein levels in indomethacin-treated cells. Both MKN28 and AGS were treated with 200 μM indomethacin for various time periods. Cells were harvested and whole cellular protein was electrophoresed in SDS-PAGE gel. Western blot was performed using monoclonal antibodies against p21$^{waf1/cip1}$, p53, and c-Myc. The figure is representative of three different experiments.

of mutant p53 protein expression was reduced by sulindac and sulindac sulfide, while its mRNA level was not analyzed [40, 41]. However, the significance of such an induction of mutant-type p53 is still unclear in indomethacin-mediated growth inhibition and apoptosis of MKN28 cells. Missense p53 mutant proteins appear to increase the expression of the multiple drug resistance gene, *mdr1*, through a transcriptional mechanism [42, 43]. Thus, the lower sensitivity to growth inhibition and apoptosis induction in the MKN28 cells compared to the AGS cells may be in part explained by the effect of *p53* mutation.

Being one of the downstream targets of p53, p21$^{waf1/cip1}$ contains p53-binding sites with high homology to the p53 consensus motif [5, 6]. Similar to a previous study [44], our data also showed that the MKN28 cell line expressed basal p21$^{waf1/cip1}$ mRNA at a relatively high level. Indomethacin treatment had no effect on p21$^{waf1/cip1}$ mRNA or protein level in either cell line, suggesting that indomethacin induced apoptosis in human gastric cancer cells through a p21$^{waf1/cip1}$-independent pathway. However, in HT-29 colon cancer cells with mutant *p53*, sulindac and sulindac sulfide enhanced p21$^{waf1/cip1}$ mRNA expression. This was not associated with intestinal cell differentiation and occurred independently of the ability of these compounds to induce apoptosis [40]. Further study is required to identify the role of p21$^{waf1/cip1}$ in NSAID-induced apoptosis in human gastrointestinal epithelial cells.

Hermeking *et al.* [21] demonstrated that unregulated c-myc expression led to apoptosis of mouse fibroblasts expressing wild-type p53, but not in *p53*-null fibroblasts. Another study in the rat hepatocellular carcinoma cell line (FAA-HTC1) also indicated that wild-type p53 and c-myc could cooperate in generating apoptosis [45]. However, experiments performed in rat kidney epithelial cells showed c-myc could induce apoptosis by both p53-dependent and p53-independent mechanisms [46]. In this study, overexpression of *c-myc* protooncogene was observed in the wild-type p53 bearing AGS cells following indomethacin treatment. On the contrary, in mutant *p53* bearing MKN28 cells, c-myc expression was unchanged and there was a much lower apoptotic response. Thus, wild-type p53 and/or overexpression of c-myc in AGS cells was associated with differential apoptosis induction in indomethacin-treated cells. We could not demonstrate significant induction of p53 mRNA and protein following indomethacin treatment, which indicated that a normal level of wild-type p53 is sufficient to confer susceptibility to c-myc-mediated apoptosis. This finding was in agreement with the results found within primary embryo fibroblasts [47].

It is still unclear whether the high concentrations of indomethacin used here can be reached in human stomach. However, before we interpret the results obtained in *in vitro* cell culture and correlate their relevance to human, several factors need to be taken into account. Firstly, although the plasma levels of indomethacin in patients receiving continuous treatment of the drug were less than 80 μM [48], a peak serum concentration as high as 150–450 μM by oral administration [48] or about 110 μM by rectal administration has been attained [49]. Secondly, indomethacin, as one of the acidic NSAIDs, can accumulate in mildly acidic compartments such as stomach [50]. It is conceivable that concentration in gastric mucosa may be higher than in serum. Thirdly, indomethacin is mainly in unionized form at pH of the gastric juice and will thus readily diffuse into the mucosal cells, where, at a pH of about 7.4, it will be largely ionized and unlikely to diffuse out of the cells again. Indomethacin, then, can rapidly build up at very high intracellular concentrations, resulting in cell death [51]. Finally, there was a close similarity in cellular effect in gastric cancer cells between a high concentration of indomethacin and short exposure and a lower dose and longer exposure (data not shown).

In conclusion, differential apoptosis by indomethacin, rather than cell cycle checkpoint, contributes to differential growth inhibition in human gastric cancer cells. Constitutive expression of wild-type p53 and/or upregulation of c-myc may be associated with differential apoptosis in indomethacin-treated gastric cancer cells.

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