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Reactive oxygen species are involved in the apoptosis induced by nonsteroidal anti-inflammatory drugs in cultured gastric cells

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Abstract

We previously reported the induction of apoptotic DNA fragmentation by nonsteroidal anti-inflammatory drugs (NSAIDs) in cultured rat gastric cells, and indicated that prostaglandin-synthesis is only marginally involved in the apoptotic process. In the present study, we examined whether the generation of reactive oxygen species is critically involved in NSAID-induced apoptosis. Indomethacin, sodium diclofenac, flurbiprofen, zaltoprofen, etodolac, but not mofezolac, enhanced apoptotic DNA fragmentation and mRNA expression for cyclooxygenase-2 in AGS cells, a cell line derived from human gastric epithelium. The apoptotic effect of indomethacin was then confirmed by fluorescent staining of the cells with annexin V. Apoptotic DNA fragmentation induced by indomethacin and flurbiprofen was suppressed by incubation of the cells with the anti-oxidants pyrrolidine dithiocarbamate, diphenyleneiodonium chloride, and *N*-acetyl-L-cysteine. These two NSAIDs also enhanced release from the cells of 8-isoprostane, a nonenzymatic product by free-radical-mediated peroxidation of arachidonic acid. Further, lucigenin chemiluminescence showed that the intracellular production of reactive oxygen species increased in cells treated with indomethacin. The present data thus indicate a crucial association between the generation of reactive oxygen species and NSAID-induced apoptosis in gastric epithelial cells. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Apoptosis; Cyclooxygenase; Gastric epithelial cell; Mofezolac; Nonsteroidal anti-inflammatory drug; Reactive oxygen species

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) suppress cellular formation of prostaglandins through inhibition of cyclooxygenase, a rate-limiting enzyme in the biosynthesis pathway (Vane, 1971). Although their favorable anti-inflammatory, anti-pyretic, and analgesic properties have placed them in wide clinical use, their oral administration is accompanied by a high incidence of gastrointestinal side-effects. Given that prostaglandins confer cytoprotective effects in the gastrointestinal toxicity, this may largely result from the suppression of gastrointestinal prostaglandin synthesis (Wallace, 1994; Vane and Botting, 1996). Against this, however, gene disruption of cyclooxygenase-1, a constitutive isoform normally present in gastric tissue which possibly contributes to the production of cytoprotective prostaglandins, did not induce gastric ulcers in mice (Langenbach et al., 1995). The gastrointestinal toxicity of NSAIDs therefore requires further investigation, especially with regard to the mechanisms by which it is induced.

We recently reported the apoptotic effect of NSAIDs in cultured rat gastric mucosal cells (Kusuhara et al., 1998). Induction of apoptotic DNA fragmentation was accompanied by enhanced expression of mRNA for cyclooxygenase-2, an inducible isoform, and suppressed by caspase inhibitors. In contrast, the apoptotic effect showed little connection with NSAID's inhibitory activity against cyclooxygenase-1, and was not prevented by exogenous supplementation of 16, 16-dimethyl prostaglandin E_2. NSAID-induced apoptosis is thus considered unrelated to the suppressive effect of these drugs on cellular prostaglandin synthesis. Further, the apoptotic effect of NSAIDs indomethacin and sodium diclofenac, with a high incidence of gastrointestinal toxicity, was much stronger than that of less toxic drugs, indicating that apoptotic effect of NSAIDs is closely related to gastrointestinal toxicity. However, the signaling pathway leading to apoptotic cell death remains unclear.

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Apoptosis induced by anti-cancer drugs including camptothecin and etoposide was accompanied by the generation of reactive oxygen species in several cell lines (Verhaegen et al., 1995; Shimizu et al., 1998). Generation of reactive oxygen species was also involved in the apoptosis induced by glycochenodeoxycholate, a hydrophobic bile salt, in cultured hepatocytes (Patel and Gores, 1997). Together, these findings indicate that oxidative stress through the generation of reactive oxygen species is a key mediator of apoptosis induced by various chemicals and toxicants. Furthermore, reactive oxygen species have also been suggested to play a critical role in the receptor-mediated apoptosis induced by transforming growth factor β1 and tumor necrosis factor-α (Cossarizza et al., 1995; Lafon et al., 1996). The recent discovery that the anti-apoptotic *bcl*-2 gene product has anti-oxidant properties lends further support to the importance of oxidative events in apoptosis (Hockenbery et al., 1993; Kane et al., 1993). Reactive oxygen species have thus been shown to play an essential role in apoptotic pathways.

The purpose of the present study is to investigate whether reactive oxygen species are also involved in the apoptosis induced by NSAIDs, using six kinds of NSAIDs in clinical use as test drugs and AGS cells, a cell line derived from the human gastric epithelium, as target cells of apoptosis.

2. Materials and methods

2.1. Reagents

Mofezolac (3,4-bis(4-methoxyphenyl)isoxazole-5-acetic acid) and 1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acid (etodolac) were chemically synthesized, and 2-(10,11-dihydro-10-oxodibenzo[*b*,*f*]thiepin-2-yl)propionic acid (zaltoprofen) was extracted and purified from commercially available tablets at Yoshitomi Pharmaceutical Industries (Fukuoka, Japan). 1-(*p*-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid (indomethacin), sodium 2-(2,6-dichloroanilino)phenylacetic acid (sodium diclofenac), and 2-(2-fluoro-4-biphenyl)propionic acid (flurbiprofen) were purchased from commercial sources. The drugs were dissolved and diluted in dimethyl sulfoxide (DMSO). The caspase-3 inhibitor *N*-acetyl-Asp-Glu-Val-Asp-aldehyde (Ac-DEVD-CHO) was purchased from Takara Biomedicals (Shiga, Japan) and dissolved in sterilized water. Pyrrolidine dithiocarbamate, diphenyleneiodonium chloride, *N*-nitro-L-arginine methyl ester, and *N*-acetyl-L-cysteine were purchased from Sigma (St. Louis, MO). These reagents were dissolved and diluted in DMSO. Human erythrocyte catalase and bovine erythrocyte superoxide dismutase were also purchased from Sigma. 8-Isoprostane (8-epi prostaglandin $F_{2\alpha}$), a nonenzymatic product by free-radical-mediated peroxidation of arachidonic acid, was purchased from Cayman Chemical (Ann Arbor, MI).

2.2. Cell culture

The human gastric epithelial cell line AGS was obtained from the American Type Culture Collection (Rockville, MD) and maintained in Ham's F12 medium supplemented with 10% fetal calf serum in an atmosphere of 5% CO_2 at 37°C. This cell line expresses wild-type p53 and has been widely used as a host for *Helicobacter pylori* infection (Barranco et al., 1983; Chen et al., 1997; Keates et al., 1997; Sharma et al., 1998). The cells were seeded at a density of 1×10^5 per ml of Ham's F12 medium supplemented with 10% fetal calf serum and grown to confluency in six- or 96-well culture plates, or 75-cm^2 culture flasks according to experimental purpose. The cells were then washed, immersed in Ham's F12 medium without serum, and subjected to assay. Drugs and reagents were added to the medium at the onset of culture and maintained thereafter until the termination of incubation. Final concentration of DMSO in the medium was 0.1–0.2%.

2.3. Detection of apoptotic DNA fragmentation

AGS cells were grown in 96-well culture plates. The cells were incubated with drugs and/or reagents for 30 min or 6 h. Those treated for 30 min were then washed and further incubated for 5.5 h in serum-free Ham's F12 medium. Apoptotic DNA fragmentation was determined using a commercially obtained enzyme-linked immunosorbent assay (ELISA) kit from Boehringer Mannheim (Mannheim, Germany). This assay is based on a quantitative sandwich enzyme-immunoassay directed against cytoplasmic histone-associated DNA fragments (Bonfoco et al., 1995; Slomiany et al.,1997; Kusuhara et al., 1998). Briefly, the cells were incubated in 200 μl of lysis buffer provided in the kit, the lysates were centrifuged, and 20 μl of the supernatant containing cytoplasmic histone-associated DNA fragments were reacted overnight at 4°C in streptavidin-coated microtitrator wells with 80 μl of the immunoreagent mix containing biotinylated anti-histone antibody and peroxidase-conjugated anti-DNA antibody. After washing, the immunocomplex-bound peroxidase was probed with 2,2′-azino-di[3-ethylbenzthiazoline sulfonate] for spectrophotometric detection at 405 nm.

2.4. Measurement of mRNA expression by semi-quantitative reverse transcription-linked polymerase chain reaction (RT-PCR)

AGS cells grown in six-well culture plates were incubated with drugs for 6 h. Total RNA was isolated from the cells by the guanidinium thiocyanate procedure (Chomczynski and Sacchi, 1987). The amount of mRNA for cyclooxygenase-1, -2, and glyceraldehyde 3-phosphate dehydrogenase was determined using a RT-PCR kit from Takara Biomedicals according to the protocol of the supplier. Briefly, cDNA was made from RNA samples using antisense oligonucleotide primers and reverse transcriptase derived from avian myeloblastosis virus. Next, cDNA was

amplified with additional sense oligonucleotide primers and Taq DNA polymerase in a Thermal Cycler Type MP (Takara Biomedicals). RT-PCR was carried out under conditions in which the PCR product increased logarithmically depending on the amount of RNA and the number of cycles. A total of 100 ng of RNA was used in each RT reaction. PCR was initiated in the thermal cycler programmed for 94°C, 30 s; 60°C, 30 s; and 72°C, 90 s. PCR was performed for 35 cycles. Primers for human cyclooxygenase-1 and -2 were as previously described (Topley et al., 1994). Those for human glyceraldehyde 3-phosphate dehydrogenase were purchased from Clontech (Palo Alto, CA). The PCR product was separated by electrophoresis through a 3% NuSieve 3:1 agarose gel (FMC BioProducts, Rockland, ME) containing ethidium bromide, scanned under UV illumination in an image analyzer (ChemiImager 4000: Alpha Innotech, San Leandra, CA) and semi-quantitated.

2.5. Flow cytometric analysis of apoptosis

Annexin V binding to cellular phosphatidylserine was employed as an indicator of apoptotic alteration of lipid organization in the plasma membrane (Koopman et al., 1994; Shenker et al., 1997). AGS cells grown in 75-cm^2 culture flasks were incubated with or without indomethacin at 1 mM for 6 h. The cells were then scraped off and washed in binding buffer (10 mM HEPES/NaOH, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4). An aliquot containing 1×10^5 cells in 100 μl of binding buffer was incubated with 10 μl of fluorescein-conjugated annexin V reagent (R&D Systems, Minneapolis, MN) for 15 min at room temperature. The cells were then diluted with the binding buffer and analyzed on a flow cytometer (EPICS XL: Coulter, Miami, FL).

2.6. Measurement of 8-isoprostanes

AGS cells grown in 96-well culture plates were incubated with drugs and/or reagents for 6 h. 8-Isoprostanes released into the culture media were measured using an ELISA kit (Cayman Chemical) according to the manufacturer's directions.

2.7. Assay for reactive oxygen species production

Generation of reactive oxygen species was assessed using lucigenin. AGS cells grown in 75-cm^2 culture flasks were incubated for 6 h with indomethacin at 1 mM in the presence or absence of 100 μM pyrrolidine dithiocarbamate. The cells were then scraped off and washed in cold Hank's buffer. An aliquot containing 1×10^6 cells in 100 μl of Hank's buffer was mixed in microtitrator wells with 100 μl of lucigenin (bis-*N*-methylacridium nitrate: Sigma) prepared at a concentration of 40 μM. Light emission was detected using a Berthold LB96V luminometer (Wildbad, Germany) for 3 min.

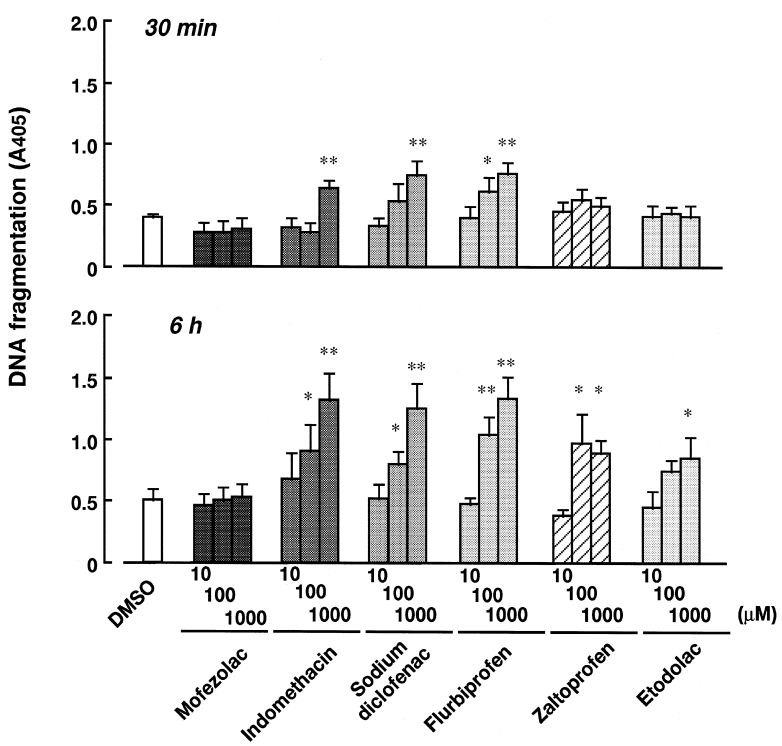

Fig. 1. Effect of NSAIDs on apoptotic DNA fragmentation in AGS cells. The cells were incubated with the indicated concentrations of drugs for 30 min and 6 h. Cytoplasmic histone-associated DNA fragments were determined using a commercial ELISA kit. Results are the means ± SE from five independent determinations: $^*P < 0.05$; $^{**}P < 0.01$ vs. DMSO controls (Dunnett method, two-way layout).

2.8. Statistical analysis

Significance of the data was analyzed using the Dunnett method (two-way layout), *t*-test, or paired *t*-test.

3. Results

3.1. Induction of apoptotic DNA fragmentation by NSAIDs in AGS cells

The effect of six kinds of NSAIDs at concentrations from 10 μM to 1 mM on apoptotic DNA fragmentation in AGS cells is shown in Fig. 1. Among these, indomethacin, sodium diclofenac, and flurbiprofen were found to significantly induce DNA fragmentation within 30 min after the onset of incubation. This fragmentation was exacerbated when the incubation period was prolonged to 6 h. Zaltoprofen and etodolac caused almost no fragmentation within 30 min, but did induce moderate fragmentation on incubation for 6 h. Among NSAIDs tested, only mofezolac caused no significant DNA fragmentation in the time periods examined. Thus, indomethacin, sodium diclofenac, and flurbiprofen were found to be the strongest inducers of apoptotic DNA fragmentation, followed by zaltoprofen, etodolac, and then mofezolac.

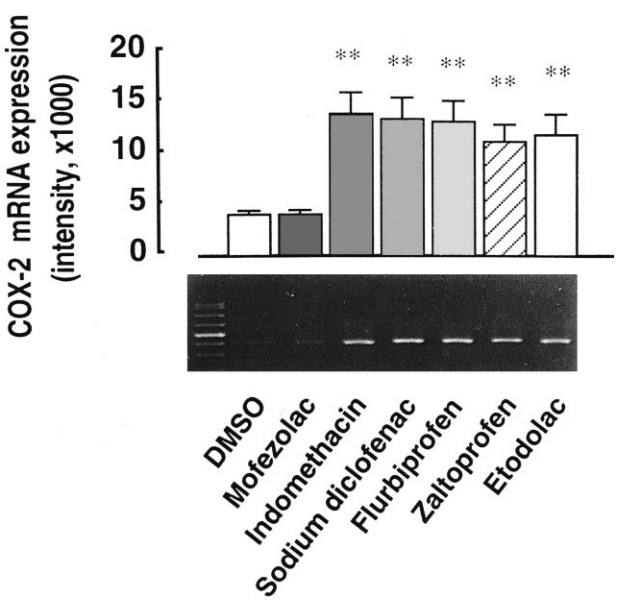

Fig. 2. Effect of NSAIDs on mRNA expression for cyclooxygenase (COX)-2 in AGS cells. The cells were incubated for 6 h with the indicated drugs at a concentration of 1 mM. Total RNA was then isolated and RT-PCR was performed using oligonucleotide primers specific to the nucleotide sequence of the cyclooxygenase-2 gene. Typical patterns of agarose gel electrophoresis of the PCR products and a summary after image analysis are shown. Molecular standards are 2000, 1500, 1000, 700, 525, 500, 400, 300, 200, 100, and 50 base pairs. Scores are the means ± SE from five independent determinations: $^{**}P < 0.01$ vs. DMSO controls (Dunnett method, two-way layout).

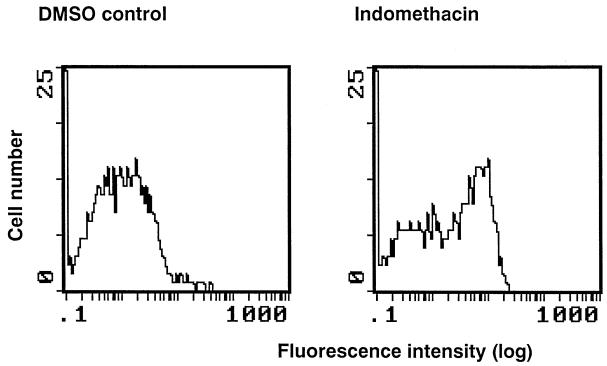

Fig. 3. Flow cytometric analysis of apoptosis induced by indomethacin. AGS cells were incubated with or without 1 mM indomethacin for 6 h. The cells were then collected, incubated with the fluorescein-conjugated annexin V reagent for 15 min at room temperature and analyzed on a flow cytometer. Typical patterns of five preparations are shown.

3.2. Induction of mRNA expression for cyclooxygenase-2 by NSAIDs in AGS cells

Expression of cyclooxygenase-2 mRNA in AGS cells incubated for 6 h with NSAIDs is shown in Fig. 2. The mRNA expression was detected in only small amounts in the untreated cells. In contrast, significant expression was induced by treatment with indomethacin, sodium diclofenac, flurbiprofen, zaltoprofen, and etodolac at a concentration of 1 mM. Mofezolac had almost no effect on this variable. Expression for cyclooxygenase-1 and glyceraldehyde 3-phosphate dehydrogenase mRNA was constitutive, with the various drug treatments showing almost no effect (data not shown).

3.3. Effect of indomethacin on annexin V-binding of AGS cells

Flow cytometric patterns of AGS cells stained with fluorescein-labeled annexin V are shown in Fig. 3. Incubation with indomethacin at 1 mM generated a new fluorescent peak, indicating apoptotic alteration of lipid organization in the plasma membrane. The percentage of apoptotic cells was approximately 10–20%.

3.4. Effect of anti-oxidants on NSAID-induced DNA fragmentation

The effect of various agents acting as inhibitors of reactive oxygen species generation on apoptotic DNA fragmentation induced by indomethacin or flurbiprofen is shown in Fig. 4. Pyrrolidine dithiocarbamate, a potent metal chelator, diphenyleneiodonium chloride, an inhibitor of flavin-containing enzymes such as NADPH oxidase, and *N*-acetyl-L-cysteine, a radical scavenger, suppressed the DNA fragmentation induced by both drugs in a concentration-dependent manner. *N*-Nitro-L-arginine methyl ester did not suppress DNA fragmentation, indicating that endogenous nitric oxide production is not involved in NSAID-induced apoptotic DNA fragmentation. Catalase and superoxide dismutase also did not suppress DNA

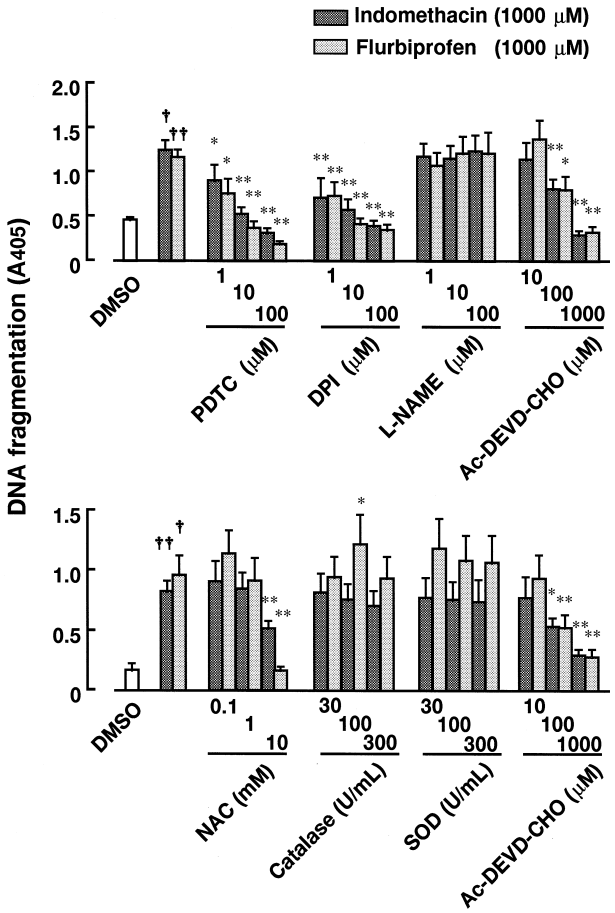

Fig. 4. Effect of antioxidants on NSAID-induced DNA fragmentation. AGS cells were incubated for 6 h with indomethacin or flurbiprofen at a concentration of 1 mM in the presence of the indicated agents acting as inhibitors of reactive oxygen species generation. Cytoplasmic histone-associated DNA fragments were determined using a commercial ELISA kit. The caspase-3 inhibitor Ac-DEVD-CHO was used as a control inhibitor of apoptosis: PDTC, pyrrolidine dithiocarbamate; DPI, diphenyleneiodonium chloride; NAC, *N*-acetyl-L-cysteine; NAME, *N*-nitro-L-arginine methyl ester; SOD, superoxide dismutase. Results are the means ± SE from five independent determinations: $^{*}P < 0.05$, $^{**}P < 0.01$ vs. cells treated with indomethacin or flurbiprofen only (Dunnett method, two-way layout); †$P < 0.05$, ††$P < 0.01$ vs. DMSO controls (paired *t*-test).

fragmentation, indicating that extracellular generation of reactive oxygen species does not participate in the apoptotic process. The caspase-3 inhibitor Ac-DEVD-CHO suppressed DNA fragmentation, and thus successfully acted as a control inhibitor of DNA fragmentation.

3.5. Enhancement of 8-isoprostane release from AGS cells by NSAIDs

The effect of three kinds of NSAIDs on 8-isoprostane release from AGS cells is shown in Fig. 5A. Indomethacin and flurbiprofen, potent inducers of apoptotic DNA fragmentation in AGS cells, enhanced 8-isoprostane release at a concentration of 1 mM. Enhancement by indomethacin was prevented by co-incubation with pyrrolidine dithiocarbamate at 100 μM, but not with caspase-3 inhibitor at 1 mM, indicating that reactive oxygen species are generated before the execution of apoptosis. Mofezolac, with low apoptotic DNA fragmentation inducing activity, had no effect on 8-isoprostane release.

The effect of 8-isoprostane on apoptotic DNA fragmentation in AGS cells is shown in Fig. 5B. 8-Isoprostane hardly induced any fragmentation at concentrations up to 100 pg/ml. This finding indicates that the induction of DNA fragmentation by NSAIDs was not mediated by 8-isoprostane per se, but rather was mediated by reactive oxygen species, which, in turn, participated in generation of 8-isoprostane.

3.6. Effect of indomethacin on the production of reactive oxygen species in AGS cells

The effect of indomethacin on the production of reactive oxygen species in AGS cells, as assessed with lucigenin chemiluminescence, is shown in Fig. 6. Chemiluminescence was significantly enhanced by incubation with indomethacin at 1 mM. This enhancement was prevented by co-incubation with pyrrolidine dithiocarbamate at 100 μM. These results demonstrate the generation of reactive

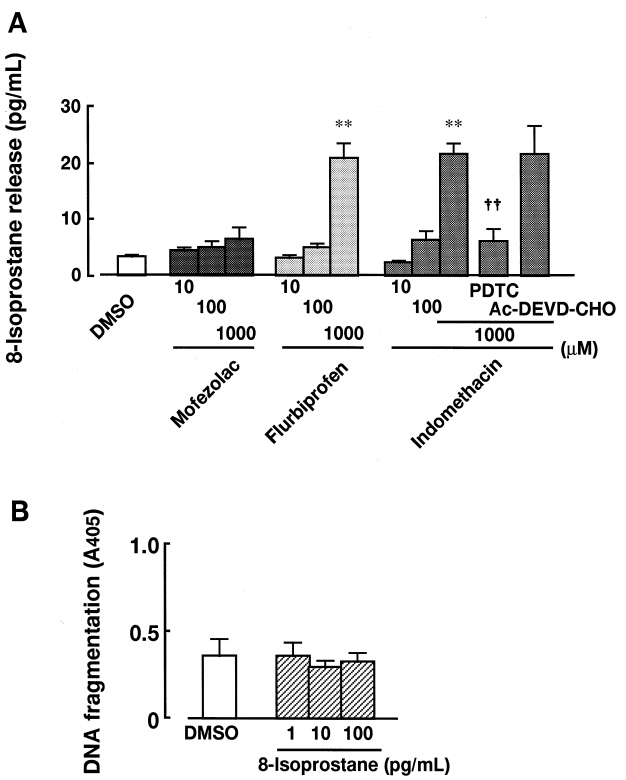

Fig. 5. Effect of NSAIDs on the release of 8-isoprostane. (A) AGS cells were incubated for 6 h with the indicated concentrations of NSAIDs in the presence of PDTC at 100 μM, caspase-3 inhibitor at 1 mM, or none. 8-Isoprostane released into the medium was determined using a commercial ELISA kit. Results are the means ± SE from five independent determinations: $^{**}P < 0.01$ vs. DMSO controls (Dunnett method, two-way layout); ††$P < 0.01$ vs. cells treated with only indomethacin (paired *t*-test). (B) Cells were incubated for 6 h with the indicated concentrations of 8-isoprostane. Cytoplasmic histone-associated DNA fragments were determined using a commercial ELISA kit. Results are the means ± SE from five independent determinations.

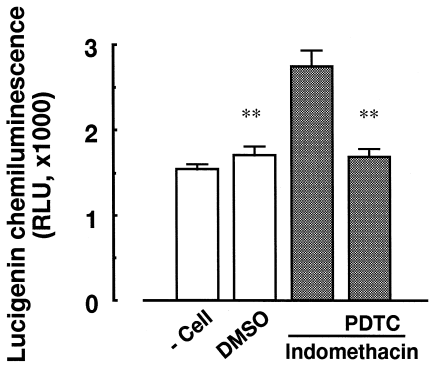

Fig. 6. Effect of indomethacin on the generation of reactive oxygen species. AGS cells were incubated for 6 h with indomethacin at 1 mM in the presence or absence of PDTC at 100 μM. Lucigenin-associated chemiluminescence was measured for 3 min with a luminometer. Results are the means ± SE from five independent determinations: $^{**}P < 0.01$ vs. cells treated with indomethacin only (*t*-test).

oxygen species in cells under incubation with indomethacin.

4. Discussion

In the present study, the participation of reactive oxygen species in the apoptotic effect of NSAIDs was examined in cultured human gastric epithelial cells. DNA fragmentation, a characteristic feature of cells undergoing apoptotic cell death (Carson and Ribeiro, 1993; Williams and Smith, 1993), was significantly induced in AGS cells by incubation with indomethacin, sodium diclofenac, flurbiprofen, zaltoprofen, and etodolac. These apoptotic effects were incubation time- and drug concentration-dependent. These NSAIDs also enhanced mRNA expression for cyclooxygenase-2 in AGS cells. Overexpression of cyclooxygenase-2 mRNA is known to occur in NSAID-induced apoptosis in Rous sarcoma virus-infected chicken embryo fibroblasts and gastric cells (Lu et al., 1995; Kusuhara et al., 1998). Mofezolac had no effect on either DNA fragmentation or cyclooxygenase-2 expression. Despite its potent inhibitory activity against cyclooxygenase-1 (Kusuhara et al., 1997; Goto et al., 1998), the apoptotic effect of this NSAID has also been found to be weak in RGM1 cells, a rat gastric mucosal cell line (Kusuhara et al., 1998). The up-regulation of cyclooxygenase-2 expression in AGS cells was thus a specific indicator of the apoptotic effect of NSAIDs in the present study. In addition, the apoptotic effect of indomethacin was further confirmed by fluorescence staining of cells with annexin V.

Apoptotic DNA fragmentation induced by indomethacin or flurbiprofen in AGS cells was significantly suppressed by various agents acting as inhibitors of reactive oxygen species generation. Among these, pyrrolidine dithiocarbamate, a potent metal chelator, has been shown to act as an anti-oxidant in blocking reactive oxygen species-associated apoptosis although this reagent inhibits many enzymes and its specificity may be low (Verhaegen et al., 1995; Johnson et al., 1996; Orrenius et al., 1996). Diphenyleneiodonium chloride, an inhibitor of flavin-containing enzymes such as NADPH oxidase, has also been used to block production of reactive oxygen species in a variety systems (Polyak et al., 1997; Shimizu et al., 1998). In addition, *N*-acetyl-L-cysteine, a radical scavenger, has been shown to block reactive oxygen species-associated apoptosis (Cossarizza et al., 1995; Johnson et al., 1996; Lafon et al., 1996; Shimizu et al., 1998). The suppressive effect of these three agents on NSAID-induced DNA fragmentation indicates the involvement of reactive oxygen species in the apoptotic process. Further, exogeneously added catalase and superoxide dismutase did not suppress DNA fragmentation, indicating that this generation of reactive oxygen species during NSAIDs treatment is intracellular. Since *N*-nitro-L-arginine methyl ester did not suppress DNA fragmentation, endogenous nitric oxide production does not appear to be required in NSAID-induced apoptosis, but its involvement through peroxynitrite formation cannot be absolutely ruled out. Taken together, these pharmacological observations strongly suggest the intracellular production of reactive oxygen species during apoptosis induced by NSAIDs in gastric cells.

The influence of NSAIDs on cellular reactive oxygen species production was more directly evaluated using 8-isoprostane release and lucigenin chemiluminescence assays. 8-Isoprostane is generated from arachidonic acid by nonenzymatic and free-radical-mediated peroxidation, and its release has been shown to be crucially associated with reactive oxygen species-mediated apoptosis (Morrow and Roberts, 1996; Patel and Gores, 1997). Indomethacin and flurbiprofen, potent inducers of DNA fragmentation in AGS cells, enhanced 8-isoprostane release from the cells. Conversely, mofezolac, which induces only slight fragmentation, hardly affected 8-isoprostane release. In addition, enhancement of 8-isoprostane release by indomethacin was blocked by pyrrolidine dithiocarbamate. The effect of NSAIDs on apoptotic DNA fragmentation thus appears to be closely related to their effect on cellular 8-isoprostane release, although the effect on DNA fragmentation was more sensitively exerted at lower concentrations. In our experiments, 8-isoprostane was found to have no apoptotic effect on DNA fragmentation, indicating that the induction of DNA fragmentation by NSAIDs is not mediated by 8-isoprostane per se, but rather by reactive oxygen species, which participate in the generation of 8-isoprostane. Further, enhancement of 8-isoprostane release by indomethacin was not blocked by a caspase-3 inhibitor, even though this inhibitor clearly suppressed the DNA fragmentation induced by the drug. reactive oxygen species production linked to 8-isoprostane release is thus suggested to be caused before the execution of apoptosis which should be suppressed by the caspase-3 inhibitor.

Finally, investigation of the enhancement of reactive oxygen species production by indomethacin in AGS cells

using a lucigenin chemiluminescence assay showed that chemiluminescence was significantly enhanced by incubation with indomethacin. Moreover, this enhancement was blocked by pyrrolidine dithiocarbamate. These findings demonstrate the generation of reactive oxygen species in AGS cells under incubation with indomethacin.

In conclusion, this study is the first we are aware of to demonstrate the apoptotic effect of NSAIDs in cultured human gastric epithelial cells. This NSAID-induced apoptosis appears to be closely associated with intracellular production of reactive oxygen species. In our experiments, the drugs were used at relatively high concentrations but these are the concentrations to which the gastric mucosa would normally be exposed after oral administration of these drugs to experimental animals and humans. The molecular mechanisms by which NSAIDs induce oxidative stress in gastric cells remains to be elucidated.

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