Effect of a Selective Cyclooxygenase-2 Inhibitor, Nimesulide, on the Growth of Lung Tumors and Their Expression of Cyclooxygenase-2 and Peroxisome Proliferator-Activated Receptor-γ

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ABSTRACT

Purpose: The objectives of this study were to evaluate the effect of a cyclooxygenase (COX)-2 inhibitor, nimesulide, on the growth inhibition of s.c. human lung A549 adenocarcinoma tumors and to assess the effect of nimesulide on the expression of COX-2 and peroxisome proliferator-activated receptor (PPAR)-γ in lung tumors harvested from mice.

Experimental Design: Female nu/nu mice were xenografted with s.c. A549 lung tumors, and 1 day after tumor implantation, the mice were fed with a diet containing nimesulide at 250–1500 ppm doses. Tumor dimensions were monitored twice weekly, and tumor samples isolated from mice were used to determine prostaglandin E2 (PGE2) levels by enzyme immunoassay, expression of COX-2 and PPAR-γ by Western blotting and immunohistochemistry. Furthermore, the induction of apoptosis in tumor specimens was determined by terminal deoxynucleotidyl transferase-mediated nick end labeling staining.

Results: Nimesulide treatment showed a dose-dependent growth-inhibitory effect of A549 tumors with a maximum of 77.7% inhibition at 1500 ppm of nimesulide. Western blotting experiments showed similar expression of COX-2 in both control and nimesulide (250–1500 ppm)-treated mouse tumor tissues. PPAR-γ was found to be overexpressed as a result of 1500 ppm nimesulide treatment and was not detected in tumors from control or 250-1000 ppm nimesulide-treated mice. Nimesulide (1500 ppm) significantly reduced intratumor PGE2 levels (P < 0.001) and induced apoptosis in 25% of tumor cells as compared with control tumors.

Conclusions: Nimesulide (1500 ppm) induced growth inhibition of A549 lung tumors is associated with the reduction of intratumor PGE2 levels but without affecting the expression of COX-2. Nimesulide-induced enhancement of the expression of PPAR-γ may also contribute to its antitumor effect, which needs to be further investigated.

INTRODUCTION

The role of inducible enzyme cyclooxygenase (COX)-2 in the process of carcinogenesis is being actively studied. COX-derived prostaglandins (PGs) have been shown to modulate cell proliferation, apoptosis, angiogenesis, and immune surveillance (1–3). It has been reported that COX-2 is overexpressed in human tumors of various organs such as colon, breast, prostate, esophagus, pancreas, liver, head, neck, and lungs (4–11). Selective inhibitors of COX-2 such as celecoxib and NS-398 reduced the formation of colon, breast, skin, bladder, and lung tumors (12–16) and suppressed the growth of established tumors including colorectal, lung, breast, and prostate in animals (17–21).

The expression of COX-2 in lung tumors may serve as a potential target for the chemoprevention and treatment of lung cancer by COX-2 inhibitors. Nonselective COX inhibitors such as aspirin, indomethacin, and ibuprofen were found to decrease the growth of non-small cell lung cancer (NSCLC) cell lines NCI-H157 and NCI-H1264 in vitro (22). The selective COX-2 inhibitors meloxicam and NS-398 have been shown to have antiproliferative effect against the human lung adenocarcinoma cell line, A549, in vitro (23, 24). Celecoxib (highly selective COX-2 inhibitor) was found to have the greatest potency in a panel of lung cancer cell lines in vitro (25). Both selective and nonselective COX-2 inhibitors, indomethacin and celecoxib, have been shown to be effective in vivo in i.v and s.c. Lewis lung carcinoma tumor models, respectively (18, 26). Hida et al. (27) reported that a selective COX-2 inhibitor, JTE-522, alone and in combination with anticancer drugs, inhibits the growth of a lung cancer cell line in vivo. Chan et al. (28) reported that the metabolite of sulindac (Exisulind) enhances the in vivo antitumor effect of docetaxel in an orthotopic A549 lung tumor model in rats. A Phase II clinical trial, combining celecoxib and two cycles of paclitaxel and carboplatin before surgery for IB-IIIA NSCLC, showed a promising response (29). Similarly, Phase II clinical data on the use of celecoxib along with docetaxel in lung cancer...
patients indicate that prostaglandin E2 (PGE₂) levels were significantly reduced in lung tumor biopsies, thus indicating a role for COX-2 in lung cancer (30).

The mechanism of action of COX-2 inhibitors for their in vitro antiproliferative effect and in vivo growth inhibition has not yet been clearly elucidated. Both COX-dependent and -independent mechanisms have been proposed for the in vitro effects of COX-2 inhibitors against various cancer cell lines (17, 20). Little is known about the mechanism involved in the in vivo antitumor effect of COX-2 inhibitors. Williams et al. (19) have reported that there is no correlation in the dose required for the in vitro and in vivo growth inhibition activity of celecoxib. The authors have proposed that the low levels of COX-2 localized to microvascular endothelial cells and/or stromal fibroblasts may not be detectable on Western blots of whole tumor lysates but may mediate celecoxib-directed therapeutic effect. Recent studies by Nikitakis et al. (31) and Wick et al. (32) indicate the role of peroxisome proliferator activating receptor (PPAR)-γ in the in vitro effects of sulindac sulfide in oral squamous cell carcinomas and NSCLC cell lines. However, the effect of COX-2 inhibitors on the expression of PPAR-γ in tumor xenografts has not been reported thus far.

We have earlier shown (33) that the COX-2 inhibitor nimesulide shows antiproliferative effect against human lung A549 and colon SW 620 cell lines and also enhances the antiproliferative effect of doxorubicin. Hida et al. (34) reported that nimesulide exhibits in vitro growth-inhibitory activity against a variety of lung cancer cell lines. Nimesulide was also found to have a chemopreventive activity against colon, urinary bladder, breast, tongue, and liver carcinogenesis (35–39). The hypothesis of the present study is that nimesulide may inhibit the growth of s.c. A549 human lung tumor xenografts, in nude mice, possibly through induction of apoptosis. We also hypothesize that nimesulide treatment may reduce PGE₂ levels and also enhance the expression of PPAR-γ in tumor tissues. Therefore, the objectives of the present study were to determine: (a) the growth-inhibitory effect of nimesulide (administered p.o. as a drug mixed in the diet) against s.c. A549 lung tumor xenografts in nude mice, possibly through induction of apoptosis. We also hypothesize that nimesulide treatment may reduce PGE₂ levels and also enhance the expression of PPAR-γ in tumor tissues. Therefore, the objectives of the present study were to determine: (a) the growth-inhibitory effect of nimesulide (administered p.o. as a drug mixed in the diet) against s.c. A549 lung tumor xenografts in nude mice; (b) the effect of nimesulide on apoptosis in tumor tissue samples; (c) the effect of nimesulide on COX-2 expression and PGE₂ levels in tumor tissues; and (d) the effect of nimesulide treatment on PPAR-γ expression in tumor tissue samples. Our results indicate that nimesulide shows a dose-dependent growth-inhibitory effect against A549 lung tumors. We observed that nimesulide induces apoptosis, decreases PGE₂ levels in tumor tissues, and has no effect on the expression of COX-2 but enhances the PPAR-γ expression in tumor tissues.

**MATERIALS AND METHODS**

**Cell Culture.** Nimesulide and antibodies against COX-2 and PPAR-γ were purchased from Cayman Chemical Company (Ann Arbor, MI). The PPAR-γ polyclonal antibody was raised in rabbits against a peptide corresponding to amino acids 82–101 of human PPAR-γ1. The antibody reacts with human and murine PPAR-γ1 and PPAR-γ2. The A549 cell line was obtained from American Type Culture Collection (Manassas, VA). All of the tissue culture chemicals and anti-β-actin antibody were obtained from Sigma Chemical Company (St. Louis, MO).

A PGE₂ high-sensitivity colorimetric enzyme immunoassay assay kit was purchased from Assay Designs (Ann Arbor, MI). DeadEnd Colorimetric Apoptosis Detection kit was obtained from Promega Corporation (Madison, WI). All other chemicals were either reagent or tissue culture grade.

**Determination of Antitumor Activity of Nimesulide against A549 Lung Tumor Xenografts.** Female Nu/Nu (six-week-old; Harlan, Indianapolis, IN) were preselected for s.c. A549 tumors, and xenografts were transplanted by s.c. administration of 5 × 10⁵ A549 cells in the right hind leg. One day after tumor implantation, the mice were treated with nimesulide in the diet¹ (250, 500, 1000, or 1500 ppm) until the end of the study (35 days). Untreated mice were used as control. The tumor dimensions were measured twice a week using a linear caliper, and tumor volume was calculated using the equation $V = \pi/6 \times a \times b^2$, where $a$ is the largest diameter and $b$ is the smallest diameter (19). The mice were fed with food and water ad libitum. Drug-mixed diet was replaced every other day and the initial and final weight of the diet was recorded to calculate the intake of drug by mice. All animal handling procedures were in accordance with the approved guidelines of the institution. At least two mice were sacrificed on 21 and 28 days post-tumor implantation to collect plasma and tumor samples for subsequent analysis. At the end of the study, all of the mice were sacrificed, and plasma and tumor tissue samples were collected and stored at −80°C until analyzed. The tumor tissue samples were snap-frozen in liquid nitrogen before their storage at −80°C.

**Determination of Plasma Levels of Nimesulide.** The high performance liquid chromatography method by Khaksar and Udupa (40) was used to quantify the plasma levels of nimesulide with minor modifications. The method has been reported to have a minimum detectable nimesulide concentration of 30 ng/ml in plasma.

**Western Blotting of Tumor Tissues.** Tumor tissues, from control and nimesulide-treated mice were cut into small pieces and homogenized in PBS. The homogenate was centrifuged at top speed for 10 min to sediment the tissue fragments. Next, the lysate buffer (25 mM HEPES, Triton-X 0.1%, 300 mM NaCl, 20 mM β-glycerophosphate, 1.5 mM MgCl₂, 0.2 mM EDTA, and 25 mM DTT) containing protease inhibitors (4 mM sodium orthovanadate, 400 mM sodium fluoride, 20 mM benzamidine, 2 µg/ml leupeptin, 4 µg/ml aprotinin, and 500 µM phenylmethylsulfonyl fluoride) were added. Samples were vortexed, incubated on ice for 30 min, and centrifuged again; and the supernatant was stored at −80°C. For Western blotting, equal amounts of supernatant protein (30 µg) from the control and different nimesulide treatments were denatured by boiling for 5 min in SDS sample buffer, separated by 8% SDS-PAGE, and transferred to polyvinylidene difluoride membranes for immunoblotting. The membranes were blocked with 5% skim milk in Tris-buffered saline with Tween 20 (10 mM Tris-HCl (pH 7.6), 150 mM NaCl, and 0.5% Tween 20) and probed with COX-2 or

¹ Harlan Teklad LM-485 Mouse/Rat sterilizable diet was used to prepare nimesulide diet. The drug-mixed diet was prepared twice a week and autoclaved before use in mice.
PPAR-γ antibodies (1:1000). Bound antibodies were revealed with horseradish peroxidase-conjugated secondary antibodies (1:500) using an enhanced chemiluminescent solution (Pierce, Rockford, IL).

**Immunohistochemistry of Tumor Tissues.** Tumor tissue samples, both with or without treatment, were frozen in cryomatrix and later sectioned in a cryotome (Shandon Cryotome 0620; Thermo Shandon, Pittsburgh, PA). Peroxidase method was used for the immunostaining of COX-2 and PPAR-γ. In brief, the 10-µm cryosections were washed three times with PBS to remove the freezing matrix. Next, the endogenous peroxidase activity was quenched by incubating the slides in 0.3% H₂O₂ solution. After this, the slides were washed again three times with PBS and were incubated with primary antibodies COX-2 or PPAR-γ (1:50) for 1 h at room temperature in a humidified chamber. Location of the primary antibodies was achieved by application of horseradish peroxidase-conjugated secondary antibodies for 30 min at room temperature and diaminobenzidine substrate. The slides were counterstained with hematoxylin. Specific staining for each protein was categorized as either positive or negative based on the presence of brown color staining. Clear staining of the cytoplasm and cell membrane was the criterion for a positive reaction. All of the immunostaining slides were observed with an Olympus BX40 light microscope equipped with computer-controlled digital camera (QiImaging, Burnaby, BC, Canada) and imaging software. Fields of view were captured in different fields taken for analysis as mentioned earlier. Immunostaining slides were observed with an Olympus BX40 microscope, and pictures of its magnified view were captured with Scion Image software (Scion Corporation, Frederick, MD).

**Determination of PGE₂ Levels in Tumor Tissues.** PGE₂ content in tumor tissue samples were determined using the method suggested by Trifan et al. (41) and analyzed using a colorimetric enzyme immunoassay method. Briefly, the tumor tissue samples were homogenized in prostaglandin extraction buffer (70% ethanol and 30% of 0.1 M sodium phosphate (pH 4.0)) and incubated on wet ice for 30 min. The samples were centrifuged, and the supernatant was collected. A known volume of supernatant (typically 250 μl) was dried under nitrogen and resuspended in assay buffer and was analyzed as per the manufacturer’s recommendations (Assay Designs, Ann Arbor, MI).

**Effect of Nimesulide on mRNA Levels of PPAR-γ in A549 Cells.** A549 cells (1×10⁶ in 25-cm² flask) were treated with nimesulide (10, 20, 40, 80, and 160 μg/ml) or vehicle (DMSO in medium) for 48 h, and the total RNA was eluted using the Eppendorf Perfect RNA Mini kit (Brinkman Instruments, Westbury, NY). Reverse transcription was performed with Moloney-murine leukemia virus reverse transcriptase (MuLV-RT; Applied Biosystem, Foster City, CA) according to the manufacturer’s protocol with some modifications. The PCR reaction was performed under hot-start condition (94°C, 2 min) with PPAR-γ (sense, 5′-TCTGGCCACCACTTTGT-3′; antisense, 5′-CTTCACAAGCATGAACTCCA-3′) and β-actin (sense, 5′-GATCATGGTGTAGACCTTTC-3′; antisense, 5′-GTCAGCGAGCTCGTAG-3′) primer pairs and ATAQ DNA polymerase (Applied Biosystem) for 30 cycles of 94°C, 60°C, and 72°C (1 min each). Statistical analysis was performed using GraphPad PRISM version 2.0 software (San Diego, CA).

**Results**

**There Is a Nimesulide Dose-Dependent Inhibition of Tumor Growth.** Fig. 1 shows the tumor volume profiles after the administration of mice with 250-1500 ppm dose of nimesulide administered in diet. It is evident from Fig. 1 that nimesulide treatment shows a dose-dependent inhibition of s.c. A549 lung tumor growth. At the end of the study period (35 days), there was 31.1 ± 11.0, 47.3 ± 9.9, 67.8 ± 8.9, and 77.7 ± 6.3% (expressed as mean ± SE) inhibition of tumor growth after the administration of nimesulide at 250, 500, 1000, and 1500 ppm dose, respectively, and the tumor volumes at 500-1500 ppm dose were significantly smaller than in the control mice (P < 0.007). The estimated dose of nimesulide (mg/kg/day) based on the consumption of drug-mixed diet was found to be 38, 84, 166, and 222 mg/kg/day for 250, 500, 1000, and 1500 ppm doses, respectively.² The mean nimesulide plasma levels in the period between 21 and 35 days post-tumor implantation were found to

²Dose = [Initial weight of drug-mixed diet − Final weight of drug-mixed diet] × mg drug per g diet × 1000 g divided by [No. of days × No. of mice in the cage × Body-weight of mice (g)]. Actual intake of drug by mice was less than the calculated dose because we did not account for wastage of drug-mixed diet in the bedding in this method.
Antitumor Activity of Nimesulide

COX-2 Protein Expression Is Unchanged Whereas PPAR-γ Expression Is Significantly Enhanced in Nimesulide-Treated Tumors. Different doses of nimesulide in the diet did not have any effect on the expression of COX-2 protein in the tumors. As shown in Fig. 2A, tumor tissues harvested from mice treated with 250 ppm, 500 ppm, 1000 ppm, and 1500 ppm nimesulide showed similar levels of COX-2 expression compared with the control (without any treatment) after 35 days, as detected by Western blot analysis. Immunohistochemical localization of COX-2 in control and 1500 ppm nimesulide-treated tumor tissue sections showed no difference in expression of the COX-2 protein (Fig. 3, A and B), thus reflecting the same pattern as observed by the immunoblotting experiments as described above. Furthermore, the COX-2 staining was predominantly localized to vascular regions of tumor expression at 40 and 80 μg/ml of nimesulide, respectively, as compared with the vehicle-treated control A549 cells (Fig. 2B).

Nimesulide (1500 ppm) Treatment Induced Tumor Necrosis as Evidenced from H&E Staining. Fig. 4 shows H&E-stained sections of tumor tissue samples from untreated control (A) and 1500 ppm nimesulide-treated (B) mice for 34 days. It is evident from Fig. 4A that the tumor section from control mice shows finely granular cytoplasm with evenly dispersed chromatin, typical of A549 tumor xenografts (43). However, nimesulide 1500 ppm treatment shows sparsely dispersed chromatin with necrotic tissue (Fig. 4B). This phenomenon was also observed with 1000 ppm of nimesulide-treated sections (data not shown).

Nimesulide Treatment Demonstrated Evidence of A549 Xenograft Tumor Cell Death, Detected by DNA Nick End Labeling Reaction. To determine whether tumor growth inhibition by 1500 ppm nimesulide treatment is attributable to induction of cell death, we evaluated the increase in percentage of apoptotic nuclei in A549 xenograft tumor tissue sections with nimesulide treatment over the control. Fig. 5 shows the results

Fig. 2 A. Western blotting of tumor tissue lysates for cyclooxygenase (COX)-2, peroxisome proliferator-activated receptor (PPAR)-γ, and β-actin. Lanes 1, 2, 3, 4, and 5, tumors obtained from control, 250, 500, 1000, and 1500 ppm nimesulide-treated mice, respectively. The tumors were harvested 35 days post-tumor implantation, and lysates were prepared as described in the “Materials and Methods” section. Thirty μg of protein were electrophoresed in each lane. B, reverse transcription (RT)-PCR of PPAR-γ in A549 cells. Lanes 1, 2, 3, 4, 5, and 6, vehicle, 10, 20, 40, 80, and 160 μg/ml nimesulide treatments, respectively. The cells were treated with vehicle or the indicated concentrations of nimesulide for 48 h and processed for RT-PCR as described in the “Materials and Methods” section. The PCR product sizes for PPAR-γ (360 bp) and β-actin (390 bp) were determined using a 100-2680-bp DNA ladder (Fisher Scientific Co., Atlanta, GA).
of terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) staining of control and 1500 ppm nimesulide-treated sections. It is evident from the fragmented DNA labeling experiment that, whereas the control tumor tissue showed no apoptotic cells, nimesulide treatment resulted in apoptosis in at least 25% of the cells in the tumor tissues. Tumor tissues obtained from mice treated with 250 or 500 ppm nimesulide have shown negligible apoptosis (<5%), whereas about 10% apoptosis was seen in tumor tissues harvested from mice treated with 1000 ppm of nimesulide.

**Fig. 3** Immunohistochemical staining for cyclooxygenase (COX)-2 (A and B) and peroxisome proliferator-activated receptor (PPAR)-γ (C and D) in tumors from control (A and C) and nimesulide 1500 ppm (B and D)-treated mice. The tumors were collected 35 days post-tumor implantation, as has been studied for Western blotting in Fig. 2. ×10. *Insets to the figures.* ×40. It is evident from A and B that COX-2 staining is mostly localized in the endothelial and stromal cells. It is evident from C that PPAR-γ staining is predominantly cytoplasmic in control tumor tissue. Nimesulide-treated tumor tissue shows both cytoplasmic and nuclear staining for PPAR-γ (D). The nuclear staining for PPAR-γ in nimesulide-treated tissues is more, as compared with the control.

**Fig. 4** H&E staining of tumor tissues from control (A) and 1500 ppm nimesulide-treated (B) mice. The tumors were harvested 35 days post-tumor implantation. ×40.
Nimesulide Treatment Significantly Reduced PGE$_2$ Level in the Tumor Tissues. The involvement of PGE$_2$ in the nimesulide-treated A549 xenograft tumor regression was addressed next. The tumors obtained from control mice expressed very high levels (326.7 ± 27.5 ng/g) of PGE$_2$, whereas the level in tumors from mice treated with 1500 ppm nimesulide was significantly ($P < 0.001$) reduced to 75.0 ± 11.5 ng/g (Fig. 6). This indicates a 77.0% reduction in PGE$_2$ level in the nimesulide-treated tumors as compared with the untreated controls. Intratumor PGE$_2$ levels were reduced by 4, 15, and 30% in mice treated with nimesulide at 250, 500, and 1000 ppm doses, respectively.

DISCUSSION

Extensive studies have shown that nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the colon tumor xenografts in mice (17–19, 41, 44, 45). Limited studies have been reported thus far on the use of human lung tumor xenograft models for evaluation of antitumor properties of COX inhibitors (27, 32). In this study, we used a s.c. A549 lung tumor xenograft model and demonstrated that oral administration of nimesulide significantly inhibits the growth of A549 tumors in nude mice (Fig. 1). In our study, nimesulide at 500-1500 ppm dose range was found to produce a statistically significant inhibition of A549 tumor, with the maximum inhibition observed at 1500 ppm dose. The nimesulide plasma levels observed in mice with 1500 ppm dose (9.3 ± 2.6 M) were comparable with the steady-state plasma levels in humans (10 ± 2 M) after the administration of 100 mg nimesulide tablet twice daily for 7 days (46), thus indicating that it may be possible to use nimesulide at a clinically acceptable dose range. Recently, Hida et al. (27) showed that oral administration of a selective COX-2 inhibitor, JTE-522, at a dose of 5000 ppm (100 mg/kg/day) produces a 31% inhibition of s.c. ACC LC-31 lung tumor xenografts in mice. Wick et al. (32) have shown that i.p administration of sulindac sulfide inhibits the growth of s.c. A549 tumors in mice. Furthermore, celecoxib was found to inhibit the Lewis lung carcinoma tumors in a dose-dependent manner (18). The significant growth inhibition by nimesulide as observed in our study along with the other recently published reports (18, 27, 32) indicate the potential of COX inhibitors in the treatment and or prevention of lung cancer.

Studies have shown that human tumor xenografts in nude mice express COX-2 in only angiogenic blood vessels but not in tumor epithelial cells, irrespective of COX-2 expression status in the tumor cell lines (18, 45). In contrast to this, others have shown that human colon or gastric tumor xenografts express COX-2 in mouse tumors (17, 47). In the present study, COX-2 was expressed in A549 xenografts grown s.c. in nude mice and retained its expression as observed in its cultured cells. Our Western blotting experiments demonstrate that nimesulide at different doses from 250 ppm to 1500 ppm failed to inhibit COX-2 expression in s.c. A549 tumor tissues (Fig. 2A). At the highest dose of nimesulide (1500 ppm) used in our study, intratumor PGE$_2$ levels were reduced by 77.0% (Fig. 6), in comparison with the control group. Likewise, treatment of an immunogenic sarcoma (FSA) in mice with a selective COX-2 inhibitor, SC'-236 was shown to have no effect on the COX-2 expression pattern in treated and untreated control tumors.
**in vivo.** However, the drug treatment produced a significant reduction in tumor PGE2 levels (48). In a recent study, celecoxib was found to inhibit the growth of human head and neck squamous cell carcinoma xenograft without altering the expression of COX-1 or COX-2 in tumor tissues. Furthermore, celecoxib treatment significantly reduced the intratumor PGE2 levels (49). Similarly, the selective COX-2 inhibitor, JTE-522, was shown to have no effect on the COX-2 expression but significantly reduced PGE2 levels in a rat esophageal tumorigenesis model (50). It has been shown that the expression of COX-2 in tumor neovascular and stromal cells support tumor growth by angiogenesis via COX-2-derived PGs (45, 51). The inhibition of COX-2 activity, as demonstrated by the reduction in the intratumor PGE2 levels in our study, may contribute to the reduction in the tumor growth by inhibition of angiogenesis and induction of apoptosis of stromal cells (45, 49).

PPAR-γ is a ligand-activated transcription factor belonging to the steroid receptor family. The binding of agonist ligands to the receptor results in changes at the expression level of mRNA encoded by PPAR target genes. The PPARs are activated by a large number of structurally diverse compounds including prostanooids, long-chain fatty acids, the fibrate class of hypolipidemic drugs, leukotriene antagonists, and antidiabetic thiazolidinediones. Although chemically diverse, these compounds share certain structural features including a lipophilic backbone and an acid moiety, usually a carboxylate. Several NSAIDs share some of these broad structural features and have been shown to activate PPARs (52). Furthermore, differential response of PPAR activation in rat hepatoma (H4IEC3) cells by some NSAIDs has also been reported (53). Wick et al. (32) have demonstrated that sulindac sulfide increases the PPAR-γ promoter activity in A549 lung tumor cells. However, recent data by Nixon et al. (54) indicate that nonselective COX inhibitors are potent activators of PPAR-γ, whereas specific COX-2 inhibitors like NS-398 and celecoxib have little or no ability to activate PPAR-γ in HCT-116 human colorectal carcinoma cell lines. The 15-lipooxygenase metabolite, 15(S)-HETE, has also been shown to activate PPAR-γ in colon and prostate tumor cell lines (54, 55). It has also been shown that 15(S)-HETE binds to PPAR-γ nuclear receptors and induces apoptosis in A549 cells (56). To investigate the possible involvement of PPAR-γ in the antitumor effect of nimesulide, we studied its expression in tumor tissues obtained from control and nimesulide (250–1500 ppm)-treated mice by Western blotting. We observed an enhanced PPAR-γ protein expression at 1500 ppm nimesulide dose (Fig. 2B). This was further confirmed by immunohistochemistry, which showed an intense staining for PPAR-γ in tumor tissue sections from 1500 ppm nimesulide-treated group (Fig. 3D) as compared with the negligible expression in control tumor (Fig. 3C). We also showed that the nimesulide dose dependently increases the mRNA levels of PPAR-γ in A549 cells (Fig. 2B) with a maximum expression at 80 μg/ml. These results are supported by the findings of Nikitakis et al. (31) in which the treatment of oral squamous cell carcinoma cell lines with sulindac sulfide has been shown to increase the mRNA and protein expression of PPAR-γ. Furthermore, it has been reported that PPAR-γ expression was negligible in human lung adenocarcinoma, HTB-175, whereas normal lung fibroblast cells lines have been shown to express PPAR-γ. Similarly, nontumor tissues expressed PPAR-γ, whereas significantly lower expression has been observed in tumor tissues of NSCLC patients (57). Furthermore, overexpression of PPAR-γ in H2122 lung cancer cell lines resulted in the failure of soft agar colony formation even in the absence of NSAID (32), thus indicating that the nimesulide antitumor effect as observed in our study may be mediated via the enhanced expression of PPAR-γ. However, it remains to be seen whether selective COX-2 inhibitors such as nimesulide and celecoxib have any effect on PPAR-γ activation in NSCLC cell lines. On the basis of the recent literature, it may be speculated that nimesulide-mediated intratumor PGE2 reduction may lead to the accumulation of arachidonic acid [as proposed by Chan et al. (58)], which may be involved in the PPAR activation (59). Alternatively, lipoxygenase metabolites of arachidonic acid may also contribute to the PPAR-γ activation (54–56). However, these speculations need to be supported by experimental data.

Histological studies clearly demonstrate that the control tumor manifested the general features of distinct cellular integrity with granular cytoplasm typical of A549 tumor xenografts, whereas the treated tumors showed clear signs of cellular necrosis (Fig. 4). There was a loss of distinct cellular organization with sparse cytoplasm and pleomorphic nucleus. This indicates an antiproliferative effect of nimesulide on the A549 tumor xenografts, which was further confirmed by study of apoptosis using the TUNEL method (Fig. 5). Apoptosis has been implicated for the in vitro antiproliferative effect of various NSAIDs including nimesulide against lung cancer cell lines (24, 34), and we demonstrate in our study that nimesulide treatment induces apoptosis in lung tumor xenografts, similar to that of other COX-2 inhibitors in human colon and gastric tumor xenografts (45, 47).

In conclusion, it should be noted that in the present study, nimesulide has been shown to inhibit tumor progression, decrease PGE2 levels, and induce apoptosis in s.c. A549 lung tumors. Furthermore, nimesulide treatment has been found to have no effect on the expression of COX-2 but, at the 1500 ppm dose, enhanced the expression of PPAR-γ. However, additional studies are needed to understand the molecular pathways involved in the nimesulide-induced PPAR-γ expression and its contribution to tumor growth inhibition.

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