Cyclooxygenase-2 Inhibitor Induces Apoptosis and Enhances Cytotoxicity of Various Anticancer Agents in Non-Small Cell Lung Cancer Cell Lines[1]

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ABSTRACT

In recent years, a combination of two demographic phenomena, an increase in the number of older people in the population and an increase in the incidence of lung cancer with age, has made it mandatory to develop therapeutic modalities with less toxicity for the treatment of inoperable elderly patients with lung cancer. Our study shows that a cyclooxygenase (COX)-2 inhibitor, nimesulide, can inhibit proliferation of non-small cell lung cancer cell lines *in vitro* in a dose-dependent manner, in part by inducing apoptosis even at clinically achievable low concentrations. Our observations also suggest that the responsiveness of non-small cell lung cancer to COX-2 inhibitors does not require the presence of wild-type p53, but may be influenced by the degree of COX-2 expression. In addition, we found that nimesulide, when used in combination at clinically achievable concentrations, reduced the IC_{50} values of various anticancer agents by up to 77%, although the level of reduction varied considerably. Because our previous studies have indicated a significantly increased COX-2 expression in up to 70% of adenocarcinoma cases, the present findings are of great clinical interest. In conjunction with the recent development of next generation, highly selective COX-2 inhibitors, they can be expected to lead to even greater efficacy of their use as adjuncts to various anticancer agents for the treatment of high-risk patients without compromising their quality of life.

INTRODUCTION

Despite unceasing efforts of clinicians in their fight against lung cancer, it remains one of the principal causes of cancer-related deaths (1). Although combination chemotherapy constitutes a major part of the treatment program for patients with inoperable lung cancer, improvements in treatment efficacy, even with newly developed anticancer agents, have been unsatisfactory (2). In recent years, a combination of two demographic phenomena, an increase in the number of older people in the population and an increase in the incidence of lung cancer with age, has made it mandatory to develop therapeutic modalities with less toxicity for the treatment of inoperable elderly patients with lung cancer.

Accumulating evidence suggests that an increase in the expression of COX[3]-2, a key inducible enzyme involved in the production of prostaglandins and other eicosanoids, may play a significant role in carcinogenesis in addition to its well-known role in inflammatory reactions (3–8). Whereas previous studies have been largely confined to colorectal tumorigenesis, we recently reported that a significantly increased expression of COX-2 is also frequently seen in a specific type of lung cancer (*i.e.*, adenocarcinoma), in contrast to the scattered weak reactivity seen in normal peripheral airway epithelial cells (9). In addition, we have shown that such an increase in COX-2 expression may be a clinically significant prognostic factor for patients undergoing surgical resection of early-stage adenocarcinomas (10). Although previously available nonsteroidal anti-inflammatory drugs were mostly nonselective and inhibited both constitutive COX-1 and inducible COX-2, a sulfonanilide compound, nimesulide, has been proven to selectively inhibit COX-2 (11, 12). Nimesulide was also found to be well tolerated by adult, elderly, and pediatric patients in both clinical trials and large postmarketing surveillance studies (13).

In this study, we examined whether nimesulide can inhibit the proliferation of NSCLC cells and whether sensitivity to nimesulide is related to COX-2 expression levels and the *p53* gene status. We also investigated whether the adjunct use of nimesulide could enhance the efficacy of anticancer agents or irradiation.

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[3] The abbreviations used are: COX, cyclooxygenase; NSCLC, non-small cell lung cancer; SM-5887, amrubicin; VP-16, etoposide; CDDP, cisplatin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling.

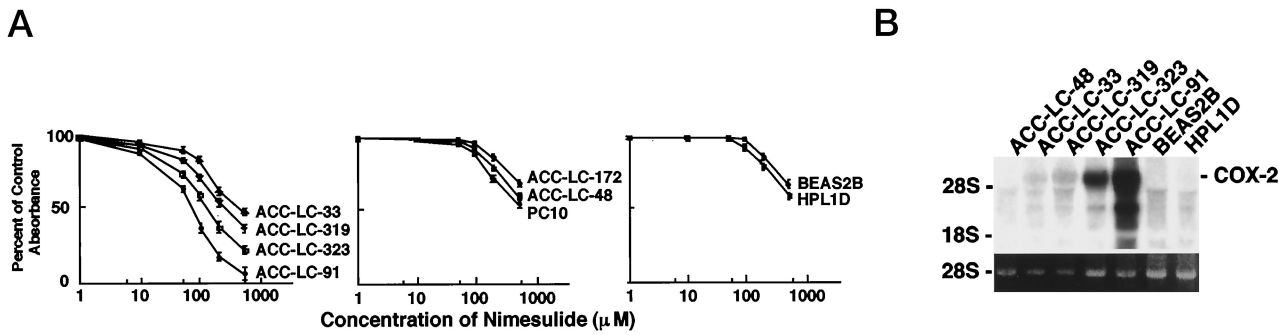

Fig. 1 Sensitivity to a COX-2 inhibitor, nimesulide, measured by MTT assay (*A*) and in relation to COX-2 expression (*B*). *A*, dose-dependent inhibition of the proliferation of NSCLC lines in response to increasing amounts of nimesulide. ACC-LC-33, -319, -323, and -91 as well as PC-10 are NSCLC cell lines, ACC-LC-172 and -48 are SCLC cell lines, and BEAS2B and HPL1D are normal lung epithelial cell lines. Each data point represents the mean of quadruplicate determinations (*bars*, SE) in a representative experiment. Similar results were obtained in four independent experiments. *B*, Northern blot analysis of COX-2 expression using 10 μg of total RNA. A substantial association between sensitivity to nimesulide and COX-2 expression levels is observed. 28S rRNAs, which are visualized by ethidium bromide staining, show loading of similar amounts of RNA in each *lane*.

MATERIALS AND METHODS

Cell Lines. The four NSCLC cell lines (ACC-LC-33, -91, -319, and -323) and two SCLC cell lines (ACC-LC-48 and -172) were established in our laboratories at Aichi Cancer Center (14) and have been maintained in RPMI 1640 supplemented with streptomycin (100 μg/ml), penicillin (100 units/ml), 2 mM glutamine, and 5% FCS. ACC-LC-323 and -319 cells are histologically associated with adenocarcinoma, ACC-LC-91 and -33 cells with large cell carcinoma, and PC-10 cells with squamous cell carcinoma (a generous gift from Dr. Yoshihiro Hayata, Tokyo Medical University, Tokyo, Japan). HPL1D, a human epithelial cell line derived from normal peripheral lung, was also established in our laboratories (15), whereas BEAS2B, a human bronchial epithelial cell line, was kindly donated by Dr. Curtis C. Harris (National Cancer Institute, Bethesda, MD; Ref. 16).

Agents. Nimesulide was provided by Hisamitsu Pharmaceutical Co. (Tosu, Japan). SM-5887–13-OH (the active substance of SM-5887 *in vivo*) was provided by Sumitomo Pharmaceutical Co. (Osaka, Japan); SN-38 (active substance of CPT-11 *in vivo*) by Daiichi Pharmaceutical Co. (Tokyo, Japan); CDDP and VP-16 by Bristol Myers Squibb Co. (Tokyo, Japan); and taxotere by Chugai Pharmaceutical Co. (Tokyo, Japan). Nimesulide and SN-38 were dissolved in DMSO, and SM-5887–13-OH was resolved in water and taxotere in 0.9% saline. DMSO was present in all experiments at a final concentration of 0.5%.

MTT Assay for Chemosensitivity and Radiation Sensitivity. For the evaluation of chemosensitivity, a MTT assay was performed using the Cell Titer 96 kit (Promega Corp., Madison, WI). Briefly, cells were plated in 96-well plates and exposed continuously for 4 days to a range of concentrations of nimesulide and/or anticancer agents. At least three independent experiments were carried out in quadruplicate. Radiation sensitivity was also measured with the MTT assay subsequent to irradiation with the MBR-1520R experimental irradiation apparatus (Hitachi Ltd., Hitachi, Japan), as described previously (17).

Northern Blot Analysis. Extraction of RNA from cell lines and Northern blotting using 10 μg of total RNA were conducted according to standard procedures. A human COX-2 cDNA probe was generated by PCR with the aid of a sense primer, 5′-TTCAAATGAGATTGTGGGAAAATTGCT, and an antisense primer, 5′-AGATCATCTCTGCCTGAGTATCTT (18).

Molecular Analyses of the Status of the *p53* Gene. Reverse transcription-PCR-single-strand conformational polymorphism and sequencing analyses were performed to search for *p53* mutations, as described previously (19, 20).

Detection of Apoptotic Cell Death. Nucleosomal-length DNAs were extracted essentially according to the method described by Hockenbery *et al.* (21). In brief, after a 48-h incubation of 1×10^7 cells with nimesulide, they were incubated in a lysis buffer containing 0.5% Triton X-100 on a rotator for 20 min at 4°C. After centrifugation at 8000 × *g* for 15 min, soluble DNAs were recovered from the supernatants by phenol/chloroform extraction and ethanol precipitation. These DNAs were then incubated with 20 μg/ml RNase A at 37°C for 30 min, and each preparation in its entirety was subjected to electrophoresis on a 1.5% agarose gel containing ethidium bromide. As an independent method to detect apoptotic cell death, the TUNEL method was used by using an *in situ* cell death detection kit (Boehringer Mannheim Gmbh, Mannheim, Germany).

Western Blot Analysis. Anti-bcl-2 and bax antibodies were purchased from Medical and Biological Laboratories Co. (Nagoya, Japan). Cells were lysed in Laemmli's sample buffer, and 10 μg of the solubilized proteins were electrophoresed on 15% SDS-polyacrylamide gels and transferred to an Immobilon-P transfer membrane (Millipore Corporation, Bedford, MA). The filters were first incubated with the primary antibodies and then with horseradish peroxidase-conjugated secondary antibody (Bio-Rad, Hercules, CA) and visualized with the aid of the enhanced chemiluminescence system (Amersham, Buckinghamshire, United Kingdom).

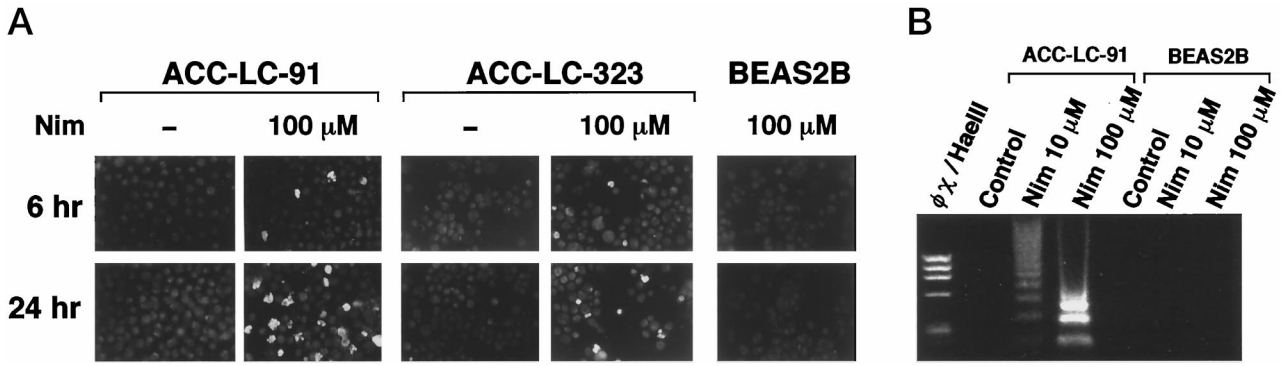

Fig. 2 Detection of apoptosis induced by incubation with nimesulide. *A*, detection of apoptosis with the TUNEL method. TUNEL-positive ACC-LC-91 cells are detectable even 6 h after the initiation of nimesulide treatment at 100 μM, whereas a significant increase in the proportion of TUNEL-positive cells is evident at 24 h. Similarly, induction of apoptosis can be seen in nimesulide-treated ACC-LC-323 cells, whereas virtually no effects are observed in BEAS2B cells. *B*, detection of DNA fragmentation. DNA fragmentation, a hallmark of apoptosis, is evident in ACC-LC-91 cells but not in BEAS2B cells, both of which were treated with nimesulide at concentrations of 10 μM and 100 μM for 48 h. Similar results were obtained in three independent experiments.

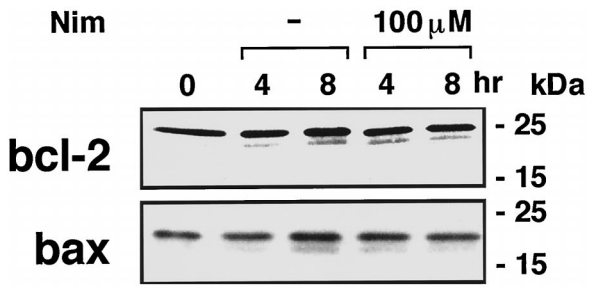

Fig. 3 Western blot analysis of bcl-2 and bax in relation to nimesulide treatment. Expression levels of bcl-2 and bax in ACC-LC-91 are not changed by the treatment with nimesulide at 100 μM for up to 8 h.

RESULTS AND DISCUSSION

Sensitivity to Nimesulide in Relation to COX-2 Expression and p53 Status. The effects of the COX-2 inhibitor nimesulide on the growth of the SCLC and NSCLC cell lines, as well as of normal lung epithelial cell lines, were examined with the MTT assay. We found that nimesulide could induce dose-dependent inhibition of the proliferation of lung cancer cell lines (Fig. 1*A*). ACC-LC-91 cells were the most sensitive to nimesulide, followed by ACC-LC-323, -319, and -33 cells, in that order, whereas ACC-LC-172, ACC-LC-48, and PC-10 cells were rather resistant to nimesulide. In marked contrast, normal lung epithelial cell lines BEAS2B and HPL1D did not show significant growth inhibition even with 100 μM nimesulide. The maximum plasma concentration of nimesulide in normal human has been reported to be about 10 mg/l or 30 μM after oral administration of 200 mg to healthy adult volunteers (13). Thus, our finding indicates that NSCLC cell lines *in vitro* are sensitive to nimesulide and considerable inhibition can be achieved within clinically achievable concentrations (up to 30 μM), which seem to be lower than the maximum tolerated dose (22).

We also investigated whether the antiproliferative effect of this compound could be accounted for, in part, by its effect on the rate of cell death. DNA fragmentation, a biochemical hallmark of apoptosis, was observed in ACC-LC-91 and -323 cells as a result of treatment with nimesulide at doses of 10 μM and 100 μM but not with DMSO, a solvent of nimesulide (Fig. 2*B*, data not shown for ACC-LC-323 cells). The TUNEL method was also used as an independent technique to visualize apoptotic cell death of ACC-LC-91 and -323 cells and showed TUNEL-positive cells as early as 6 h after the initiation of nimesulide treatment (Fig. 2*A*). After a 24-h incubation in the presence of nimesulide, 30% of ACC-LC-91 cells and 12% of ACC-LC-323 cells had become apoptotic. In marked contrast, no significant increase of apoptosis was detected in BEAS2B cells by either method (Fig. 2, *A* and *B*). These findings indicate that one of the mechanisms responsible for nimesulide-induced cell death in lung cancer cells is apoptosis.

We next investigated whether sensitivity to nimesulide may be associated with COX-2 expression. Northern blot analysis showed that COX-2 expression was highest in ACC-LC-91 cells and relatively high in ACC-LC-323 cells, whereas ACC-LC-319 and -33 cells showed modest to weak expression of COX-2 (Fig. 1*B*). In contrast, ACC-LC-48, ACC-LC-172, and PC-10 cells, as well as the normal lung epithelial cell lines HPL1D and BEAS2B, expressed only negligible amounts of COX-2 mRNA. Comparisons of COX-2 expression with sensitivity to nimesulide, thus, suggest a substantial association between COX-2 expression level and sensitivity to nimesulide.

Reverse transcription-PCR-single-strand conformational polymorphism analysis was also used to determine whether sensitivity to nimesulide may be related to the status of the *p53* gene. A missense mutation (Tyr to Cys) was detected at 126 in ACC-LC-172 cells, Gly to Cys at 245 in PC-10 cells, Arg to Leu at 248 in ACC-LC-323 cells, Arg to Ser at 249 in ACC-LC-48 cells, and Val to Leu at codon 274 in ACC-LC-91 cells. Frameshift mutations were identified in ACC-LC-319 and ACC-LC-33 cells (*i.e.*, the former carried a combined 1-bp deletion and a missense mutation occurring at codon 72, while the latter had a 25-bp deletion harboring codons 260–268). These findings indicate that nimesulide-induced apoptosis can be elicited in the absence of wild-type p53.

Table 1 Chemosensitivity to five anticancer agents used in combination with nimesulide

Cell line	SM-5887-13-OH		SN-38		Taxotere		VP-16		CDDP	
	IC_{50} (μg/ml)	RR[a]	IC_{50} (μg/ml)	RR	IC_{50} (μg/ml)	RR	IC_{50} (μg/ml)	RR	IC_{50} (μg/ml)	RR
NSCLC cell lines										
ACC-LC-91	0.015 ± 0.003[b]	1.00	0.0065 ± 0.0010	1.00	0.0032 ± 0.0004	1.00	0.21 ± 0.03	1.00	2.51 ± 0.42	1.00
ACC-LC-91/NIM 10[c]	0.008 ± 0.002	0.53	0.0039 ± 0.0008	0.60	0.0025 ± 0.0003	0.78	0.17 ± 0.02	0.80	2.11 ± 0.26	0.84
ACC-LC-91/NIM 30[c]	0.006 ± 0.002	0.40	0.0015 ± 0.0003	0.23	0.0015 ± 0.0002	0.46	0.14 ± 0.02	0.66	1.90 ± 0.23	0.75
ACC-LC-323	0.025 ± 0.004	1.00	0.0055 ± 0.0008	1.00	0.0028 ± 0.0002	1.00	0.24 ± 0.02	1.00	0.45 ± 0.06	1.00
ACC-LC-323/NIM 10	0.023 ± 0.003	0.92	0.0050 ± 0.0007	0.90	0.0022 ± 0.0003	0.78	0.21 ± 0.03	0.87	0.37 ± 0.04	0.82
ACC-LC-323/NIM 30	0.021 ± 0.003	0.84	0.0043 ± 0.0006	0.78	0.0017 ± 0.0003	0.60	0.20 ± 0.02	0.83	0.35 ± 0.03	0.77
ACC-LC-319	0.038 ± 0.005	1.00	0.0061 ± 0.0009	1.00	0.0060 ± 0.0009	1.00	0.50 ± 0.09	1.00	2.10 ± 0.33	1.00
ACC-LC-319/NIM 10	0.034 ± 0.004	0.89	0.0055 ± 0.0008	0.90	0.0051 ± 0.0008	0.85	0.45 ± 0.08	0.90	2.01 ± 0.28	0.95
ACC-LC-319/NIM 30	0.028 ± 0.004	0.73	0.0049 ± 0.0009	0.80	0.0042 ± 0.0009	0.70	0.43 ± 0.09	0.86	1.89 ± 0.24	0.90
ACC-LC-33	0.080 ± 0.010	1.00	0.0091 ± 0.0010	1.00	0.0082 ± 0.0009	1.00	0.82 ± 0.11	1.00	2.88 ± 0.38	1.00
ACC-LC-33/NIM 10	0.078 ± 0.009	0.97	0.0088 ± 0.0009	0.96	0.0073 ± 0.0008	0.89	0.80 ± 0.10	0.97	2.68 ± 0.35	0.93
ACC-LC-33/NIM 30	0.064 ± 0.009	0.80	0.0078 ± 0.0009	0.85	0.0070 ± 0.0009	0.85	0.73 ± 0.09	0.89	2.43 ± 0.40	0.84
Normal lung epithelial cell lines										
BEAS2B	0.024 ± 0.003	1.00	0.0061 ± 0.0007	1.00	0.0062 ± 0.0006	1.00	0.31 ± 0.02	1.00	1.31 ± 0.22	1.00
BEAS2B/NIM 10	0.023 ± 0.004	0.95	0.0064 ± 0.0005	1.04	0.0070 ± 0.0008	1.12	0.32 ± 0.03	1.03	1.52 ± 0.34	1.16
BEAS2B/NIM 30	0.025 ± 0.002	1.04	0.0064 ± 0.0006	1.04	0.0064 ± 0.0008	1.03	0.33 ± 0.02	1.06	1.50 ± 0.26	1.14
HPL1D	0.042 ± 0.005	1.00	0.0110 ± 0.0010	1.00	0.0092 ± 0.0009	1.00	0.64 ± 0.08	1.00	1.73 ± 0.12	1.00
HPL1D/NIM 10	0.040 ± 0.006	0.95	0.0099 ± 0.0008	0.90	0.0088 ± 0.0011	0.95	0.60 ± 0.07	0.93	1.71 ± 0.21	0.98
HPL1D/NIM 30	0.040 ± 0.007	0.95	0.0098 ± 0.0007	0.89	0.0090 ± 0.0010	0.97	0.59 ± 0.10	0.92	1.92 ± 0.10	1.10

[a] RR, relative resistance.
[b] Mean ± SD of the results obtained in three independent experiments.
[c] NIM 10 and NIM 30, simultaneous exposure to 10 μM and 30 μM nimesulide, respectively. As a single agent, nimesulide yielded 15% growth inhibition at 10 μM and 28% at 30 μM in ACC-LC-91 cells, 12% and 23% in ACC-LC-323 cells, 8% and 13% in ACC-LC-319 cells, 6% and 10% in ACC-LC-33 cells, 0% and 0% in BEAS2B cells, and 0% and 1% in HPL1D cells (see Fig. 1).

To gain an insight into the potential mechanism of COX-2-induced apoptosis in lung cancer cells, we performed Western blot analysis of the apoptosis-related proteins, including bcl-2 and bax in ACC-LC-91 cells, because reduced bcl-2 protein expression has been suggested to be involved in the COX-2 inhibitor-induced apoptosis of prostate cancer cells (23). In our case, however, neither bcl-2 nor bax protein showed any significant changes in expression levels both in the presence and in the absence of nimesulide (Fig. 3).

Effects of Adjunct Use of Nimesulide on Sensitivity to Anticancer Agents or Irradiation. We further investigated whether adjunct use of nimesulide at clinically achievable concentrations can enhance chemosensitivity of NSCLC cells *in vitro*. The effect of nimesulide at 0, 10, or 30 μM was evaluated in combination with SM-5887, CPT-11, taxotere, VP-16, or CDDP, which are known to be effective in the management of NSCLC. Various degrees of reduction of IC_{50} were observed in NSCLC cell lines but not in either BEAS2B or HPL1D (Table 1). It was noted that lung cancer cell lines with high COX-2 expression levels were generally more sensitive to the adjunct use of nimesulide, as was expected from the observed correlation between COX-2 expression and sensitivity to nimesulide as a single agent. In the case of ACC-LC-91 cells, for example, the use of 30 μM nimesulide in combination with SN-38 and SM-5887–13-OH resulted in the reduction of IC_{50} by 77% and 60%, respectively. A 54% reduction of IC_{50} was observed in ACC-LC-91 cells treated with taxotere as a result of the addition of 30 μM nimesulide. In addition, the use of 30 μM nimesulide as an adjunct yielded 25–40% reductions in IC_{50} in ACC-LC-91 cells treated with VP-16 and CDDP, as well as in ACC-LC-323 and ACC-LC-319 cells treated with taxotere. An isobologram was constructed based on the dose-response curves for each individual agent, including nimesulide, to examine its synergistic effects with various anticancer agents (24, 25). It clearly showed that supra-additive effects could be obtained in ACC-LC-91 cells by using SM-5887–13-OH or SN-38 in combination with nimesulide (Fig. 4). A nearly supra-additive effect was observed in ACC-LC-91 cells for the combination of taxotere and nimesulide, whereas nimesulide was shown to have an additive effects with VP-16 and CDDP. Similarly, isobolograms showed nearly supra-additive effects for nimesulide in the combinatorial treatment of ACC-LC-323 cells with taxotere, whereas other combinations were found to be additive (data not shown). These results indicate that chemosensitivity to various anticancer agents can be enhanced in NSCLC cells *in vitro* by the adjunct use of nimesulide at a clinically achievable concentration.

The last question addressed by us was whether the adjunct use of nimesulide can reduce surviving fractions of NSCLC cells after irradiation *in vitro*. Radiation therapy is usually delivered as a series of 1.8–3.0-Gy fractions, 5–6 days a week for 5–8 weeks, whereas hyperfractionation uses relatively small doses per fraction, usually 1.0–1.2 Gy, which are administered two or three times a day (26). The surviving fractions of NSCLC cell lines after exposure to 1 or 2 Gy were found to have been consistently reduced in the presence of 10–30 μM nimesulide, although the observed effects appeared to be simply additive (Table 2). In contrast, neither BEAS2B nor HPL1D cells showed such reduction in the presence of nimesulide, suggesting that any adjunct administration of nimesulide in patients is unlikely to adversely affect the cell survival of normal lung epithelial cells within the field of irradiation.

Our study has shown that the COX-2 selective inhibitor

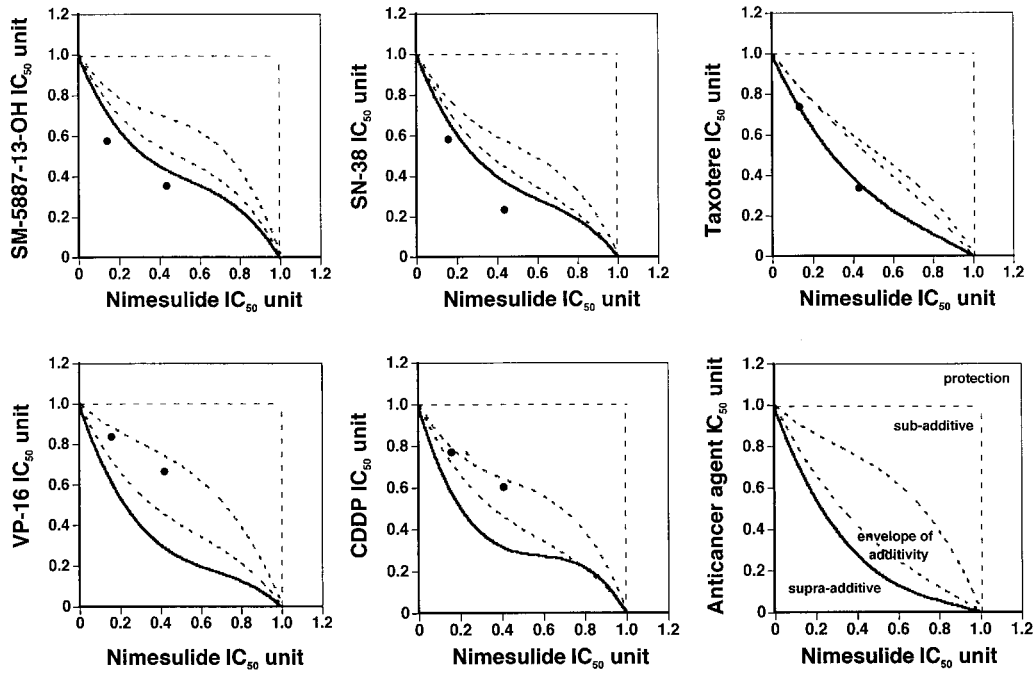

Fig. 4 IC_{50} isobolograms of the use of nimesulide as an adjunct with SM-5887–13-OH, SN-38, taxotere, VP-16, or CDDP in ACC-LC-91. Supra-additive enhancement of chemosensitivity to SM-5887 and SN-38 is seen as a result of the adjunct use of nimesulide at both 10 μM and 30 μM, whereas the combination of taxotere and 30 μM nimesulide shows a nearly synergistic effect. *Solid* and *broken lines* represent Mode I and Mode II in the IC_{50} isobologram, which were constructed from dose-response curves of nimesulide and each chemotherapeutic agent, respectively (24, 25). The area delineated by Mode I and Mode II lines represents the envelope of additivity, whereas the area below this envelope represents the supra-additive area and the area above it the sub-additive area.

Table 2 Radiation sensitivity in combination with nimesulide

Cell line	SF1[a]	SF2[a]	SF0[a]
NSCLC cell lines			
ACC-LC-91	0.90 ± 0.01	0.81 ± 0.02	1.00
ACC-LC-91/NIM 10	0.79 ± 0.03	0.72 ± 0.03	0.85 ± 0.02
ACC-LC-91/NIM 30	0.72 ± 0.03	0.69 ± 0.03	0.80 ± 0.02
ACC-LC-323	0.91 ± 0.02	0.80 ± 0.02	1.00
ACC-LC-323/NIM 10	0.85 ± 0.02	0.74 ± 0.03	0.91 ± 0.01
ACC-LC-323/NIM 30	0.68 ± 0.03	0.63 ± 0.03	0.84 ± 0.02
ACC-LC-319	0.98 ± 0.01	0.97 ± 0.01	1.00
ACC-LC-319/NIM 10	0.90 ± 0.02	0.90 ± 0.02	0.92 ± 0.02
ACC-LC-319/NIM 30	0.88 ± 0.02	0.87 ± 0.02	0.90 ± 0.02
ACC-LC-33	0.87 ± 0.02	0.83 ± 0.02	1.00
ACC-LC-33/NIM 10	0.80 ± 0.03	0.77 ± 0.03	0.95 ± 0.01
ACC-LC-33/NIM 30	0.72 ± 0.03	0.67 ± 0.03	0.91 ± 0.02
Normal lung epithelial cell lines			
BEAS2B	0.90 ± 0.01	0.82 ± 0.02	1.00
BEAS2B/NIM 10	0.91 ± 0.02	0.84 ± 0.02	1.01 ± 0.01
BEAS2B/NIM 30	0.90 ± 0.02	0.85 ± 0.02	0.99 ± 0.01
HPL1D	0.89 ± 0.01	0.84 ± 0.02	1.00
HPL1D/NIM 10	0.90 ± 0.02	0.85 ± 0.02	0.99 ± 0.01
HPL1D/NIM 30	0.90 ± 0.02	0.84 ± 0.02	0.98 ± 0.02

[a] Surviving fractions for 1 Gy, 2 Gy, and 0 Gy.
[b] The values represent means ± SD of a representative experiment. Similar results were obtained in three independent experiments.

nimesulide can inhibit proliferation of NSCLC cell lines *in vitro*. Similar findings have been reported showing sensitivity of lung cancer cells to nonselective, nonsteroidal anti-inflammatory drugs such as sulindac and sulindac sulfone, which inhibit both COX-1 and COX-2 (27, 28). Importantly, the results present here show for the first time that selective inhibition of COX-2 by nimesulide can induce apoptosis even at clinically achievable low concentrations and that the level of COX-2 expression in NSCLC cells may affect their responsiveness to COX-2 inhibitors. Previous studies of ours indicate that a significantly increased COX-2 expression is present in up to 70% of adenocarcinoma cases, showing its potential association with tumor progression (9, 10). It is, therefore, possible that a significant proportion of adenocarcinomas *in vivo* may be sensitive to COX-2 inhibitors.

The most interesting finding of the present study is perhaps that the use of nimesulide as an adjunct at a clinically achievable concentration reduced the IC_{50} values of various anticancer agents by up to 77%, although the levels of reduction varied considerably. It is, therefore, important to clarify which pathway is used in the induction of apoptosis by nimesulide and how the synergistic effects in combination with various anticancer agents are exerted. We note that while this study was under review, Milas *et al.* (29) recently reported that the adjunct use of another COX-2 inhibitor, SC-′236, markedly enhanced therapeutic effects of irradiation in a sarcoma cell line transplanted in C3Hf/Kam mice, in contrast to the simply additive effects of nimesulide *in vitro* observed in the present study. It will be interesting to investigate whether the effects of nimesulide on host cells, such as the inhibition of angiogenesis, can further enhance its synergistic effects in combination with anticancer agents.

NSCLC now accounts for approximately 75–85% of lung cancer cases, and more than 60–65% of patients are diagnosed as having locally advanced or metastatic diseases with dismal prognosis (30, 31). Unfortunately, a considerable proportion of such patients are not eligible for intensive chemotherapy because of age, performance status, or comorbid conditions. The present findings are, therefore, of great clinical interest because the administration of nimesulide as an adjunct with anticancer agents or irradiation may well be feasible without serious side effects in view of previously obtained favorable data on its use as an anti-inflammatory drug. Furthermore, the recent development of next generation, highly selective COX-2 inhibitors can be expected to lead to even greater efficacy of their use as adjuncts with various anticancer agents for the treatment of such high-risk patients without compromising their quality of life.

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