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ORIGINAL ARTICLE

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Superior effectiveness of ibuprofen compared with other NSAIDs for reducing the survival of human prostate cancer cells

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Abstract *Purpose:* Although NSAIDs (nonsteroidal antiinflammatory drugs) appear to be effective in the prevention and treatment of prostate cancer, very little information exists on the comparative effects of common nonprescription NSAIDs. In the present investigation, we evaluated the effects of widely used nonprescription NSAIDs on human prostate cancer cells in vitro. *Materials and methods:* Using in vitro models of androgen-sensitive and androgen-insensitive human prostate cancer cells, we evaluated the effects of acetaminophen, aspirin, naproxen, and ibuprofen on cell survival, cell cycle and the induction of apoptosis. We also compared the effects of these drugs with that of the selective cyclooxygenase-2 (COX-2) inhibitor, NS-398. *Results:* Ibuprofen was significantly more effective against human prostate cancer cells in vitro than the other tested nonprescription NSAIDs. MTT analysis indicated that clinically relevant concentrations of ibuprofen significantly reduced the survival of LNCaP human prostate tumor cells. TUNEL analysis demonstrated that this was due in part to a significant number of LNCaP cells undergoing apoptosis. Ibuprofen also induced the same amount of apoptosis of an androgen-independent human prostate cancer cell line (DU-145), but had little effect on normal mouse fibroblast (3T3) cells. Cell cycle analysis indicated that ibuprofen caused LNCaP cells to shift from the S and G_2/M phases to the G_0/G_1 phases of the cell cycle. Another propionic acid NSAID, naproxen, had an effect similar to but overall less than that of ibuprofen. Suprapharmacological concentrations of aspirin and acetaminophen did not induce levels of apoptosis in LNCaP cells similar to those induced by clinically relevant concentrations of ibuprofen. The selective COX-2 inhibitor NS-398 mirrored the effectiveness of ibuprofen against LNCaP cells in vitro. However, when the pharmacokinetics of selective COX-2 inhibitors and other NSAIDs reported to be effective against prostate cancer were taken into consideration, ibuprofen appeared to be one of the most effective NSAIDs at clinically relevant concentrations. *Conclusions:* These observations support the use of ibuprofen in future in vivo studies and in clinical trials designed to test the effectiveness of NSAIDs against human prostate cancer.

Keywords NSAIDs · Ibuprofen · Prostate cancer · Apoptosis

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Introduction

Prostate cancer is one of the leading causes of cancer-related deaths in men in the United States [27]. Estimates indicate that in the year 2002 there will be over 189,000 new cases of prostate cancer and approximately 30,200 prostate cancer-related deaths [21]. Because androgen ablation therapy and chemotherapy have not resulted in a significant survival benefit in patients with metastatic disease or advanced local disease [50], new approaches are needed.

An increasing number of studies have demonstrated that nonsteroidal antiinflammatory drugs (NSAIDs) are effective in the prevention and treatment of many common cancers including prostate cancer [53, 55]. In vivo studies using rodents have indicated that NSAIDs can decrease the size of prostatic tumors [10, 33] and suppress the metastasis of prostatic cancer [10, 44]. NSAIDs have also been shown to induce apoptosis in both androgen-sensitive and androgen-insensitive prostate tumor cell lines [20, 22, 31, 33, 63], and reduce the

invasiveness of human prostatic tumor cells [3]. Retrospective studies have indicated that there is a significantly reduced risk of prostate cancer associated with regular use of NSAIDs [40, 41, 46]. Finally, it has been reported that there is an overexpression of cyclooxygenase-2 (COX-2) in human prostate adenocarcinoma [12, 13, 24, 25, 28, 59], a potential target of some NSAIDs. With these and other studies recommending the use of NSAIDs in the prevention and treatment of prostate cancer, it is important to determine which NSAIDs may be most effective against prostate cancer.

Very little information is currently available on the comparative effects of nonprescription NSAIDs on human prostate cancer cells. In the present series of investigations, we evaluated the effectiveness of aspirin, ibuprofen, naproxen, and acetaminophen on human prostate cancer cells in vitro. We also compared the effectiveness of these nonprescription NSAIDs with the selective COX-2 inhibitor, NS-398 [*N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide]. Our findings indicate that ibuprofen is able to effectively suppress proliferation and induce apoptosis of prostate cancer cells at clinically relevant concentrations.

Materials and methods

Cell culture

LNCaP, DU-145 and 3T3 cell lines were purchased from the American Type Culture Collection (Rockville, Md.). The LNCaP cell line is an androgen-responsive human prostate adenocarcinoma, which was isolated from a biopsy of a lymph node aspirated from a patient with a confirmed diagnosis of metastatic prostate carcinoma [19]. The DU-145 cell line is an androgen-insensitive human prostate cancer cell line originally isolated from the brain of a patient with metastatic carcinoma of the prostate [52]. The 3T3 cell line is a contact-inhibited embryonic mouse fibroblast cell line first established in 1962 by Todaro and Green [54]. All cells were grown in flasks containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Mediatech, Herndon, Va.), and were seeded into six-well cluster dishes. LNCaP cells were grown in the presence of 10^{-9} *M* dihydrotestosterone (maintenance level). Culture dishes were kept in a humidified atmosphere of air containing 10% CO_2 at a temperature of 37°C. All NSAIDs added to the culture medium (acetaminophen, aspirin, ibuprofen, naproxen, and NS-398) were purchased from Sigma Chemical Company (St. Louis, Mo.).

MTT analysis for cell proliferation

Cellular proliferation was determined using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay according to the procedure provided by the manufacturer (Roche Diagnostics Corporation, Indianapolis, Ind.) and as reported by Mosmann [38]. The principle of the assay is that MTT, a nontoxic pale-yellow substrate, is taken up by living cells to yield a dark blue formazan product. The process requires active mitochondria; thus, dead cells will not form formazan. The formazan formed is read spectrophotometrically (570 nm absorbance) and is directly proportional to the number of viable cells. Since no medium or cells are removed from the culture wells during the MTT analysis, there is no loss of cells (i.e. dead or floating cells) as a result of these procedures. For this assay, cells were seeded in 96-well plates and allowed to incubate for 48 h. Medium containing selected concentrations of the different NSAIDs were then added to the wells and the cells incubated for an additional 48 h. Following incubation with NSAIDs, 10 µl of labeling reagent (MTT) was added to each well, and the cells incubated for an additional 4 h at 37°C. The cells were then solubilized by incubation overnight at 37°C in 100 µl of a solubilization solution (10% sodium dodecyl sulfate in 0.01 *M* HCl). The absorbance at 570 nm was determined in a microtiter plate reader (Bio-Rad Laboratories, Richmond, Calif.). The results are expressed as the percentage of viable cells in relation to nontreated controls.

TUNEL analysis of apoptotic cells

The cells were preserved for TUNEL assay by the addition of 10% neutral formalin to each of the six-well plates (2 ml per well). The fixed cells were harvested by scraping and analyzed using an in situ apoptosis detection kit (ApopTag; Intergen Company, Purchase, N.Y.) according to the manufacturer's instructions. Labeled cells were examined and counted using an OM-2 Olympus microscope equipped for fluorescence. Two independent observers undertook cell counts blindly and statistical analysis was performed using ANOVA. The significance of differences between groups was determined using Student's *t*-test with *P*-values < 0.05 being considered statistically significant.

Cell cycle analysis

The cells were washed twice in phosphate-buffered saline and trypsinized. The cells ($1–2\times10^6$) were pelleted in triplicate by centrifugation and resuspended in 100 µl citrate buffer (40 m*M* trisodium citrate·$2H_2O$, 250 m*M* sucrose, and 5% DMSO, pH 7.6). Nuclei were prepared for flow cytometric cell cycle analysis at the Vincent T. Lombardi Cancer Research Center Flow Cytometry Core Facility (Georgetown University Medical Center, Washington DC) using the method of Vindelov et al. [57], with propidium iodide as the stain for nucleic acid. Cell cycle analysis was performed using a FACStar Plus fluorescence-activated cell sorter (Becton Dickinson Immunocytochemistry Systems, Mountain View, Calif.) equipped with the ModFit cell cycle analysis program (Verity Software House, Topsham, Me.).

Results

Ibuprofen suppresses the proliferation of LNCaP cells

Except for acetaminophen, all the NSAIDs tested induced a significant reduction in proliferation of LNCaP cells ($P < 0.05$). However, incubation with 1 m*M* ibuprofen resulted in significantly greater suppression of proliferation than incubation with aspirin or naproxen and the suppression of proliferation was similar to that induced by incubation with 0.1 m*M* NS-398 (Fig. 1).

At clinically relevant concentrations, only the propionic acid category of NSAID induces significant apoptosis of LNCaP cells

The percentage of apoptosis of LNCaP cells resulting from incubation with selected NSAIDs for 48 h is summarized in Fig. 2. Only suprapharmacological concentrations (5 m*M*) of aspirin and acetaminophen induced statistically significant increases in apoptosis

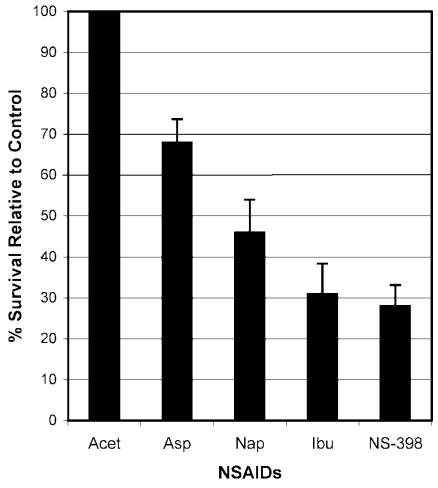

Fig. 1. MTT analysis of cell numbers (i.e. survival) of LNCaP cells following 48 h of incubation with 1 m*M* acetaminophen (*Acet*), aspirin (*Asp*), naproxen (*Nap*) and ibuprofen (*Ibu*) or 0.1 m*M* of NS-398. Ibuprofen and the selective COX-2 inhibitor NS-398 produced the greatest reduction in number of viable cells (*error bars* SD)

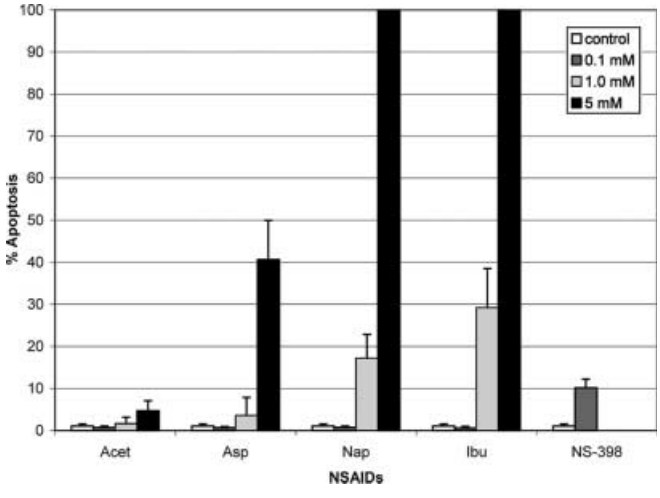

Fig. 2. TUNEL analysis of LNCaP cells following 48 h of incubation with various concentrations of acetaminophen (*Acet*), aspirin (*Asp*), naproxen (*Nap*), ibuprofen (*Ibu*) and NS-398. While aspirin and even acetaminophen induced apoptosis at supraphar-macological concentrations (5 m*M*), ibuprofen and naproxen induced significant apoptosis at clinically relevant concentrations (1 m*M*) (*error bars* SD)

of LNCaP cells relative to controls ($P < 0.05$). The propionic acid NSAIDs, ibuprofen and naproxen, however, induced 100% apoptosis at 5 m*M*, and a significant level of apoptosis at 1 m*M*. NS-398 also induced significant apoptosis at 0.1 m*M*. Additional studies indicated that 48 h incubation with 2 m*M* ibuprofen also induced 100% apoptosis, while concentrations of ibuprofen as low as 0.5 m*M* caused statistically significant levels of apoptosis ($P < 0.05$; data not shown in Fig. 2).

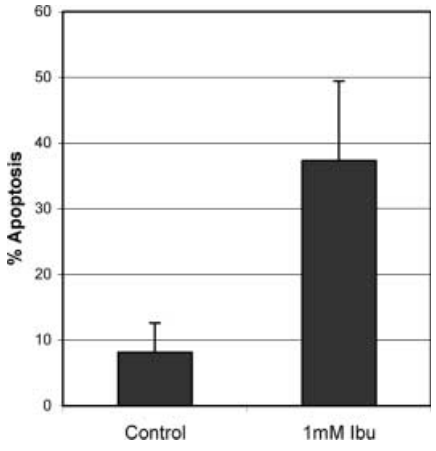

Fig. 3. TUNEL assays preformed on DU-145 cells following 48 h of incubation in the presence and absence (controls) of 1 m*M* ibuprofen. Note that 1 m*M* ibuprofen induced a similar increase in the percentage apoptosis of DU-145 as of LNCaP cells (Fig. 2) (*error bars* SD)

Ibuprofen induces a similar percentage of apoptosis in an androgen-independent human prostate cancer cell line

In this study, we evaluated the extent of apoptosis of DU-145 cells following 48 h of incubation with 1 m*M* ibuprofen (Fig. 3). Because of its greater effectiveness against androgen-dependent human prostate cancer cells (i.e. LNCaP cells), this study focused on ibuprofen. Ibuprofen at 1 m*M* induced a similar increase in apoptosis of DU-145 cells (approximately 29% more than controls) as of LNCaP cells (approximately 28% more than controls).

Of the NSAIDs tested, only acetaminophen induces extensive apoptosis of normal fibroblastic cells

We evaluated the ability of the different NSAIDs to induce apoptosis of a rapidly proliferating non-tumorigenic cell line derived from a different tissue type (3T3 cells). Of the NSAIDs added at 1 m*M* to the medium of nonconfluent normal fibroblastic 3T3 cells and incubated for 48 h, only acetaminophen induced extensive apoptosis (Fig. 4). The effect of acetaminophen at 1 m*M* was impressive with over 40% of the cells being apoptotic. Even lower concentrations of acetaminophen (0.1 m*M*) induced a statistically significant level of apoptosis of the 3T3 cells (data not shown). Of the other NSAIDs tested, only NS-398 induced a statistically significant increase in the percentage apoptosis when compared with controls ($P < 0.05$).

Ibuprofen causes LNCaP cells to shift from the S and G_2/M phases to the G_0/G_1 phases of the cell cycle

We used flow cytometry to determine the effect of selected NSAIDs on the cell cycle of LNCaP cells

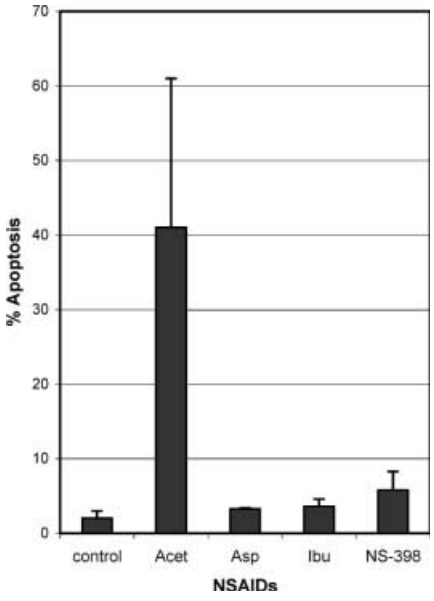

Fig. 4. Percentage apoptosis resulting from incubation of 3T3 cells with selected NSAIDs. Note that of the NSAIDs tested, acetaminophen and to a lesser extent NS-398 induced a significant increase in apoptosis of 3T3 cells compared with controls ($P < 0.05$) (*error bars* SD)

following 48 h of incubation. As shown in Table 1, ibuprofen (1 m*M*) and NS-398 (0.1 m*M*) induced the largest statistically significant shift of LNCaP cells out of the S and G_2/M phases into the G_0/G_1 phases of the cell cycle.

Discussion

In this study ibuprofen was significantly more effective in reducing the survival of human prostate cancer cells in vitro than the other nonprescription NSAIDs tested. Ibuprofen appeared to accomplish this by inducing apoptosis and causing cancer cells to shift from mitotic to resting stages of the cell cycle. Ibuprofen proved effective against both androgen-dependent (LNCaP) and androgen-independent (DU-145) human prostate cancer cells, with no effect on normal (3T3) cells. These observations are in line with the findings of other investigations, which have shown that NSAIDs are effective in reducing the survival of both androgen-dependent and -independent human prostate cancer cells, but have little effect on normal cell lines [20, 22, 30, 31]. Of particular significance in our study, however, is that ibuprofen was able to induce these effects at clinically relevant concentrations. Pharmacokinetic studies following oral administration of the maximum clinically acceptable dosage of ibuprofen (3200 mg/day) have indicated that ibuprofen would be present in the blood at the levels shown to be effective in this study (0.5 to 1 m*M* ibuprofen) [26, 34, 37]. Symptoms of ibuprofen overdose are unlikely after ingestion of up to 100 mg/kg body weight, and are usually not life-threatening unless more than 400 mg/kg is ingested [51].

Relevant to patients with prostate cancer is that advanced age has very little effect on the pharmacokinetics of ibuprofen [2]. Also, of the non-selective COX inhibitors, ibuprofen is one of the least-damaging to the gastric mucosa [58], and patients can be maintained on high doses of ibuprofen for years without serious adverse effects [26]. Since this circulating ibuprofen would be effective against distant metastatic sites as well as the localized cancer, additional studies of ibuprofen and its analogs are warranted in order to determine the possible clinical significance of these observations, especially in view of the poor prognosis of patients who have developed androgen-independent metastatic disease.

How does ibuprofen compare with prescription and experimental NSAIDs that have been tested against human prostate cancer cells in vitro? Lim et al. [30] have examined the activity of two metabolites of sulindac (an acetic acid category of NSAID), sulindac sulfide and sulindac sulfone. These investigators reported that both metabolites induce marked growth inhibition and apoptosis of both androgen-sensitive and androgen-insensitive human prostate cancer cell lines. More interesting is the fact that sulindac sulfone causes growth inhibition and apoptosis despite the fact that it lacks COX inhibitory activity. Treatment of LNCaP cells for 3 days with 0.5 and 0.6 m*M* sulindac sulfide and sulindac sulfone resulted in 23% and 16% apoptosis, respectively. However, pharmacokinetic studies have indicated that these concentrations represent blood serum levels that are over ten times those obtainable following administration of maximum recommended dosages [45].

Zhu et al. [63] have reported the effects of a number of NSAIDs on LNCaP cells. Using the MTT assay for cell growth, they found that salicylic acid, fenoprofen, indomethacin, sulindac and piroxicam at 0.2 m*M* have little effect on LNCaP cells following 5 days incubation. However, they reported that lower concentrations (0.1 m*M* and less) of the NSAIDs flufenamic acid and tolfenamic acid significantly decrease the proliferation of

Table 1. Cell cycle analysis of LNCaP cells following 48 h of incubation in the presence of various NSAIDs. The results indicate that high concentrations of ibuprofen (1 m*M*) and NS-398 (0.1 m*M*) caused LNCaP cells to shift from the S/G_2-M to the G_0/G_1 phases of the cell cycle

	G_0/G_1		G_2/M		S	
	%	SD	%	SD	%	SD
Control	63.43	1.73	16.32	0.68	20.1	1.38
Aspirin 1 m*M*	67.76	2.77	14.23	0.76	17.86	2.39
Aspirin 0.1 m*M*	66.99	1.97	16.21	0.71	16.78	1.32
Acetaminophen 1 m*M*	61.81	0.20	14.68	0.41	23.44	0.24
Acetaminophen 0.1 m*M*	62.08	1.86	16.14	0.82	21.79	1.05
Ibuprofen 1 m*M*	82.00*	0.68	9.20*	0.62	8.80*	1.01
Ibuprofen 0.1 m*M*	66.83	4.83	15.60	0.50	17.57	4.35
NS-398 0.1 m*M*	82.27*	2.05	11.17*	0.22	6.56*	2.07

*$P < 0.05$ vs control

LNCaP cells. Although these represents clinically relevant concentrations of these NSAIDs [29], this decrease in cell numbers did not begin until after 48 h of incubation. In the present study, we found significant decreases in both cell survival and apoptosis following 48 h of incubation with both ibuprofen and naproxen.

In recent years, selective COX-2 inhibitors (rofecoxib, celecoxib) have become popular as the "next generation" of NSAIDs due to their ability to selectively inhibit COX-2 while not affecting the protective "housekeeping" effects of COX-1 (i.e. maintenance of gastrointestinal tract lining and renal function) [53, 56]. In the present study, we compared the effectiveness of an experimental selective COX-2 inhibitor (NS-398, which is not for use in humans) with other widely used nonprescription NSAIDs. Our finding that 0.1 m*M* NS-398 reduced the number of viable LNCaP cells by about 28% after 2 days of incubation is similar to the results of Liu et al. [31], who reported an approximately 32% decrease in the number of viable LNCaP cells following incubation for 2 days with NS-389 at the same concentration. These researchers also reported that NS-398-induced apoptosis is associated with a downregulation of Bcl-2 protein expression, indicating a possible mechanism of action of NS-398.

Kamijo et al. [22] compared the effects of NS-398 with those of a clinically available selective COX-2 inhibitor, Etodolac, in LNCaP and PC-3 human prostate cancer cells. Using the MTT analysis, they found a time- and dose-dependent response to both selective COX-2 inhibitors in reducing survival of these prostate cancer cells, but they did not affect a normal prostate stromal cell line (PRSC). They also found that NS-398 reduced survival more than Etodolac, despite the fact that Etodolac is a more effective COX-2 inhibitor than NS-398. Recently, Hsu et al. [20] have reported that the selective COX-2 inhibitor celecoxib is more effective against LNCaP and PC-3 human prostate cancer cell lines then piroxicam (from the oxicam family of NSAIDs), as well as the selective COX-2 inhibitors NS-398, rofecoxib and DuP697. Using trypan blue exclusion to evaluate cell viability, they found that concentrations of celecoxib as low as 10 μ*M* result in a reduction in cell viability. However, pharmacokinetic studies have indicated that even supratherapeutic concentrations of celecoxib (e.g. 800 mg/day) result in less than 10 μ*M* of this drug in the blood [9]. These researchers also provide evidence that the action of celecoxib is not associated with Bcl-2 expression, but rather involves blocking the activation of the antiapoptotic kinase Akt (protein kinase B).

Therefore, when considering the data reported thus far, it would appear that a less-expensive nonprescription NSAID (i.e. ibuprofen) might be one of the most useful NSAIDs against prostate cancer, because more effective blood serum levels are obtainable following administration of maximum dosages. However, additional in vivo as well as in vitro comparisons between potentially effective NSAIDs such as ibuprofen, flufenamic acid and celecoxib are clearly needed in order to determine their relative effectiveness against prostate and other cancers.

The importance of comparing clinically relevant dosages of ibuprofen with other NSAIDs is illustrated by a recent in vivo study in which the effectiveness of celecoxib was compared with that of ibuprofen in a model of breast cancer [14]. In this study, female rats were fed diets containing equal concentrations of celecoxib or ibuprofen (1500 mg/kg) and then were treated with dimethylbenz(a)anthracene to induce breast tumors. While both NSAIDs produced striking reductions in the incidence, multiplicity, and volume of breast tumors relative to control groups, the mean blood serum levels at the end of the experiments were 5.1 μg/ml for celecoxib and 8.0 μg/ml for ibuprofen. Although these investigators concluded that celecoxib appeared more effective than ibuprofen, the blood serum levels of celecoxib are higher than can be achieved with maximum dosages in humans, while the blood serum levels of ibuprofen are dramatically lower than can be achieved with acceptable high dosages in humans [26, 34, 37]. Therefore, contrary to the investigators conclusions, this study would suggest that ibuprofen may be more effective against human breast cancer when clinically relevant dosages are taken into consideration.

Rotem et al. [49] evaluated the effects of aspirin on LNCaP cells. They observed that aspirin decreased cell proliferation of prostate cancer cells, but had no effect on cell death. Our findings regarding survival of LNCaP cells following incubation with 1 m*M* aspirin mirror the results of this group. Our results on cell cycle analysis also indicate, in agreement with those of Rotem et al., no statistically significant effects following incubation with 1 m*M* aspirin. Rotem et al. also found that a higher dose of aspirin (2 m*M*) did not increase cell death compared with the control group using the trypan blue exclusion procedure [49]. However, using the TUNEL procedure we found that a suprapharmacological concentration of aspirin (5 m*M*) [1] induced a significant increase in apoptosis of LNCaP cells when compared with controls.

The mechanisms associated with the effectiveness of NSAIDs against cancer cells remain under investigation. One of the proposed mechanisms is inhibition of the production of COX-2, one of two isoforms of COX. COX-2 is induced by growth factors, cytokines, oncogenes, and tumor promoters, and is upregulated in transformed cells and malignant tumors [16, 36]. In the present study, the effectiveness of the nonprescription NSAIDs correlated with their ability to inhibit COX-2 activity. That is, the ability of the NSAIDs to inhibit COX-2 activity decreased in the order ibuprofen, naproxen, aspirin, acetaminophen [23, 58]. However, there is accumulating evidence indicating that the effectiveness of NSAIDs against cancer cells is due mechanisms independent of COX-2. As noted above, sulindac sulfone is effective against prostate cancer cells despite having no COX-2-inhibitory ability [30]. NSAIDs still have antineoplastic effects when used against COX-1- and COX-2-null mouse embryo fibroblasts [62]. The

concentrations of NSAIDs that inhibit growth are often 10 to 100 times higher than are required to inhibit COX activity [15].

Finally, while a number of studies have indicated an overexpression of COX-2 in prostate cancer [12, 13, 24, 25, 28, 59], this conclusion is not supported by the findings of a recent study which indicate that prostate cancer cells lines (LNCaP, DU-145, PC-3 and TSU) do not express detectable levels of COX-2 protein under basal conditions [60]. These and other studies have provided evidence for a number of alternative mechanisms to explain the anticancer effects of NSAIDs. As noted above, Liu et al. [31] have indicated the possible involvement of Bcl-2, while Hsu et al. [20] have indicated the possible involvement of Akt. Others have suggested that the mechanisms underlying the anticancer action of NSAIDs may involve the expression of the apoptotic proteins Bax and Bcl-xl [61], inhibiting the expression of peroxisome proliferator-activated receptor beta (PPAR-beta) [15], induction of arachidonic acid elevation leading to the production of ceramide [5, 7], or inhibiting the transcription factor NF-κB by blocking the phosphorylation and degradation of the NF-κB inhibitor IκBa [43]. Although the present investigation does not shed light on the mechanistic aspects of NSAIDs, the fact that high concentrations of ibuprofen were found to be increasingly effective despite the fact that these concentrations were well above those required to inhibit COX activity [58], argues for a non-COX-2 mechanism for this NSAID as well.

Although acetaminophen is considered a relatively weak NSAID [4], it was tested because of its widespread use and in view of recent evidence indicating its effectiveness against brain [6] and ovarian cancers [8, 39]. In our study, acetaminophen was the least effective of the NSAIDs against human prostate cancer cells in vitro. The toxicity of acetaminophen to normal mouse fibroblasts was an unexpected finding. Acetaminophen is known to be toxic to hepatocytes due to generation of the toxic metabolite *N*-acetyl-*p*-benzoquinoneimine, leading to depletion of intracellular glutathione, alteration of redox potential and ultimately cell death. However, acetaminophen has other toxic effects including inhibition of ribonucleotide reductase [17] and nucleotide excision repair [18]. Recently, Rocha et al. [47] have reported that acetaminophen is toxic to inner medullary collecting duct cells in vitro, indicating its involvement in the papillary necrosis that results from chronic ingestion of combinations of NSAIDs. Despite the toxicity of acetaminophen to 3T3 cells, ibuprofen and aspirin did not produce statistically significant increases in the apoptosis of 3T3 cells. Although NS-398 induced a statistically significant increase in apoptosis when compared with controls, this increase was not dramatic. As noted above, others have reported that most NSAIDs that have been found to decrease the survival of prostate cancer cells appear to have little or no effect on normal cell lines [20, 22, 30, 31]. Nevertheless, the variable effects of NSAIDs on 3T3 cells seen in the present study, underlines the specificity of the toxic effects of different NSAIDs to different cell lines.

It should be added, that in addition to their ability to inhibit proliferation and induce apoptosis of prostate cancer cells, NSAIDs also appear to inhibit the metastasis of these cells [3] and angiogenesis associated with prostate tumors [32, 35]. Attiga et al. [3] have reported that both ibuprofen and NS-398 inhibit human prostate cancer cells (DU-145 and PC-3) from passing though Matrigel. This inhibition is associated with a decrease in secretion of metalloproteinases, key enzymes in the proteolysis of Matrigel during invasion. Liu et al. [32] have presented data indicating that NS-398 inhibits the angiogenesis of human prostate tumors induced in mice by injection of PC-3 cells. These researchers have provided evidence that this antiangiogenic effect may be due to a downregulation of tumor vascular endothelial growth factor (VEGF) expression [32].

Finally, NSAIDs appear to work synergistically with other chemotherapeutic drugs such as the anthracyclines (e.g. doxorubicin) and vincristine to enhance the apoptosis of cancer cells [11, 48]. Also, ibuprofen has been shown to have a combined antitumor effect when used with radiation on human prostate cancer cells [42]. Since this enhanced radiation response requires ibuprofen concentrations higher than those reported to inhibit prostaglandin synthesis, Palayoor et al. also concluded that other molecular mechanisms might be responsible for this effect [42].

In summary, recent studies have suggested that NSAIDs can decrease the risk of prostate cancer and may be useful in the treatment of this disease. The present in vitro investigation indicates that, among the NSAIDs tested, ibuprofen should be considered in planning future clinical trials and in vivo studies designed to test the effectiveness of NSAIDs against human prostate cancer.

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