Nitric Oxide-releasing Nonsteroidal Anti-inflammatory Drugs (NSAIDs) Alter the Kinetics of Human Colon Cancer Cell Lines More Effectively than Traditional NSAIDs: Implications for Colon Cancer Chemoprevention

Jennie L. Williams, Simona Borgo, Izhar Hasan, Eddy Castillo, Frank Traganos, and Basil Rigas


Abstract

Nitric oxide-releasing nonsteroidal anti-inflammatory drugs (NO-NSAIDs), consisting of a known nonsteroidal anti-inflammatory drug (NSAID) and a nitric oxide (NO)-releasing group, are reported safer than NSAIDs. To assess their potential in colon cancer chemoprevention, we studied in vitro the effect of NO-aspirin, NO-sulindac, and NO-ibuprofen on colonocyte kinetics. These three NO-NSAIDs reduced the growth of cultured HT-29 colon adenocarcinoma cells much more effectively than the corresponding NSAIDs; e.g., at 24 h, their IC50 values were as follows: (a) aspirin, >5000 μM; (b) NO-aspirin, 1 μM; (c) sulindac, 750 μM; (d) NO-sulindac, 150 μM; (e) ibuprofen, >1000 μM; and (f) NO-ibuprofen, 42 μM. This effect was due to inhibition of proliferation and induction of apoptosis and perhaps to the induction of novel cell changes, characterized by extensive DNA degradation. NO-NSAIDs also blocked the G0-G1 to S cell cycle transition. Their superior effectiveness compared with traditional NSAIDs, combined with their reported safety, makes them promising candidates for chemopreventive agents against colon cancer.

Introduction

Ample evidence supports the notion that chemoprevention has the potential to be a major component of the control of colorectal cancer, one of the most common human malignancies in the Western world (1). NSAIDs3 are prototypical chemopreventive agents against colorectal cancer (reviewed in Refs. 2 and 3), but side effects are a major obstacle to their large-scale application to prevent colorectal cancer. NSAID toxicity includes (a) GI side effects, which range from dyspepsia to GI bleeding, obstruction, and perforation; (b) renal side effects, including a wide range of tubular, interstitial, glomerular, and vascular lesions; and (c) a large number of additional side effects, some of them serious, ranging from hypersensitivities reactions to the distinct salicylate intoxication (summarized in Ref. 4). Among patients using NSAIDs, up to 4% per year suffer serious GI complications; in 1998, the number of deaths in the United States from NSAID-induced GI complications was 16,550, virtually identical to the 16,685 deaths from AIDS (5, 6). The synthesis of NO-NSAIDs represents a novel approach to reduce the side effects of NSAIDs, especially their gastric toxicity (7–9). NO-NSAIDs consist of a known NSAID molecule and a NO-releasing group (typically –NO2) linked to it via a chemical spacer (Fig. 1). The rationale for their development was based on the observation that NO possesses some of the same properties as PGs within the gastric mucosa. NO increases mucosal blood flow, mucous release, and repair of the mucosa, whereas it inhibits neutrophil activation and adherence. These effects can theoretically compensate for gastric PG reduction. Coupling a NO-releasing moiety to a NSAID might deliver NO to the site of NSAID-induced damage and thus decrease gastric toxicity. Existing data, mostly from animal studies, indicate that this prediction may be true (7–9).

Like all cancers, colon cancer reflects one or more disturbances in tissue homeostasis. Changes in the rates of colonocyte proliferation, apoptosis, or both participate in colonic tumorigenesis (2). Work by us and others has established that traditional NSAIDs exert a major effect on cell kinetics (e.g., Ref. 10). In vitro, their effects include inhibition of cell proliferation, induction of apoptosis, and a significant block in cell cycle transitions leading to cell quiescence. There is evidence that at least some of these changes occur in animals and humans. To assess the chemopreventive potential of these novel compounds against colon cancer, we evaluated the effect of three NO-NSAIDs, namely, NO-aspirin, NO-sulindac, and NO-ibuprofen, on colonocyte kinetics. Their parent NSAIDs represent important structural classes of NSAIDs and have been studied extensively for their effect on colon cancer. This report presents our findings, which indicate that in vitro NO-NSAIDs are much more effective on colonocyte kinetics than their parent compounds.

Materials and Methods

Cell Lines. HT-29 and HCT-15 human colon adenocarcinoma cell lines (American Type Culture Collection, Manassas, VA) were grown as monolayers in McCoy 5A medium and RPMI 1640, respectively, and supplemented with 10% FCS (Mediatech, Herndon, VA), penicillin (50 units/ml), and streptomycin (50 μg/ml; Life Technologies, Inc., Grand Island, NY). Cells were seeded at a density of 1.5 × 106 cells/100-cm2 culture dish and incubated at 37°C in 5% CO2 and 90% relative humidity. Single-cell suspensions were obtained by trypsinization (0.05% trypsin/EDTA), and cells were counted using a hemacytometer. Viability was determined by the trypan blue dye exclusion method.

Reagents. NO-sulindac (NCX1102; (Z)-5-fluoro-2-methyl-1-[(4-(methyl-sulfinyl)phenyl)methylene]-1H-indene-3-acetic acid 4-(nitrooxy)butyl ester), NO-ibuprofen (NCX2210; trans-3-[4-[(3-methyl-2-propenoyl)benzene- acetyl]oxy]-3-methoxyphenyl]-2-propenoic acid 4-nitrooxy)butyl ester), and NO-aspirin (NCX4040; 2-acetoxylbenzoic acid 4-(nitrooxy methyl)phenyl ester) were gifts of Dr. F. Del Soldato (Nicox, SA, France). Stock (100 mM) solutions of NO-NSAIDs and NSAIDs were prepared in DMSO (Fisher Scientific, Fair Lawn, NJ). All compounds were added to the culture medium immediately before plating. Final DMSO concentration was adjusted in all media to 1%.

Flow Cytometry. Cell cycle phase distributions of control and treated colon cancer cell lines were obtained using a Coulter Profile XL equipped with a single argon ion laser. For each subset, we analyzed ≥10,000 events. All parameters were collected in listmode files. Data were analyzed on an XL Elite

Received 2/12/01; accepted 2/23/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

3 Supported by a grant from the A. Weinstein Foundation.
4 To whom requests for reprints should be addressed, at American Health Foundation, One Dana Road, Valhalla, NY 06595. Phone: (914) 789-7295; Fax: (914) 592-6317.
5 The abbreviations used are: NSAID, nonsteroidal anti-inflammatory drug; NO, nitric oxide; NO-NSAID, NO-releasing NSAID; DAPI, 4',6-diamidino-2-phenylindole; GI, gastrointestinal; FBS, fetal bovine serum; PCNA, proliferating cell nuclear antigen; PG, prostaglandin; COX, cyclooxygenase; ASA, aspirin.

The Kinetics of Human Colon Cancer Cell Lines More Effectively than Traditional NSAIDs: Implications for Colon Cancer Chemoprevention
Work station (Coulter) using the software programs Multigraph and Multi-
cycle.

Cell Proliferation and Cell Cycle Analysis. Cells (0.5 \texttimes 10^6) were fixed
in 100% methanol for 10 min at 220°C, pelleted (5000 rpm for 10 min at 4°C),
resuspended, and incubated in PBS containing 1% FBS/0.5% NP40 on ice for
5 min. Cells were washed twice in PBS/1% FBS, pelleted, and resuspended in
50 \mu l of a 1:10 dilution of the anti-PCNA primary antibody (PC-10; all
antibodies were from Santa Cruz Biotechnology, Santa Cruz, CA) in PBS/1%
FBS for 60 min at room temperature. Nonspecific IgG1/IgG2 was used as an
isotypic control. Cells were then washed and incubated with goat antimouse
phycoerythrin antibody (diluted 1:50) for 60 min at room temperature in the
dark. Cells were washed again in 500 \mu l of PBS/1% FBS containing 40 \mu g/ml
propidium iodide and 200 \mu g/ml RNase type IIA and analyzed within 30 min
by flow cytometry. The percentage of cells in G_0-G_1, G_2-M, and S phases was
determined from DNA content histograms.

Assays for Apoptosis. The induction of apoptosis was determined by the
presence of a subdiploid (sub-G_0-G_1) peak in DNA content histograms ob-
tained by flow cytometry as described above and by fluorescence microscopy
of cells stained with DAPI (Accurate Chemical, Westbury, NY). For each
sample, at least five fields were examined. The morphological criteria used to
identify apoptosis included cytoplasmic and nuclear shrinkage, chromatin
condensation, and cytoplasmic blebbing with maintenance of the integrity of
the cell membrane.

Results

NO-NSAIDs Reduce the Number of Cultured Colon Cancer
Cells More Effectively than Their Parent Compounds. Compared
with their parent NSAIDs, all three NO-NSAIDs reduced the
number of HT-29 cells in culture at lower concentrations and after
shorter incubation periods (Table 1; Fig. 2). Remarkably, even at
the maximal concentrations used, ASA and ibuprofen failed to
reduce the number of cells by half, whereas their NO-derivatives
reduced the cell number to near zero by 24 h. Indeed, the ratio of
the IC_{50} for ASA compared with that of NO-ASA (ASA:NO-ASA)
ranged between 1270 and \textasciitilde 5000 over the 72 h of observation; for
ibuprofen:NO-ibuprofen, it was \textasciitilde 42, and for sulindac:NO-sulind-
dac, it ranged between 5 and 20. Similar ratios were obtained with
HCT-15 cells (data not shown).

NO-NSAIDs Inhibit Cell Proliferation. To evaluate the mecha-
nism(s) involved in these substantial reductions in cell number, we
determined the effect of NO-NSAIDs on cell proliferation by assaying
the expression of PCNA. As shown in Table 2, all three compounds
reduced the expression of PCNA in a concentration-dependent man-
ner. The extent of this effect, however, differed substantially between
these three compounds. For example, 48 h of exposure to 100 \mu M
NO-ASA reduced PCNA expression by 40% and the cell number by
99%, compared with control. The corresponding values were 22% and
77% for NO-sulindac and 42% and 76% for NO-ibuprofen. Further-
more, the reduction in PCNA expression was not linearly related to
reductions in cell number, suggesting that the participation of other
kinetic effects, such as apoptosis, may play a role.

![Fig. 1. The chemical structure of NO-aspirin. The three structural components of this
novel derivative of aspirin (designated NCX 4040) are indicated: the spacer molecule links
the traditional NSAID to \textasciitilde NO_2, which can release NO. The structure of traditional aspirin
is also shown for comparison.](image)

![Fig. 2. The effect of NO-NSAIDs on HT-29 cell
growth. HT-29 cells were treated with various con-
centrations of NO-NSAIDs or their corresponding
traditional NSAIDs as described in “Materials and
Methods.” Cell number was determined at 24, 48,
and 72 h.](image)

Table 1 The IC_{50} of NO-NSAIDs and NSAIDs in HT-29 cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} (\mu M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>Aspirin</td>
<td>&gt;5000^a</td>
</tr>
<tr>
<td>NO-aspirin (NCX 4040)</td>
<td>1</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>NO-ibuprofen (NCX 2210)</td>
<td>42</td>
</tr>
<tr>
<td>Sulindac</td>
<td>750</td>
</tr>
<tr>
<td>NO-sulindac (NCX 1102)</td>
<td>150</td>
</tr>
</tbody>
</table>

* Exceeded the maximum concentration used in these studies.

Downloaded from cancerres.aacrjournals.org on April 9, 2017. © 2001 American Association for Cancer Research.
NO-NSAIDs Induce Apoptosis. NO-NSAIDs induced apoptosis, which was detected by morphological evaluation of DAPI-stained cells and also by determination of cell DNA content. All three NO-NSAIDs induced apoptosis in a concentration-dependent manner (Table 2). The effect appeared maximal at 48 h.

Microscopic examination of DAPI-stained cells revealed that, compared with controls, cultures treated with NO-NSAIDs had an increased number of apoptotic cells, as defined by standard morphological criteria such as cytoplasmic and nuclear shrinkage or chromatin condensation (Table 2; Fig. 3). For example, exposure of cells to 100 μM NO-ASA for 48 h increased the frequency of apoptotic cells from 2% in controls to 46%; 1 μM NO-ASA had no such effect. The other two compounds had a similar but less pronounced effect on apoptosis: 48 h of exposure to 100 μM NO-ibuprofen resulted in 19% apoptotic cells, whereas NO-sulindac under identical culture conditions increased apoptosis to 34%.

An interesting phenomenon was the appearance of a new population of cells. These cells exhibited diminished or no detectable DNA. On the basis of morphological criteria, they were clearly different from apoptotic cells. Preliminary study of such cells indicates that the loss of DNA is a progressive event, which in some cells is probably accompanied by a reduction in cell size. These cells have been termed “atypical,” and their frequency is listed in Table 2. Atypical cells were quite frequent, especially in cultures treated with higher concentrations of NO-ASA and NO-ibuprofen, where they constituted the absolute majority of all cellular entities. Indeed, in some instances, atypical and classical apoptotic cells were the only two cell types observed. It would not be unreasonable to hypothesize that the progressive loss of DNA adversely affects the longevity of these atypical cells. Thus, this phenomenon may contribute to cell death (or be a

Table 2  The effect of NO-NSAIDs on HT-29 cell proliferation, apoptosis, and cell cycle

<table>
<thead>
<tr>
<th>Concentration, (μM)</th>
<th>Proliferation</th>
<th>Apoptosis</th>
<th>Cell cycle phases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCNA, % (+)</td>
<td>DAPI-stained apot/typical (%)</td>
<td>sub-G1/G0, G1/S, G2/M</td>
</tr>
<tr>
<td>NO-aspirin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>90.5</td>
<td>2.2/0.7</td>
<td>2.2</td>
</tr>
<tr>
<td>0.01</td>
<td>87.5</td>
<td>2.3/0.4</td>
<td>2.6</td>
</tr>
<tr>
<td>1</td>
<td>88</td>
<td>3.0/1.5</td>
<td>2.4</td>
</tr>
<tr>
<td>10</td>
<td>74.5</td>
<td>25.4/7.7</td>
<td>23.3</td>
</tr>
<tr>
<td>100</td>
<td>54.5</td>
<td>45.7/54.3</td>
<td>18.8</td>
</tr>
<tr>
<td>72 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>92</td>
<td>3.5/1.8</td>
<td>3.9</td>
</tr>
<tr>
<td>0.01</td>
<td>90</td>
<td>3.2/1.6</td>
<td>4.3</td>
</tr>
<tr>
<td>0.1</td>
<td>96</td>
<td>1.1/1.0</td>
<td>4.0</td>
</tr>
<tr>
<td>1</td>
<td>88</td>
<td>3.1/0.0</td>
<td>4.4</td>
</tr>
<tr>
<td>10</td>
<td>69</td>
<td>10.9/9.6</td>
<td>18.1</td>
</tr>
<tr>
<td>100</td>
<td>43</td>
<td>42.0/58.0</td>
<td>18.4</td>
</tr>
<tr>
<td>NO-Ibuprofen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>93</td>
<td>2.2/0.7</td>
<td>2.7</td>
</tr>
<tr>
<td>0.01</td>
<td>88</td>
<td>11.2/7.6</td>
<td>3.1</td>
</tr>
<tr>
<td>10</td>
<td>53</td>
<td>19.2/45.7</td>
<td>12.5</td>
</tr>
<tr>
<td>100</td>
<td>46</td>
<td>39.8/60.2</td>
<td>20.4</td>
</tr>
<tr>
<td>72 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>89</td>
<td>3.5/1.8</td>
<td>3.4</td>
</tr>
<tr>
<td>0.01</td>
<td>87</td>
<td>4.0/1.7</td>
<td>2.2</td>
</tr>
<tr>
<td>10</td>
<td>61</td>
<td>28.7/41.0</td>
<td>7.9</td>
</tr>
<tr>
<td>100</td>
<td>57</td>
<td>34.0/58.8</td>
<td>17.9</td>
</tr>
<tr>
<td>NO-Sulindac</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>91</td>
<td>2.2/0.7</td>
<td>6.8</td>
</tr>
<tr>
<td>0.01</td>
<td>81</td>
<td>6.1/1.2</td>
<td>10.4</td>
</tr>
<tr>
<td>10</td>
<td>71</td>
<td>33.9/7.4</td>
<td>13.2</td>
</tr>
<tr>
<td>100</td>
<td>55</td>
<td>36.7/5.0</td>
<td>11.5</td>
</tr>
<tr>
<td>72 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>92</td>
<td>3.2/1.9</td>
<td>6.3</td>
</tr>
<tr>
<td>0.01</td>
<td>84</td>
<td>3.1/1.2</td>
<td>6.2</td>
</tr>
<tr>
<td>10</td>
<td>55</td>
<td>40.2/13.4</td>
<td>10.0</td>
</tr>
<tr>
<td>100</td>
<td>51</td>
<td>36.9/10.4</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Fig. 3. The effect of NO-aspirin on apoptosis in HT-29 cells. A, control samples (no NO-NSAID treatment) showed rare apoptotic cells. Samples treated for 72 h with 200 μM NO-sulindac (B), 10 μM NO-aspirin (C), or 100 μM NO-ibuprofen (D) show classical apoptotic cells (cytoplasmic and nuclear shrinkage; chromatin condensation). In addition, especially in D, there are cells that maintain their basic structure but show diminished to no detectable amounts of DNA without DNA condensation or cytoplasmic shrinkage (atypical cells in Table 2).
consequence of it) and thereby to the diminished cell number observed after exposure to the various NO-NSAIDs.

As shown in Table 2, all three NO-NSAIDs also induced apoptosis in HT-29 cells when apoptosis was determined by assaying the proportion of cells with a subdiploid DNA content. For example, exposure to 10 μM NO-ASA resulted in 20% apoptotic cells. Indeed, at that concentration, the effect already appeared maximal, being around 10-fold higher than control. The relative efficacy of these compounds in inducing apoptosis can be assessed by determining the frequency of apoptotic cells after treatment of HT-29 cells with equimolar concentrations of these compounds for the same period of time. Thus, after exposure to 100 μM of each NO-NSAID for 48 h, the increase in the percentage of apoptotic cells over control was 8.7-fold for NO-ASA, 4.6-fold for NO-ibuprofen, and 1.9-fold for NO-sulindac. As expected (10, 11), the percentage of apoptotic cells was higher when apoptosis was determined by morphological criteria rather than by cell DNA content, although the changes in apoptosis detected by these two methods were always concordant.

**NO-NSAIDs Block Cell Cycle Transitions.** All three NO-NSAIDs significantly altered the cell cycle distribution of HT-29 cells, inducing a block in the G<sub>s</sub>-G<sub>1</sub> to S transition. This was evident by the increased percentage of cells in the G<sub>s</sub>-G<sub>1</sub> phase that was accompanied by corresponding reductions of the proportion of cells in S and G<sub>2</sub>-M phases. This effect was concentration dependent. NO-ASA was the most effective of the three in inducing these changes. This is evident, for example, when equimolar concentrations of the three compounds are compared, e.g., exposure to 100 μM for 48 h. NO-ASA increased the G<sub>s</sub>-G<sub>1</sub> fraction by 57% over control versus no change or a 31% increase after exposure to NO-ibuprofen and NO-sulindac, respectively. The corresponding changes for the S-phase were a 61% reduction for NO-ASA, no change for NO-ibuprofen, and a 60% reduction for NO-sulindac. For G<sub>2</sub>-M, there was a 78% reduction for NO-ASA and virtually no change for the other two.

**Discussion**

Our data demonstrate that three members of the family of the novel NO-NSAIDs inhibit cell growth much more effectively than their parent NSAIDs; their superior effectiveness reached a very high level in the case of NO-ASA.

NO-NSAIDs inhibited cell growth through an effect on cell proliferation, apoptosis, or both. Although it is difficult to accurately determine their individual contribution to these complex kinetic changes, our data provide some indication of the relative magnitude of these effects. The case of NO-ASA illustrates this point. Compared with controls, exposure to 1 μM NO-ASA for 48 h reduced the cell number by 11% and PCNA expression by 17%, whereas there was no detectable induction of apoptosis and no change in the cell cycle phase distribution of these cells. Thus, it appears that at this concentration of NO-ASA, there is only a minor antiproliferative effect. In contrast, exposure of colonic tumor cells to 10 μM NO-ASA reduced the number of cells by 83% and PCNA expression by 25% and increased apoptosis at least 10-fold, not taking into account the atypical cells. This treatment also increased the percentage of cells that accumulated in the G<sub>s</sub>-G<sub>1</sub> phase. Therefore, at this concentration, the predominant effect of NO-ASA is to induce apoptosis.

The induction of the atypical cells in response to NO-NSAIDs was intriguing. This phenomenon, which is currently under investigation, requires further assessment. It is important to note, however, that traditional NSAIDs, even at high concentrations, failed to induce this morphologically unique cell type. It is reasonable to speculate that cellular forms devoid of apparent DNA, akin to platelets, have a shortened life span, which may contribute in a unique way to the dramatic effect of NO-NSAIDs on cell kinetics.

All three NO-NSAIDs were superior to their parent NSAIDs in affecting the parameters we studied. However, they differed substantially among themselves when compared on an equimolar basis; NO-ASA was clearly the most effective in all respects. Given the structural complexity of these molecules and the paucity of information on the biological role of each of their structural components (traditional NSAID, spacer, −NO<sub>2</sub>), it is difficult to deduce plausible structure-activity correlates that could account for such pronounced differences. Remarkably, of the three parent NSAIDs, ASA has the highest IC<sub>50</sub> followed by ibuprofen and sulindac. However, of the NO derivatives, NO-ASA has by far the lowest IC<sub>50</sub>.

It is unclear at this point what accounts for the enhanced effectiveness of NO-NSAIDs compared with their NSAID counterparts. The magnitude of their enhanced activity, especially that seen with NO-ASA, is difficult to ascribe to simple changes in the physical properties of each NSAID. The spacer part of the molecule and the −NO<sub>2</sub> group, which releases NO, must contribute to this effect. Our preliminary data indicate that these compounds do not block PG synthesis, at least at concentrations that inhibit cell growth. Combined with their effect on HCT-15 cells that lack both COX isozymes (12), this suggests that the effect of NO-NSAIDs does not necessarily involve inhibition of COX, the classical target of traditional NSAIDs and of the selective COX-2 inhibitors. Rather, it appears likely that NO-NSAIDs act on targets beyond COX and may use novel modes of action. Such a notion has been considered for traditional NSAIDs as well (13).

An important biological question is whether NO-NSAIDs maintain their superior performance in vivo. Although it is difficult to extrapolate results from cultured cells to animal systems and humans, a recent study suggested that NO-NSAIDs may be more effective in vivo than traditional NSAIDs. The chemopreventive effect of aspirin versus a NO-ASA derivative (NCX 4016; a positional isomer of the one reported here) was assessed using a rat azoxymethane model of colon cancer (14). Whereas ASA reduced the number of aberrant crypt foci, the putative neoplastic lesions of the colon, by 64%; NO-ASA reduced them even more (85%). Further work, some of it already in progress, will assess this critical question. Of interest, it was recently reported that low-dose ASA (<150 mg) used for cardiac prophylaxis failed to protect patients from colon cancer (15). NO-NSAIDs, with their potential for enhanced effectiveness against colon cancer, may provide double protection against coronary artery disease and colon cancer.

In conclusion, our data demonstrate that a novel class of NSAID derivatives, the NO-NSAIDs, which promise to be less toxic than traditional NSAIDs, affects colon cancer cell kinetics in vitro in a way consistent with a chemopreventive effect. In this respect, NO-NSAIDs are severalfold more powerful than traditional NSAIDs. Their potential role in human colon cancer prevention remains to be established.

**Acknowledgments**

We thank Dr. Z. Darzynkiewicz for helpful discussions, Jan Kunicki for assistance with flow cytometry, and Dr. P. Del Soldato (Nicox, SA, Nice, France) for providing the NO-NSAIDs and for helpful discussions.

**References**

Nitric Oxide-releasing Nonsteroidal Anti-inflammatory Drugs (NSAIDs) Alter the Kinetics of Human Colon Cancer Cell Lines More Effectively than Traditional NSAIDs: Implications for Colon Cancer Chemoprevention

Jennie L. Williams, Simona Borgo, Izhar Hasan, et al.

Cancer Res 2001;61:3285-3289.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/8/3285

Cited articles
This article cites 13 articles, 1 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/8/3285.full.html#ref-list-1

Citing articles
This article has been cited by 25 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/61/8/3285.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.