

Chemopreventive Efficacy of Naproxen and Nitric Oxide–naproxen in Rodent Models of Colon, Urinary Bladder, and Mammary Cancers

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Abstract

Nonsteroidal anti-inflammatory drugs (NSAID) have been highly effective in preventing colon, urinary bladder, and skin cancer preclinically, and also in clinical trials of colon adenoma formation. However, certain NSAIDs cause gastrointestinal ulceration and may increase cardiovascular events. Naproxen seems to cause the lowest cardiovascular events of the common NSAIDs other than aspirin. Nitric oxide (NO)-naproxen was tested based on the finding that adding a NO group to NSAIDs may help alleviate GI toxicity. In the azoxymethane-induced rat colon aberrant crypt foci (ACF) model, naproxen administered at 200 and 400 ppm in the diet reduced mean ACFs in the colon by about 45% to 60%, respectively. NO-naproxen was likewise administered in the diet at roughly equimolar doses (300 and 600 ppm) and reduced total ACF by 20% to 40%, respectively. In the hydroxybutyl (butyl) nitrosamine rat urinary bladder cancer model, NO-naproxen was given at 183 or 550 ppm in the diet, and naproxen at 128 ppm. The NO-naproxen groups had 77% and 73% decreases, respectively, in the development of large urinary bladder tumors, whereas the 128 ppm naproxen group also showed a strong decrease (69%). If treatments were started 3 months after hydroxybutyl (butyl) nitrosamine, NO-naproxen (550 ppm) and naproxen (400 ppm) were also highly effective (86–94% decreases). In the methylnitrosourea-induced mammary cancer model in rats, NO-naproxen and naproxen showed nonsignificant inhibitions (12% and 24%) at 550 and 400 ppm, respectively. These data show that both naproxen and NO-naproxen are effective agents against urinary bladder and colon, but not mammary, carcinogenesis.

Perhaps the most strikingly positive group of agents tested to prevent colon, urinary bladder, and skin cancers in experimental animal models are the various specific and nonspecific cyclooxygenase (COX) inhibitors. It was shown over 20 years ago that nonspecific nonsteroidal anti-inflammatory drugs (NSAID) were highly effective in the azoxymethane (AOM)-induced rat colon cancer model and the hydroxybutyl (butyl) nitrosamine (OH-BBN)-induced rodent urinary bladder cancer model. Several years ago, our laboratories found that celecoxib, which preferentially inhibits COX-2 activity, was similarly effective in these models (1, 2). These latter findings helped validate COX-2 as a potential target for chemopreven-

tion in a number of organs, including skin cancer (3–5). The studies also helped to support clinical trials in the prevention of colon adenomas and skin cancer (6).

Although the clinical chemoprevention results with celecoxib in the colon and skin seem promising, they must be balanced against the increase in cardiovascular (CV) problems associated with the selective COX-2 inhibitors (7). Although these CV problems are clearly associated with rofecoxib at its standard dose, major problems associated with the standard dose of celecoxib are less clear, although higher doses do yield an increase in CV events. Naproxen, which seems to have fewer CV effects than most NSAIDs, might prove to be an alternative. However, a second and more common side effect of NSAIDs is gastric bleeding, perhaps to a slightly lesser degree with specific COX-2 inhibitors. In an attempt to overcome this adverse effect, nitric oxide (NO)-linked derivatives have been synthesized with various NSAIDs (7, 8). It is clear, both in preclinical studies and in more limited human studies, that this class of agents does have gut-sparing effects (9–11). Our laboratories have reported that NO-aspirin is effective in preclinical models of colon cancer (12).

The three cancer models used in the present studies are relatively standard, and have been used by numerous investigators to screen for potential chemopreventive agents. The animals in the OH-BBN-induced urinary bladder cancer model

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develop large invasive tumors that show many of the gene and protein expression changes associated with invasive bladder cancer in humans (2). The rats in the methylnitrosourea (MNU)-induced mammary cancer model develop minimally invasive estrogen receptor-positive (ER⁺) mammary cancers that by array analysis appear similar to well-differentiated human estrogen receptor-positive breast cancer (13). This model has been shown to respond to a wide variety of potential chemopreventive agents including hormonal manipulations (selective estrogen receptor modulators, aromatase inhibitors, and ovariectomy) that significantly inhibit the development of human estrogen receptor-positive breast cancer. Additionally, the AOM-induced aberrant crypt foci (AOM-ACF) assay (which examines the development of aberrant crypts) was used because it was felt to be a precursor of colon cancer in rats (14). The ACF end point has been used to screen for a wide range of agents and has previously shown a high concordance with animal colon tumor assays. The drugs were administered either shortly after the last carcinogen treatment (AOM-ACFs, MNU-mammary) or in the OH-BBN bladder model both 2 weeks after the last OH-BBN treatment and 3 months later when precancerous lesions were already present.

It is now well recognized that NSAIDs and, more recently, COX-2 preferential inhibitors are highly effective in preventing the development of urinary bladder cancer in rodent models. Most of the earlier studies initiated treatment with the chemopreventive agents beginning with the first dose of carcinogen administration. We reported with celecoxib, however, that administration beginning after the final OH-BBN administration was equally effective, implying that most of the preventive effect of NSAIDs was later in cancer development (2). This later administration of NSAIDs both overcomes questions regarding alterations in OH-BBN metabolism and more closely parallels the timing of a likely clinical intervention where treatment will be initiated later in tumor progression. Our laboratories have used the late intervention protocol as a standard for testing this class of chemopreventive agents.

Materials and Methods

Reagents

Naproxen (Fig. 1) was obtained from Sigma and NO-naproxen was provided by the National Cancer Institute Division of Cancer Prevention. The carcinogen OH-BBN was purchased from TCI America,

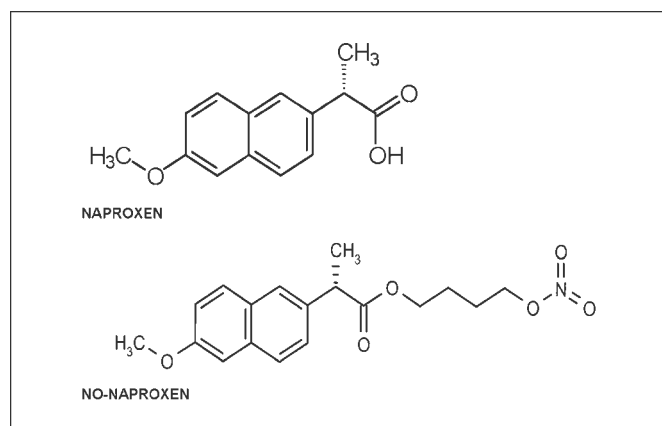


Fig. 1. Molecular structures of naproxen and NO-naproxen.

whereas MNU and AOM were purchased from Midwest Research Institute. Rats for the urinary bladder and mammary study were obtained from Harlan Sprague-Dawley, Inc. Rats for the colon studies were purchased from Harland Barrier. The diets were purchased either from Teklad (Harlan Teklad) or Bioserv, and tap water was provided *ad libitum*. Diets for colonic ACF assay were based on the modified AIN-76A containing 5% corn oil by weight (American Institute of Nutrition). Chemopreventive agents used in the colon model were premixed with a small quantity of casein and then blended into bulk diet using a Hobart Mixer. In the urinary bladder and mammary cancer studies, naproxen was mixed directly into the diet (Teklad mash diet), whereas NO-naproxen was premixed with ethanol, trioctanoin, and corn oil (12, 9, and 9 g/kg diet, respectively) to ensure a homogeneous mixture in the feed. Both control and experimental diets were prepared weekly and stored in a cold room. Agents content in the experimental diets were determined periodically in multiple samples taken from the top, middle, and bottom portions of individual diet preparations to verify uniform distribution. Rats were allowed *ad libitum* access to the respective diets.

Rat colon ACF model

Seven-week-old male Fischer-344 rats were randomized into groups ($n = 18$ rats/group) and fed AIN-76A control diet. At 8 wk of age, rats intended for carcinogen treatment were injected s.c. with AOM at a dose of 15 mg/kg BW once weekly for 2 wk, and those intended for vehicle treatment received an equal volume of normal saline. Three days after the second AOM treatment, groups of rats were fed either the control diet or experimental diet containing 200 or 400 ppm naproxen and 300 or 600 ppm NO-naproxen. These dietary regimens were continued until termination of the experiment, i.e., 8 wk after the second AOM treatment. Rats were sacrificed by CO₂ euthanasia, and all organs were examined grossly. Colons were then evaluated for ACF. For this evaluation, they were opened lengthwise from the anus to the caecum, and then fixed flat with mucosa on the upper side between filter papers in 10% buffered formalin.

Quantification of ACF

Topographical analysis of the colonic mucosa was done according to Bird (14) and is routinely performed in our laboratory (15). After a minimum of 24 h, fixed colons were stained with 0.2% methylene blue solution for 5 to 10 min, placed mucosal side up on a microscopic slide, and viewed under a light microscope. The total number of ACF in the entire colon was determined in every 2-cm section of the colon, starting from the distal (taken as 0 cm) to the proximal end of the colon. Aberrant crypts were distinguished from the surrounding normal crypts by their increased size, increased distance from lamina to basal surfaces of cells, and easily discernible pericryptal zone. The parameters used to assess the aberrant crypts were incidence and multiplicity. Aberrant crypt multiplicity was determined as the number of crypts in each focus and categorized as small (if less than four crypts/focus) or large (if containing four or more aberrant crypts/focus). ACF data are reported as mean \pm SEM. Statistical differences between control and treated groups were evaluated using unpaired *t* test with Welch's correction (to correct for unequal group variances). Differences between groups were considered significant at $P < 0.05$.

Rat urinary bladder model

The rat OH-BBN-induced urinary bladder model was performed as previously described (2, 16). Briefly, female Fischer 344 rats were obtained from Harlan Sprague-Dawley, Inc., at 4 wk of age. Beginning at 56 d of age, the rats were treated twice a week with 150 mg OH-BBN/gavage for 8 wk. There were 29 to 30 rats per group. During the studies, the rats were weighed once per week and palpated for bladder tumors biweekly. Diet supplementation with naproxen (400 or 128 ppm) or NO-naproxen (550 or 183 ppm) was initiated either 2 wk or 3 mo after the end of the OH-BBN treatments. The studies were terminated 7 or 8 mo after the last of the OH-BBN treatments. At necropsy, urinary bladders with lesions were excised and weighed. The

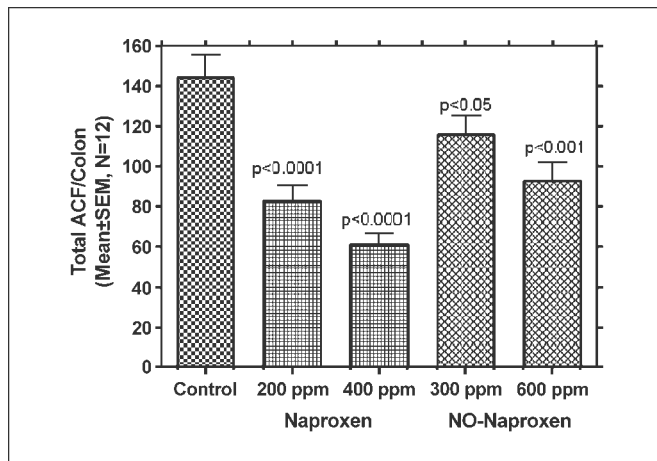


Fig. 2. Effects of naproxen and NO-naproxen on AOM-induced colonic ACF in Fischer-344 rats. *P* values noted above bars are compared with control.

weights and the number of rats with large tumors (≥ 200 mg) were recorded as end points for cancer prevention. Histopathology of all large tumors showed that they were carcinomas. A bladder with no lesions would weigh ~80 to 90 mg. Statistical analysis of bladder weights were performed by the Wilcoxon rank test and the final incidence of large urinary bladder cancers were analyzed by the Fischer's exact test (2, 16).

Rat mammary model

The MNU-induced rat mammary cancer model was performed as previously described (16). Female Sprague-Dawley rats were obtained at 4 wk of age from Harlan Sprague-Dawley, Inc. The carcinogen, MNU, was injected i.v. (75 mg/kg BW) via the jugular vein when the animals were 50 d of age. Each group had 15 rats. Following MNU, the rats were weighed 1x/wk and palpated 2x/wk for the development of mammary cancers. Because the tumors are close to the skin, they can be palpated by experienced technicians as early as 2 mm in size. All palpable tumors are recorded on a diagram of a rat in a logbook such that after histologic classification the latency period of the cancers can be determined. The incidence, multiplicity, and weight data in Table 2 reflect only cancers. The agents (400 ppm naproxen or 550 ppm NO-naproxen) were administered in the diet beginning 5 d after carcinogen treatment and continued until the end of the experiment (148 d after MNU). The mammary cancer incidence and multiplicity were analyzed using Logrank analysis and the Armitage test, respectively (17).

Results

Inhibition of colon carcinogenesis in AOM treated rats

In the AOM-induced rat colon ACF model, both naproxen and NO-naproxen were tested, at equimolar doses, and were shown to inhibit both the total number of ACF per colon and the number of multicrypt foci per colon (Figs. 2 and 3). These doses are, in fact, below the standard clinical dose of naproxen using standard animal/human scaling factors. In this assay, the parent compound (naproxen) seemed to be slightly more effective in inhibiting ACF than the NO-naproxen, but the differences were not significant. Naproxen at 200 and 400 ppm inhibited ACFs per colon by 40% and 60%, respectively, whereas NO-naproxen at 300 and 600 ppm inhibited 20% and 40%, respectively. If one considers only the larger multicrypt ACF, then the naproxen decreased larger ACFs by 55% (200 ppm) and 70% (400 ppm), and NO-naproxen reduced

large ACFs by 30% (300 ppm) and 50% (600 ppm). Importantly, both agents showed dose-dependent suppression of AOM-induced rat colonic ACF formation.

Inhibition of bladder cancer in OH-BBN-treated rats

Both early and late intervention protocols were used to examine the cancer preventive efficacy of naproxen and NO-naproxen. When administration of the agents at equimolar doses was started early (or 2 weeks post-OH-BBN), reductions in the incidence of large urinary bladder cancers were reduced by 77% for NO-naproxen at 183 ppm, 73% for NO-naproxen at 550 ppm, and 69% for naproxen at 128 ppm when compared with the controls (Table 1). Thus, the results for NO-naproxen showed a slightly higher inhibition than the parent compound. The final body weights were not significantly changed for any experimental group. Subsequently, both naproxen and NO-naproxen were tested when treatment was initiated at 3 mo after the last OH-BBN treatment. Note that the naproxen dose was higher (400 ppm) than that used in the first study (128 ppm). We used two high equimolar doses for comparison in this later intervention study, since typically efficacy is lower with later treatments. At this time, all the animals had preinvasive lesions (mostly hyperplastic) and roughly 70% had small, established cancers. Animals were then followed to our normal end points of incidence of large tumors and final bladder weights. With the later treatment, naproxen (400 ppm) reduced large tumors 94% and the NO-naproxen (550 ppm) caused a 86% reduction (Table 1). A 54% and 58% reduction in mean urinary bladder weights was also observed in the naproxen and NO-naproxen groups, respectively. These data consistently showed little differential effect between naproxen and NO-naproxen with respect to efficacy in preventing bladder tumors.

Inhibition of mammary cancer in MNU-treated rats

Both naproxen and NO-naproxen showed a nonsignificant preventive effect on MNU-induced rat mammary cancers (Table 2). The final body weights were not significantly changed for any experimental group. Naproxen at 400 ppm in the diet reduced the mammary cancer incidence by 27%, whereas NO-naproxen at 550 ppm reduced the incidence only

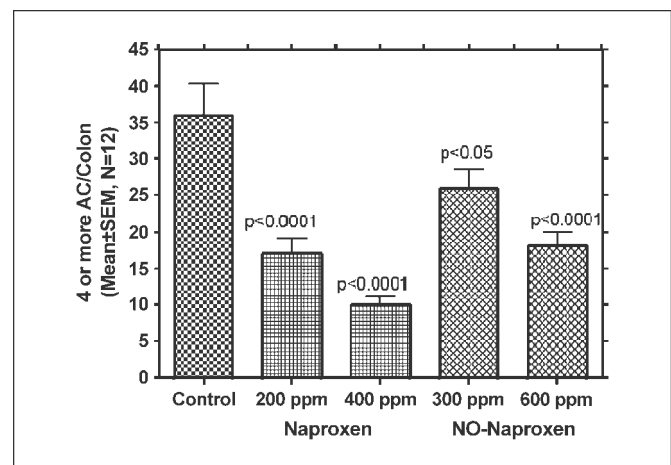


Fig. 3. Effect of naproxen and NO-naproxen on AOM-induced multicrypt foci in Fischer-344 rats. *P* values noted above bars are compared with control.

Table 1. Weights of urinary bladders with OH-BBN induced lesions in Fischer-344 rats treated with naproxen and NO-naproxen

Treatment*	Mean final body weights (g)	Urinary bladder + lesions (mg) [†]		Number of large urinary bladder cancers [‡]
		Mean	% decrease	
Agent started 2 wk post-OH-BBN				
NO-naproxen, 550 ppm in diet	232	186 ± 22	(71% ↓) [§]	7/30 (73% ↓) [§]
NO-naproxen, 183 ppm in diet	231	161 ± 11	(75% ↓) [§]	6/30 (77% ↓) [§]
Naproxen, 128 ppm in diet	225	217 ± 32	(66% ↓) [§]	8/30 (69% ↓) [§]
None	220	641 ± 104		25/29
Agent started 3 mo post-OH-BBN				
NO-naproxen, 550 ppm in diet	227	136 ± 7	(58% ↓) [§]	2/29 (86% ↓) [§]
Naproxen, 400 ppm in diet	226	148 ± 15	(54% ↓) [§]	1/30 (94% ↓) [§]
None	224	323 ± 33		15/30

*Rats received OH-BBN beginning at 56 d of age. Treatment with naproxen and NO-naproxen initiated either 2 wk or 3 mo after the final OH-BBN treatment; studies terminated 8 and 7 mo after final OH-BBN, respectively.

[†]Urinary bladder plus lesion weights were those observed at sacrifice of the rats. Values are means ± SEM (number in parenthesis is percent decrease from control).

[‡]Values represent the number of rats with urinary bladder weights of ≥200 mg divided by the number of rats in the group. Number in parenthesis is percent decrease from control.

[§]Significantly different ($P < 0.01$) from the respective control group.

13%. Neither reduction was significantly different from the controls. The average number of tumors/rat was reduced by 24% in the naproxen treated rats, whereas only a 12% reduction was seen in the NO-naproxen-treated rats (Fig. 4). Again, neither result was significantly different from control tumor multiplicity. The weight of the mammary tumors was statistically different from the controls in rats receiving NO-naproxen, although the biological significant is questionable in lieu of there being no effect on cancer incidence or multiplicity.

Discussion

In preclinical animal models, both a variety of NSAIDs and the COX-2 inhibitor celecoxib have been uniformly effective against carcinogen-induced colon and urinary bladder cancers (18–22). For a variety of reasons, including the fact that COX-2 is presumably a major chemopreventive target and that it might have fewer gastric effects, celecoxib progressed more

rapidly than other NSAIDs to large-scale clinical trials. However, when examined in a large placebo-controlled trial at doses beyond those normally recommended, celecoxib increased the incidence of CV events starting ~18 months after the initiation of treatment (23). These results, and the even more striking results with rofecoxib, raised the possibility that most NSAIDs might increase CV incidents. At this time, it is felt that the nonspecific NSAID naproxen might have the least CV effects of any of the NSAIDs. Results of studies with naproxen have been mixed, with certain studies showing limited increases in CV risk relative to placebo and certain studies showing CV protection (24, 25). Nevertheless, it has consistently caused fewer CV effects than any of the other NSAIDs and may even be cardioprotective (26, 27). In addition, a recent American Gastroenterological Association panel has recommended the use of naproxen for persons of moderate CV risk based on the initial estimation that this agent might have fewer CV problems than other NSAIDs (27). This was

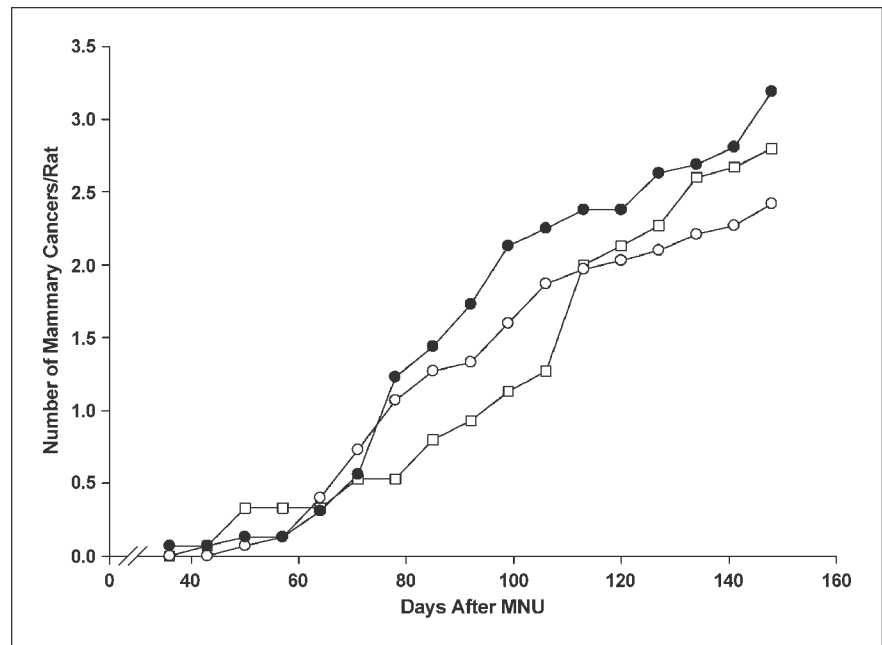
Table 2. Effects of NO-naproxen and naproxen against MNU-induced mammary cancer

Treatment*	Mean final body weights (g)	Mammary cancers [†]		
		% incidence	Number/rat	Mean weight/rat (g)
NO-naproxen, 550 ppm in diet	240	87	2.80 (12% ↓)	3.80 (59% ↓)
Naproxen, 400 ppm in diet	251	73	2.42 (24% ↓)	10.61 (13% ↑)
None	251	100	3.19	9.38

*MNU administered to rats (15/group) at 50 d of age. Chemopreventive agents initiated at 55 d of age.

[†]Incidence, number, and weight of mammary tumors at end of study (148 d after MNU). Number in parenthesis is percent decrease from control.

Fig. 4. Number of mammary cancers in MNU-treated rats following administration of NO-naproxen or naproxen. □, NO-naproxen, 550 ppm in diet; ○, naproxen, 400 ppm in diet; ●, Control. Data points are based on palpation data except the last 148-d point, in which the cancers were verified histologically.



our rationale for evaluating the efficacy of naproxen. Interestingly, this agent has infrequently been used in preclinical chemoprevention studies. The second major toxicity associated with the use of NSAIDs is the development of gastric lesions in susceptible individuals. To address this problem, we examined NO-naproxen because there is both preclinical and clinical data showing that the NO-NSAIDs have lower gastric toxicity than the parent NSAIDs (9, 10).

One of the major concerns regarding doses used in animal studies is their comparability to human doses. Although one might attempt to compare serum levels in animals and humans, this is extremely difficult when the agent is administered by diet. A reasonable approach is to use general scaling factors. The doses of naproxen used in the rat studies were between 128 and 400 ppm in the diet, which is equivalent to 12 and 36 mg/kg body weight. Using a 6-fold scaling factor to extrapolate rat doses to human doses, this would be equivalent to human doses of 2 and 6 mg/kg. Such doses would translate to human doses of 160 and 480 mg in a human weighing 80 kg. These doses are comparable with a single naproxen dosing in humans of approximately 225 and 500 mg, which would correspond to one or two standard doses of 225 mg/day. It is important to note that in the studies described here, we found that doses less than the standard human equivalent dose were still highly effective in preventing both colon and urinary bladder cancers. These doses can be compared with the classic doses of celecoxib used in animal studies (1,000-1,500 ppm), which are approximately five to eight times higher than the recommended human dose using similar scalings. The possibility that naproxen is effective at significantly less than the standard human dose (although interesting), would require detailed dose reduction studies to confirm.

The reduction in ACF per colon by naproxen and NO-naproxen are comparable with that normally seen with a variety of NSAIDs. In our studies, the parent compound naproxen inhibited total ACFs slightly more than NO-naproxen, but both were moderately (40-60% inhibition) effective. When

measuring only larger multicrypt foci, the effect is more pronounced (50-70% inhibition); again, the naproxen was slightly more effective than the NO-naproxen. These two NSAID compounds were effective in the colon because the inflammatory process is proposed to drive the cancer process in this organ. The ACF has been recognized as an early preneoplastic lesion of colon cancer (28, 29). It is generally observed that agents that inhibit colonic ACF formation will similarly exhibit chemopreventive activity against colon cancer (30). Previous studies have shown that reduction of colon crypt multiplicity of four or more ACFs/focus has been a consistent predictor of colon tumor inhibition (31, 32).

In the OH-BBN urinary bladder model, both naproxen and NO-naproxen were highly active in blocking the development of urinary bladder cancers. Again in bladder cancer the inflammatory process is known to promote cancer development. Clinical trials with NSAIDs are ongoing. Most importantly, these agents were still highly effective even when the administration was initiated after microscopic cancers already existed. This implies that most of the preventive activity seen in early intervention studies may be actually due to efficacy late in the study. Furthermore, these results show the promise that these agents may be preventive in a late or adjuvant clinical setting. This is particularly important in view of the high recurrence rate for human bladder cancer.

The mammary findings are not surprising, and replicate what is typically seen for NSAIDs in the MNU-induced rat mammary cancer model, i.e., limited to moderate activity for most NSAIDs and COX-2 inhibitors (33). The data show modest nonsignificant reductions in mammary cancer incidence and multiplicity. Because the cancer process in the mammary gland is not driven to a large extent by the inflammatory process, such agents would likely have little effect in human breast cancer prevention. This study also shows that one does not see a striking effect due to any potential release of NO in the animals.

Overall, these data provide strong evidence for the continued development of naproxen and NO-naproxen, especially

as chemopreventive agents for colon (where a variety of NSAIDs have proven clinically effective) and urinary bladder cancer. The lack of effects of these agents on mammary cancers is consistent with previous data from our laboratories regarding NSAIDs. Identifying NSAIDs that have minimal CV or GI toxicity will allow these compounds to be used clinically in the prevention of colon, urinary bladder, and skin cancer.

References

- Reddy BS, Hirose Y, Lubet RA, et al. Chemoprevention of colon cancer by specific cyclooxygenase-2 inhibitor, celecoxib, administered during different stages of carcinogenesis. *Cancer Res* 2000;60:293–7.
- Grubbs CJ, Lubet RA, Koki AT, et al. Celecoxib inhibits N-butyl-N-(4-hydroxybutyl)-nitrosamine-induced urinary bladder cancer in male B6D2F1 mice and female Fischer-344 rats. *Cancer Res* 2000;60:5599–602.
- Fischer SM, Lo HH, Gordon GB, et al. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, and indomethacin against ultraviolet light-induced skin carcinogenesis. *Mol Carcinog* 1999;25:231–40.
- Zhi H, Wang L, Zhang J, et al. Significance of COX-2 expression in human esophageal squamous cell carcinoma. *Carcinogenesis* 2006;27:1214–21.
- Zhan H, Zheng H. The role of topical cyclo-oxygenase-2 inhibitors in skin cancer: treatment and prevention. *Am J Clin Dermatol* 2007;8:195–200.
- Bertagnoli MM, Eagle CJ, Zauber AG, et al. APC Study Investigators. Celecoxib for the prevention of sporadic colorectal adenomas. *N Engl J Med* 2006;355:873–84.
- Zhang J, Ding EL, Song Y. Adverse effects of cyclooxygenase 2 inhibitors on renal and arrhythmia events: meta-analysis of randomized trials. *JAMA* 2006;296:1619–32.
- Rigas B, Kashfi K. Nitric-oxide-donating NSAIDs as agents for cancer prevention. *Trends Mol Med* 2004;10:324–30.
- Lohmander LS, McKeith D, Svensson O, et al; for the STAR Multinational Study Group. A randomized placebo-controlled, comparative trial of the gastrointestinal safety and efficacy of AZD3582 versus naproxen in osteoarthritis. *Ann Rheum Dis* 2005;64:449–56.
- Wilder-Smith CH, Jonzon B, Fornstedt-Wallin B, Hedman A, Karlsson P. Dose-effect comparisons of the CINOD AZD3582 and naproxen on upper gastrointestinal tract mucosal injury in healthy subjects. *Scand J Gastroenterol* 2006;41:264–73.
- Wallace JL, Viappiani S, Bolla M. Cyclooxygenase-inhibiting nitric oxide donors for osteoarthritis. *Trends Pharmacol Sci* 2009;30:112–7.
- Rao CV, Reddy BS, Steele VE, et al. Nitric oxide-releasing aspirin and indomethacin are potent inhibitors against colon cancer in azoxymethane-treated rats: effects on molecular targets. *Mol Cancer Ther* 2006;5:1530–8.
- Chan MM, Lu X, Merchant FM, et al. Gene expression profiling of NMU-induced rat mammary tumors: cross species comparison with human breast cancer. *Carcinogenesis* 2005;26:1343–53.
- Bird RP. Role of aberrant crypt foci in understanding the pathogenesis of colon cancer. *Cancer Lett* 1995;93:55–71.
- Janakiram NB, Rao CV. Molecular markers and targets for colorectal cancer prevention. *Acta Pharmacol Sin* 2008;29:1–20.
- Lubet RA, Yang CS, Lee MJ, et al. Preventive effects of Polyphenon E on urinary bladder and mammary cancers in rats and correlations with serum and urine levels of tea polyphenols. *Mol Cancer Ther* 2007;6:2022–8.
- Freedman LS, Midthune DC, Brown CC, Steele VE, Kelloff GJ. Statistical analysis of animal cancer chemoprevention experiments. *Biometrics* 1993;49:259–68.
- Rao CV, Reddy BS. NSAIDs and chemoprevention. *Curr Cancer Drug Targets* 2004;4:29–42.
- Rao CV, Rivenson R, Simi B, et al. Chemoprevention of colon carcinogenesis by sulindac, a nonsteroidal anti-inflammatory agent. *Cancer Res* 1995;55:1464–72.
- Reddy B, Rao CV, Rivenson A, Kelloff G. Inhibitory effect of aspirin on azoxymethane-induced colon carcinogenesis in F344 rats. *Carcinogenesis* 1993;14:1493–97.
- Sabichi AL, Lippman SM. COX-2 inhibitors and other nonsteroidal anti-inflammatory drugs in genitourinary cancer. *Semin Oncol* 2004;31:36–44.
- Steele VE, Hawk ET, Viner JL, Lubet RA. Mechanisms and applications of non-steroidal anti-inflammatory drugs in the chemoprevention of cancer. *Mutat Res* 2003;523–4:137–44.
- Solomon SD, McMurray JJ, Pfeffer MA, et al. Adenoma Prevention with Celecoxib (APC) Study Investigators. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. *N Engl J Med* 2005;352:1071–80.
- ADAPT Research Group. Cardiovascular and cerebrovascular events in the randomized, controlled Alzheimer's Disease Anti-Inflammatory Prevention Trial (ADAPT). *PLoS Clinical Trials* 2006;1:e33. doi:10.1371/journal.pctr.0010033.
- Kearney PM, Baigent C, Godwin J, Halls H, Emberson JR, Patrono C. Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. *BMJ* 2006;332:1302–8.
- Fosbol EL, Gislason GH, Jacobsen S, et al. Risk of myocardial infarction and death associated with the use of nonsteroidal anti-inflammatory drugs (NSAIDs) among healthy individuals: a nationwide study. *Clin Pharmacol Ther* 2009;85:190–7.
- Chan FK, Abraham NS, Scheiman JM, et al. Management of patients on nonsteroidal anti-inflammatory drugs: a clinical practice recommendation from the First International Working Party on Gastrointestinal and Cardiovascular Effects of Nonsteroidal Anti-Inflammatory Drugs and Anti-platelet Agents. *Am J Gastroenterol* 2008;103:2908–18.
- McLellan EA, Bird RP. Aberrant crypts: potential preneoplastic lesions in the murine colon. *Cancer Res* 1988;48:6187–92.
- McLellan E, Medline A, Bird RP. Sequential analyses of the growth and morphological characteristics of aberrant crypt foci: putative preneoplastic lesions. *Cancer Res* 1991;51:5270–74.
- Pretlow TP, O'Riordan MA, Pretlow TG, Stellato TA. Aberrant crypts in human colonic mucosa: putative preneoplastic lesions. *J Cell Biochem Suppl* 1992;16G:55–62.
- Wargovich MJ, Chen CD, Jimenez A, et al. Aberrant crypts as a biomarker for colon cancer: evaluation of potential chemopreventive agents in the rat. *Cancer Epidemiol Biomarkers Prev* 1996;5:355–60.
- Rao CV, Desai D, Simi B, Kulkarni N, Amin S, Reddy BS. Inhibitory effect of caffeic acid esters on azoxymethane-induced biochemical changes and aberrant crypt foci formation in rat colon. *Cancer Res* 1993;53:4182–88.
- Christov K, Grubbs CJ, Shilkaitis A, Juliana MM, Lubet RA. Short-term modulation of cell proliferation and apoptosis and preventive/therapeutic efficacy of various agents in a mammary cancer model. *Clin Cancer Res* 2007;13:5488–96.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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