NSAIDS AND COLORECTAL CANCER

An aspirin a day keeps the (adenomatous) polyp away?

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There is increasing interest in the potential therapeutic efficacy of aspirin, acetylsalicylic acid, as well as other non-steroidal antiinflammatory drugs (NSAIDS). Although NSAIDs have been useful for their analgesic, antipyretic, and antiinflammatory effects, more recent studies have suggested that NSAIDs, and especially aspirin, may also be useful in other conditions. Aspirin, the rather unpretentious derivative from the bark of the willow tree, is now used as an antiplatelet drug for the secondary prevention of occlusive cardiovascular disease, including myocardial infarction, transient cerebral ischemia, and stroke.[1] Aspirin may also be useful in the primary prevention of myocardial infarction, and in the prevention of cataracts, migraines, and vascular dementia.[1] Aspirin is especially receiving much publicity in the lay press as a new wonder drug.[2]

Recent studies suggest that aspirin and the other NSAIDs may have a potential role in the prevention of colorectal cancer and perhaps also other digestive tract cancers. Extensive cell culture experiments and laboratory animal studies, as well as an increasing number of epidemiologic surveys and human studies, including a few clinical trials, have shown promising results.[3]

COLORECTAL CANCER

Colorectal cancer, along with cancer of the lung and breast, are the top three cancer killers in the United States. It has been estimated that in 1994, colorectal cancer will account for 149,000 new cases of cancer and 56,000 deaths.[4] The average American has a 6-7% life time probability of developing colorectal cancer and a 3% probability of dying from the disease.

Table 1

COLORECTAL CANCER

	1994	1993
New Cases	149,000	152,000
Deaths	56,000	57,000

Figure 1. The adenoma-carcinoma sequence

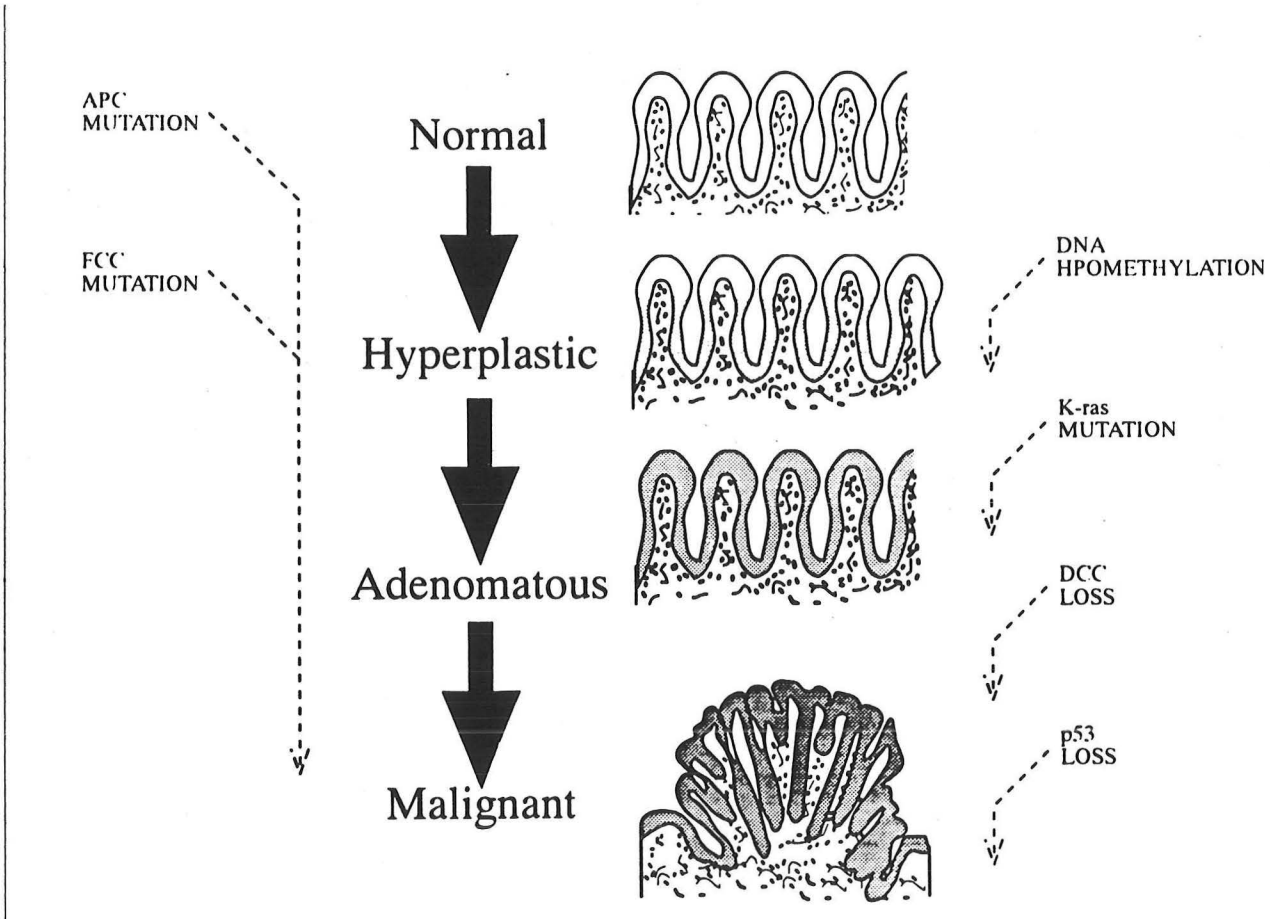


Figure 2. Structures of several common NSAIDs (Ref. 14)

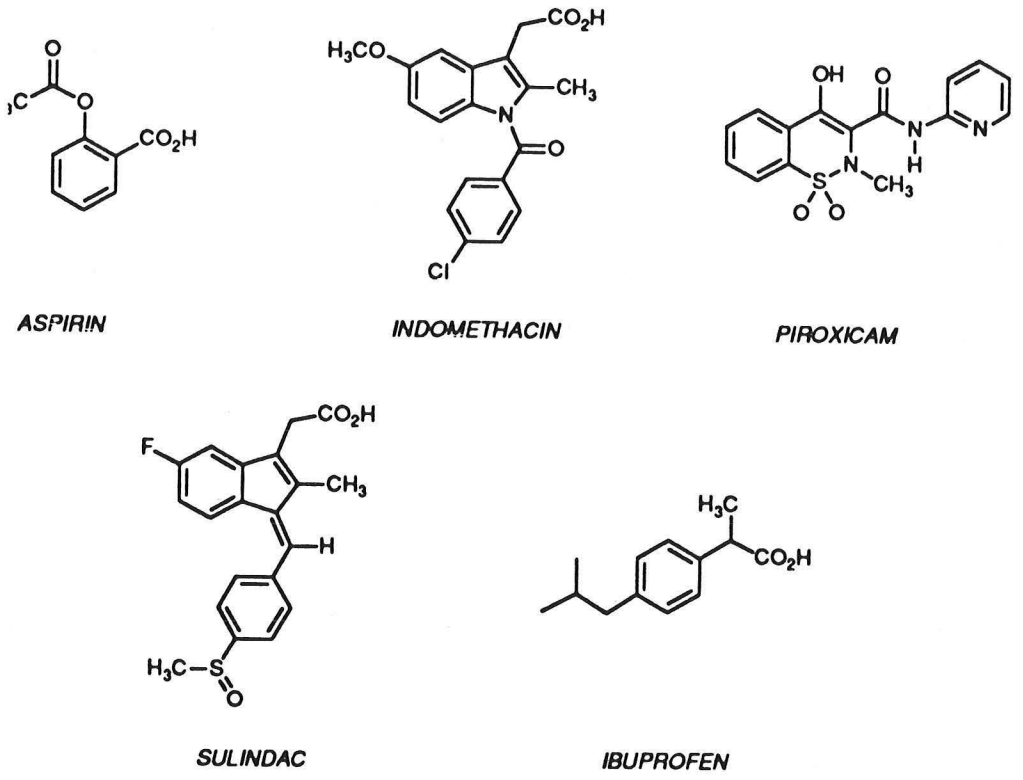
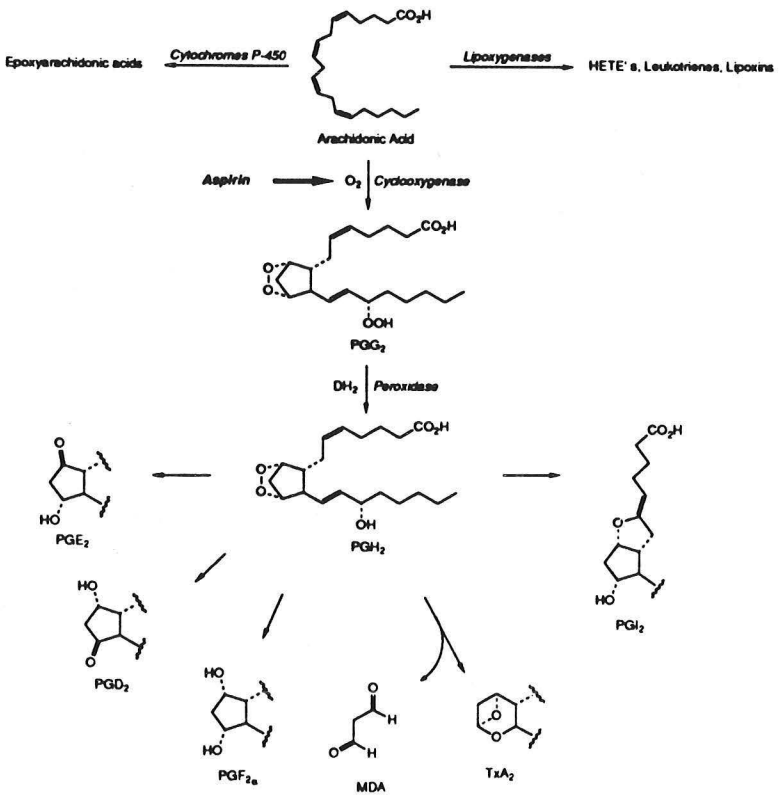


Figure 3. The cyclooxygenase pathway and arachidonic acid metabolism (Ref. 14)



It is estimated that over 6 million Americans who are alive today will die of this disease. Although the frequency of new cases has shown a small gradual decline over the last four decades, especially in women, the case mortality rate has changed little if at all.[4-7] (Table 1)

It is now widely accepted that the adenoma (adenomatous polyp) is the premalignant precursor lesion for colorectal cancer. Several lines of evidence support this adenoma-cancer sequence. These lines of evidence come from epidemiologic surveys of geographic association of adenomas and cancers, similar anatomic distribution of adenomas and cancers, the chronologic time sequence between the appearance of adenomas and cancers, and more recently the demonstration of parallel genetic alterations in adenomas and cancers, and the correlation between progressive accumulation of genetic alterations and mutations and the progression from normal mucosa to adenoma and cancer.(Figure 1) Perhaps the strongest clinical evidence is that patients who are maintained adenoma free by polypectomy are generally kept cancer free. This argument has been supported by cohort studies and intervention trials.[8,9]

With a long latency period (a decade or more) and the evolving understanding of the biology of the adenoma-cancer sequence, there is now increasing scientific and clinical interest in preventing colon cancer, by either the prevention of adenoma formation and recurrence, or by interference with neoplastic progression. Various interventions have been tried using evidence gathered from epidemiologic, animal, and human studies. The most commonly advocated strategies of chemoprevention (the use of drugs, foods, or food components in an attempt to prevent cancer) have included dietary alterations to decrease dietary fat and increase dietary fiber, the addition of vitamins such as vitamin A, C, and E, and micronutrients.[10,11] Much recent attention has also focused on the potential efficacy of NSAIDs.[12,13]

NSAIDS

The NSAIDs, non-steroidal antiinflammatory drugs, have been used for their analgesic, antiinflammatory, and antipyretic effect. Aspirin has been used for decades, and the other agents have been developed more recently. The structures of some of the common NSAIDs are shown.(Fig.2) In addition to their clinical effect, the NSAIDs also share a common property as inhibitors of cyclooxygenase (prostaglandin H synthase), one of the three key enzymes in arachidonic acid metabolism. Arachidonic acid may be converted by epoxygenase to produce epoxy-arachidonic acids. Lipoxygenases convert arachidonic acid eventually to hydroxy fatty acids, leukotrienes, and lipoxins. The cyclooxygenase pathway results in the formation of prostaglandins, thromboxanes, prostacyclin and malondialdehyde.[14] (Fig.3)

It has been recently found that mammalian cells contain two related but distinct cyclooxygenases (COX). The two isozymes share about 75% homology at the mRNA level and 60% homology at the protein level, but the catalytic sites appear to be highly con- served.[15-17] (Fig.4) The enzyme that was discovered first is termed COX-1 (prostaglandin H synthase 1, PGHS-1), and the one discovered more recently is known as COX-2 (PGHS-2).[18,19]

The NSAIDs inhibit COX by binding to the protein. Aspirin not only binds, but covalently modifies the COX protein by acetylating a serine residue at position 529 on COX-1.(Fig.5) It is believed that an analogous process occurs for COX-2 at a serine residue at position 516. The other NSAIDs bind COX, but not covalently , nor do they irreversibly modify the COX protein. Nevertheless, the binding appears to be extremely tight with an

Figure 4. Schematic protein structure of human COX-1 and COX-2 (Ref. 16)

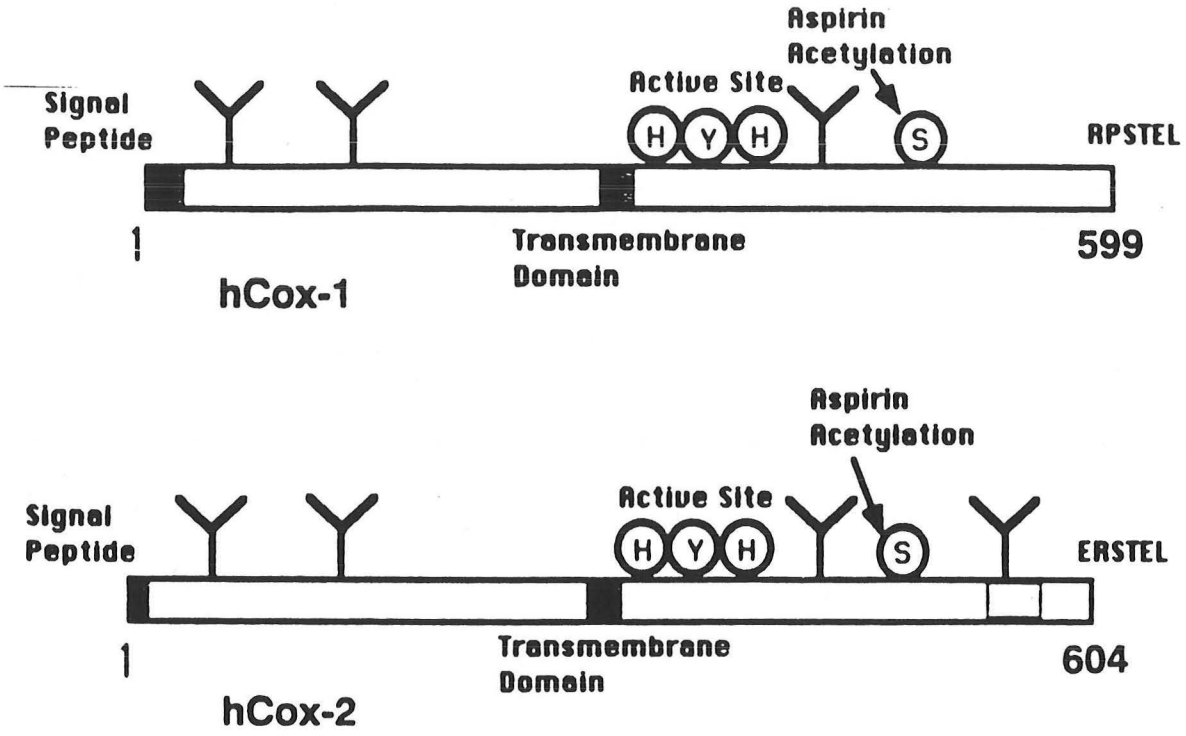


Figure 5. Aspirin acetylates serine 529 on COX-1 (Ref. 1)

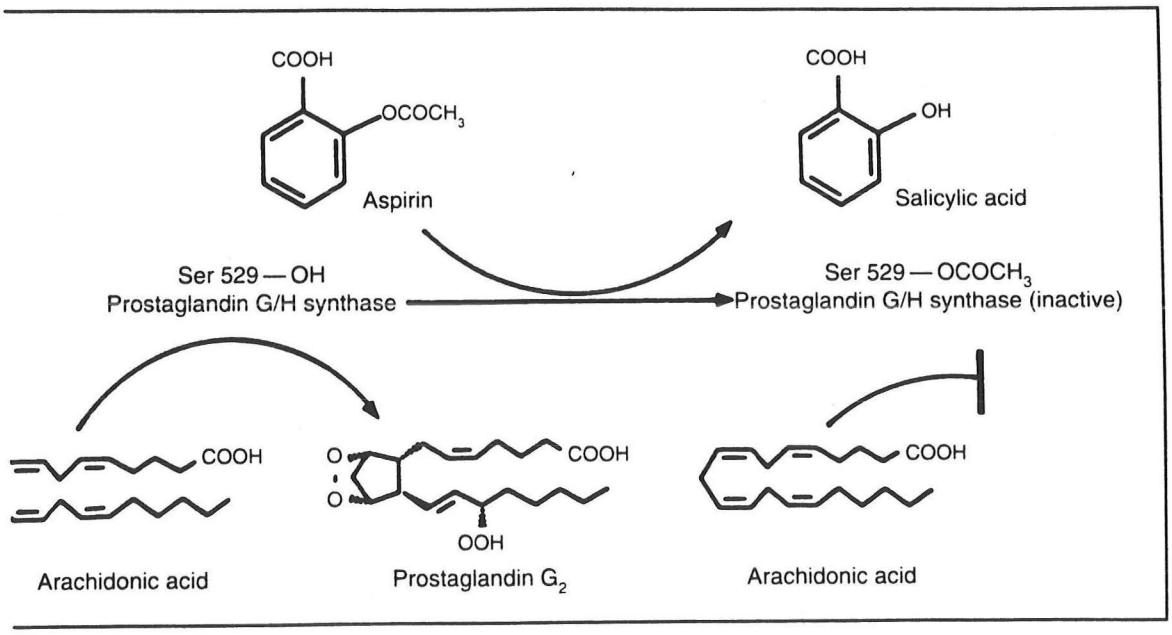
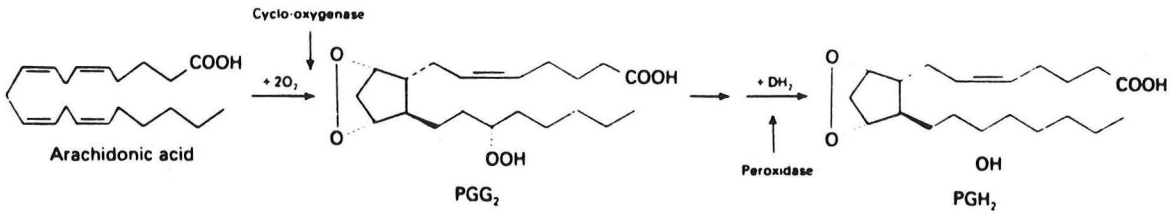


Figure 6. The two enzymatic activities of COX (PGHS) (Ref. 15)



Scheme 1

Consecutive reactions catalysed by PGHS.

Table 2

CYCLOOXYGENASE VS. PEROXIDASE ACTIVITY

Ser-529	CLO	PO	ASA Inhibition
Ser	100%	100%	Yes
Ala	90%	100%	No
Thr	8%	100%	No
Asn	5%	100%	No

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extremely slow dissociation rate. Indomethacin has one of the tightest binding constants and slowest dissociation rate.[18,19]

NSAIDS AND CANCER

Activation of carcinogens

The COX enzyme (more appropriately termed PGH synthase but termed COX in common usage) has two distinct enzymatic activities, cyclooxygenase and prostaglandin hydroperoxidase activities.(Fig.6) For PGHS-1, the cyclooxygenase active site resides near the serine 529 residue, and the hydroperoxidase activity site is near a heme binding site at the histidine 328 position. Several elegant studies including site directed mutagenesis have shown that the cyclooxygenase activity can be lost without significant effect on the hydroperoxidase activity.(Table 2)

The cyclooxygenase activity converts arachidonic acid to prostaglandin G2, a metastable cyclic endoperoxide, which is then reduced by the hydroperoxidase activity to prostaglandin H2, an alcohol. During the early peroxidase reaction, free radicals are produced which have the potential to damage cells. The peroxidase reaction also may metabolize other xenobiotics to reactive products which may be mutagenic or carcinogenic.[20] The COX enzyme has been shown to activate heterocyclic aromatic amines found in food to mutagenic reaction products [21]. The administration of the NSAID indomethacin blocks the activation of the heterocyclic aromatic amine and the associated mutagenic activity.[21] Similarly, aspirin has also been shown to inhibit the peroxidation of colon carcinogens, and suppress the formation of colon cancers induced by these carcinogens.[20,22]

Table 3

NSAID CELLULAR EFFECTS

- Block peroxidation of heterocyclic amines (? block activation of food mutagens)
- G_1-S cell cycle arrest
- Inhibit DNA synthesis
- Inhibit ornithine decarboxylase
- Enhance T-cell proliferation, lymphokine production, T- and NK-cell cytotoxicity
- Enhance immune surveillance

Figure 7. Ornithine decarboxylase and polyamine metabolism (Ref. 36)

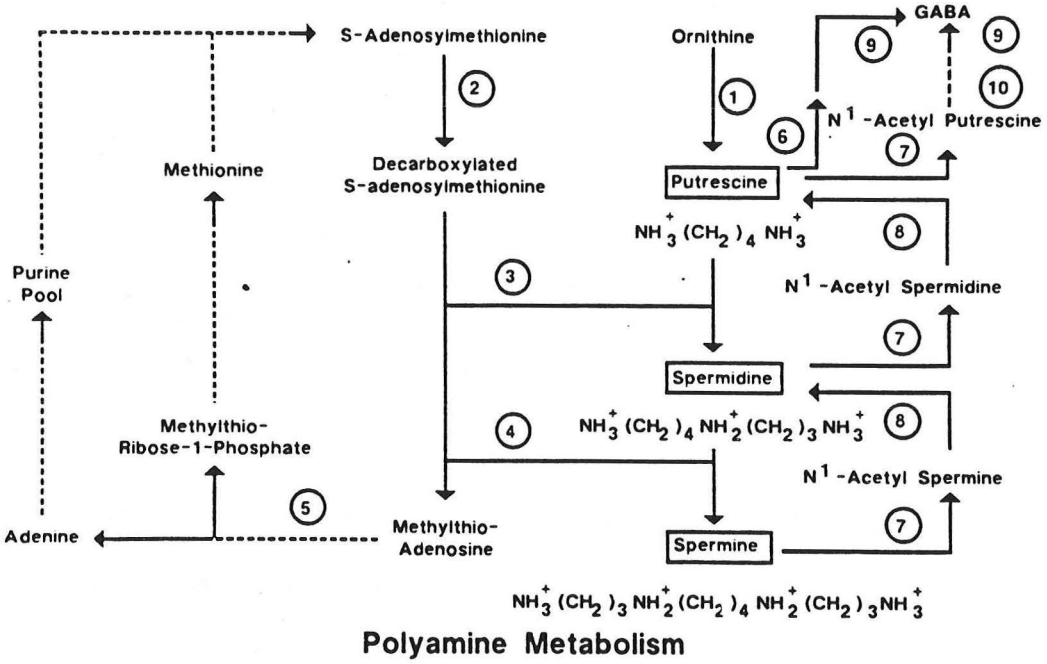


FIGURE 1. Polyamine metabolism. The enzymes indicated are: (1) ornithine decarboxylase (EC 4.1.1.17); (2) *S*-adenosylmethionine decarboxylase (EC 4.1.1.50); (3) spermidine synthase (EC 2.5.1.16); (4) spermine synthase (EC 2.5.1.22); (5) methylthioadenosine phosphorylase (EC 2.4.2.28); (6) diamine oxidase (EC 1.4.3.6); (7) spermidine/spermine-N^1-acetyltransferase; (8) polyamine oxidase; (9) acetyltransferase; (10) aldehyde dehydrogenase. (From Luk, G. D. and Casero, R. A., in *Advances in Enzyme Regulation*, Vol. 26, Webber, G., Ed., Pergamon Press, England, 1987, 91. With permission.)

Cancer cell growth (Table 3)

Several NSAIDs, primarily indomethacin, have been shown to inhibit DNA synthesis and cell growth of tumor cells in culture,[23-26] including murine colon adenocarcinoma [27], murine lung cancer[28], human stomach cancer[29], and human breast cancer cells.[30] In most studies, the effect of NSAIDs was cytostatic and not cytotoxic, cell viability was maintained and growth inhibition was reversible upon removal of NSAIDs. This growth inhibition was subsequently shown to be a result of G_1-S phase arrest during cell cycle progression and suppression of DNA synthesis.[31,32] In addition, NSAIDs have also been shown to inhibit proliferation-associated enzymes such as phosphodiesterase, cyclic AMP protein kinase,[33] pyruvate kinase,[34] and ornithine decarboxylase.[35]

Ornithine decarboxylase (ODC) is the first and often rate-limiting step in polyamine biosynthesis, and has been shown to be critical for neoplastic transformation and carcinogenesis.[36-38](Fig. 7) Administration of carcinogens increases colonic mucosal ODC prior to development of colon tumors,[39] and inhibition of ODC leads to a suppression of tumorigenesis.[40,41] Indomethacin has been shown to inhibit ODC,[35] and this inhibition of ODC may be one mechanism by which NSAIDs suppress colon tumorigenesis.

NSAIDs, other than inhibiting COX at the enzyme protein level, also inhibit the expression of COX mRNA,[42,43] and the expression of ras.[44] Thus NSAIDs appear to be able to inhibit neoplastic cell and tissue growth processes at multiple steps other than the simple depletion of prostaglandins. The precise mechanism by which NSAIDs exert their anti-neoplastic effect on colorectal cancer is under active investigation.[3,14]

Table 4

RODENT COLON CARCINOGENESIS

- Dimethylhydrazine (DMH), methylazoxymethanol (MAM), Azoxymethane (AOM)
- Carcinogen 1 dose or weekly dose x 2 to 12
- Tumors develop by wk 20 to 52 (usu. 26)
- Tumor histology analogous to human adenocarcinoma
- Adenomatous polyp stage poorly defined

Table 5

RODENT COLON CARCINOGENESIS

Initiation phase
Rx within 1 wk of carcinogen

Promotion phase (post-initiation)
Rx >4 wks after carcinogen

Tumor incidence
Percentage of animals with tumors

Tumor multiplicity
Number of tumors/animal

Immune surveillance

The prostaglandins appear to have an immune modulatory role.[45,46] PGE_2 has been shown to suppress T cell proliferation,[47] lymphokine production,[48] T cell mediated cytotoxicity,[47] antibody-dependent cellular cytotoxicity,[49] macrophage activation,[50] and NK cell mediated cytotoxicity.[51] NSAIDs, primarily indomethacin, has been shown to stimulate cellular immune function both in vitro and in vivo.[45,52,53] NSAIDs appear to be able to reverse the immune suppressive effect of high tissue levels of PGE_2.[45] In addition, NSAIDs have also been shown to enhance peripheral T cell proliferation in patients with colorectal cancer.[54]

ANIMAL STUDIES

A large number of animal studies have shown that NSAIDs, most notably indomethacin and piroxicam, suppress chemical carcinogen induced development of colon cancers in rodents. This is manifested by decreases in tumor incidence (percentage of animals with tumors) and multiplicity (tumors per animal), and therefore also total number of tumors in the entire experimental group. (Table 4 and 5)

Studies beginning in the early 1980s, after some of the early work on in vitro cell culture studies described above were published, show that administration of indomethacin in their drinking water[55,56] or by intrarectal administration[57] decreased tumor incidence and multiplicity in rats given the carcinogen azoxymethane or one of its active metabolites. Subsequent studies found that the tumor suppressive effect lasted as long as 40 weeks and that the tumor suppressive effect was present even if indomethacin was administered several weeks after carcinogen administration.[58] These results suggested that the NSAIDs' tumor suppressive effect was not

mediated entirely at the level of carcinogen activation, but might be a result of direct inhibition of tumor initiation and/or progression, or enhanced immune surveillance.

Yet other studies found that the tumor inhibitory effect of indomethacin persisted after the drug was stopped, and in fact persisted even if PGE_2 was administered.[59] It is suggested that NSAIDs have a direct and long lasting (perhaps even irreversible) direct inhibitory effect on tumor progression, propagation, or growth, at least in the rodent model. This direct antitumorigenesis effect was also suggested in other studies that showed a lower tumor incidence, but a similar number, distribution, size, location, and spread of tumors in those fewer tumor-bearing animals in the NSAID treated group.[60] However, other studies by some of these same investigators suggested that the antitumor effect of NSAIDs may be more pronounced at an earlier initiation phase than in later promotion phases, and that earlier studies may have missed the strong initiation effect because of lower carcinogen dose or other technical experimental differences. These studies suggested that the tumor inhibitory effect was partially to totally reversible after stopping indomethacin.[61,62] (Table 6)

More recent studies have utilized the NSAID piroxicam, partly because its long half-life (longer than 50 hrs) allows once daily dosing for animal studies. The results obtained with piroxicam were quite similar to those with indomethacin. Virtually all studies demonstrated a decrease in tumor incidence and multiplicity.[63-69] In addition, studies using sulindac also demonstrated tumor suppression.[70,71] (Table 7) Furthermore, some studies have used combinations of NSAIDs with other potential chemopreventive agents, and have shown antitumor efficacy with combinations with oltipraz,[72] interleukin-2,[73] and the ODC inhibitor, difluoromethylornithine.[65,67,68]

Table 6

NSAID ANIMAL STUDIES - INDOMETHACIN

Study	Design	Incidence	Multiplicity		Ref.
Pollard '80	In	↓	↓		55
Kudo '80	In	↓	↓	(Intrarectal)	57
Pollard '81	In	↓	↓		56
Narisawa '81	In/Pr	↓	↓	(In > Pr)	61
Pollard ' 83	Pr	↓	↓	(>40 wks)	58
Narisawa '83	In/Pr	↓	↓	(In > Pr)	62
Metzger '84	In	↓	-		60
Narisawa '84	In/Pr	↓	↓	(PG ineffective)	59

Table 7

NSAID ANIMAL STUDIES - PIROXICAM

Study	Design	Incidence	Multiplicity		Ref.
Pollard '83	In	↓	↓		63
Pollard '84	In	↓	↓		64
Nigro '86	In/Pr	↓	↓		65
Reddy '87	In/Pr	↓	↓	(Pr> 13 wks)	66
Reddy '90	In/Pr	↓	↓		67
Rao '91	Prev.+	↓	↓	(40% MTD)	68
Reddy '92	Prev.+	↓	↓	(40% MTD)	69

Other than colon cancers, animal carcinogenesis studies have also shown a tumor-inhibitory effect of NSAIDs on stomach cancer,[74] lung cancer,[74] breast cancer,[75,76] bladder cancer,[77,78] and fibrosarcoma.[79] As discussed in the next section, some epidemiologic surveys have suggested a protective effect of NSAIDs against stomach, lung,and breast cancer, along with colorectal cancer. Other than carcinogen-induced animal tumors, the NSAID piroxicam has been shown to decrease the frequency of naturally occurring tumors in dogs - transitional cell carcinomas, squamous cell carcinomas, and mammary adenocarcinomas.[80]

These animal tumor studies of NSAIDs take on added relevance when one considers that many human tumors, especially colorectal carcinomas, have been found to have elevated levels of tumor tissue prostaglandins.[23,81] The depletion of tissue prostaglandin levels by NSAIDs appear to be a plausible mechanism for the antitumor effects of NSAIDs. However, one study suggested that a single dose of indomethacin administered during adolescence (age 29 days) resulted in lower body weight, shorter survival, and increased numbers of tumors.[82] The tumors were testicular Leydig tumors, hepatocellular tumors, and intestinal and colonic adenocarcinomas. Nevertheless, the preponderance of scientific evidence suggests that NSAIDs have an anti-tumor and not a tumor promoting effect.

Other recent studies have focused on the potential chemopreventive effect of NSAIDs in colon tumorigenesis. These studies delayed the administration of NSAIDs for as long as 13 weeks after carcinogen administration, but at a time that was still several months before colon tumors would have eventually appeared.[66,83] These studies have shown colon tumor inhibitory and chemopreventive effects of several NSAIDs, including piroxicam, ibuprofen, ketoprofen [69] as well as aspirin.[22,83] The animal studies with aspirin represent one instance when animal

Table 8

CASE-CONTROL STUDY

Identification of persons with the disease (cases)
Identification of suitable group without disease (controls)
Cases + controls compared for existing or past attribute
or exposure thought causally related to disease

Proceeds from effect to cause
Always RETROSPECTIVE
(Even if cases/controls accumulated prospectively)

Table 9

COHORT STUDY

Identification of a group
Defined by common characteristic(s)
May have stratified sub-groups
Follow-up for appearance of disease

May be part of a clinical trial

Table 10

PITFALLS OF EPIDEMIOLOGIC STUDIES

Case-Control	Cohort
Selection bias	Latent period not considered
Recall bias	Inclusion of prevalent cases
Early Sx - ASA use	Non-adherence
Early Sx - ASA avoidance	Drop-in/Drop-out
	Loss to follow-up

Table 11

SUGGESTIONS OF CAUSALITY IN EPIDEMIOLOGY

Consistency
Strength of the association
Dose-response effect
(Exposure-response effect)
Change in risk on stopping exposure
Biological plausibility

Table 12

ESTIMATES OF STRENGTH OF ASSOCIATION

RR = (Standard) Relative Risk (Ratio)
IRR = Incidence Rate Ratio

Exposure in cases / Exposure in controls	>1	Risk factor
	<1	Protective

Cigarette smoking and lung cancer	RR = 20
Low-fat, high fiber diet and colon cancer	RR = 0.5
Flu vaccination and flu	RR = 0.05

studies follow the lead provided by epidemiologic surveys. For years aspirin was considered an innocuous and unpretentious compound by most investigators, and was seldom used in cell or animal studies. This has now changed with the tantalizing results found on epidemiologic surveys.

EPIDEMIOLOGIC SURVEYS (Tables 8-12)

In contrast to cell culture and animal studies, most epidemiologic surveys have focused on aspirin, because it is by far the one in longest use, and still the most widely and commonly used NSAID. Initial studies were done using case control methods. An Australian population-based case control study from Melbourne compared aspirin use in 715 incident cases of colorectal cancer with 727 controls. Aspirin use was determined by questioning whether each subject had used aspirin-containing medications on a regular basis. Use of aspirin was associated with a relative risk for colorectal cancer of 0.53. This protective effect persisted after controlling for potential confounding factors including diet, co-morbid illnesses, and hospitalization.[84] (Tables 13-14)

In a subsequent case control study reported from the Northeast United States, a total of 1,326 incident cases of colorectal cancer was compared with 4,891 controls, of whom 1,011 had cancers at other sites and 3,880 were patients that were hospitalized for acute infections or trauma. Use of aspirin for at least 4 days a week during the previous year was associated with a relative risk for colorectal cancer of 0.5. Previous users who had discontinued aspirin use for a year or more showed no protective effect, suggesting that aspirin's protective effect might be

Table 13

ASPIRIN USE AND COLORECTAL CANCER

Study	Design	ASA	Site	RR	Ref
Melbourne	Case Cont	Any	C+R	0.5	84
N.E. US	Case Cont	4/wk x 1 yr	C+R	0.5	85
California	Cohort	Daily	C	1.3	86
			R	1.0	
US (CPS)	Cohort	16/mo x 1 yr	C	0.6	90
			R	0.8	
Buffalo	Case Cont	1-2/d	C	0.6	91
			R	0.3	
US (NHANES)	Cohort	Past mo	C+R	0.9	92
		(Men <65 yrs)		0.4	
US (PHS)	Trial	QOD	C+R	1.2	95

Table 14

ASPIRIN USE AND COLORECTAL POLYPS

Study	Design	ASA Dose	Polyp	RR	Ref
Buffalo	Case Cont	1-2/d	All	0.4	91
Nottingham	Case Cont	Any	Adenoma	0.6	97
US (PPS)	Cohort	Any	Adenoma	0.5	101
US (PHS)	Trial	QOD	All	0.9	95

reversible, and required continued usage to be manifest.[85] (Table 15) There appeared to be an age effect, with the protective effect being stronger in younger individuals. The median age of the cases was 63. For individuals under 60 years of age, the relative risk was 0.3; for those older than 60, it was 0.6.

At about the same time a cohort study was published which appeared to contradict the above studies. Cohort studies are generally accepted to provide stronger support for a hypothesis than do case control studies, due to a lower potential for bias in assessing exposure, selection bias, recall bias, as well as confounding variables. In a cohort study of 13,987 elderly (median age 73) residents of Leisure World, a southern California retirement community, those individuals who used aspirin at least once daily were found to have a relative risk for colon cancer of 1.3 and for rectal cancer of 1.0.[86] This was based on 181 incident cases of colorectal cancer found after a median follow-up of 7 years. The cohort has been followed continuously since 1981. When the results were re-analyzed to look at mortality instead of incidence, there was also no protective effect. In a subsequent follow-up report 3 years later on the same cohort, which then included 50 more cases of colorectal cancer (accounting for a total of 231 incident cases of colorectal cancer), there was again no protective effect, with a relative risk for colon cancer of 1.5 in men and 1.0 in women.[87] It is important to note that this study differs from most other epidemiologic surveys on aspirin and colorectal cancer in several aspects. First, this study found a substantial gender difference, which was not seen in any other epidemiologic survey. In addition, the subjects were quite elderly, with a median age of 73 at the time of analysis for the initial report. As shown in two other studies, the protective effect seems to be stronger for younger individuals, with greater protective effect for those under the age of 60-65

Table 15

ASPIRIN USE AND COLORECTAL CANCER
DURATION OF USE EFFECT

	CPS (Ref. 90)		N.E. US (Ref. 85)	
Duration	>10 yr	<10 yr	past yr	not past yr
RR	0.36	0.71	0.5	1.0

Table 16

ASPIRIN USE AND COLORECTAL CANCER

Study	Median Age	Protective	Ref.
NHANES	54	Yes	92
CPS	57	Yes	90
N.E. US	62	Yes	85
Melbourne	65	Yes	84
California	73	No	86

Table 17 (Ref. 89)

ASPIRIN USE AND COLORECTAL CANCER
Dose/Frequency Effect

ASA/mo	RR
0	1.00
<1	0.88
1-15	0.77
16+	0.36

years. (Table 16) In addition, many of the subjects in this cohort study were quite health conscious, with most of the women being on calcium and estrogen. In fact, the cohort was designed to study osteoporosis, and its risk factors and protective factors, in the elderly.[88]

A subsequent cohort study was reported from the American Cancer Society in 1991 in the New England Journal of Medicine.[89] This raised great public awareness, especially coming at a time when aspirin was being shown to have significant protective effect in the secondary prevention of cardiovascular events. In this Cancer Prevention Study, a cohort of 662,424 adults had been surveyed about a variety of life style factors and medications in 1982 as part of a prospective study of cancer mortality. Aspirin use was ascertained as part of a large health and dietary habits questionnaire and recorded as usage on a monthly basis, dividing users into non-users, and those taking less than 1, 1-15, and more than 16 aspirins per month. A total of 1,111 deaths from colorectal cancer were recorded. There was a significant protective effect for aspirin users, with the relative risk for colon cancer in those who used aspirin more than 16 times per month of 0.6 for colon cancer, and 0.8 for rectal cancer. There was a protective effect at each level of use compared with non-users, and a dose-response effect was observed. (Table 17) No effect was found for acetaminophen, and the results persisted after correcting for potential confounding factors such as co-morbidity and diet.[90]

Two recent studies appear to confirm the protective effect of aspirin. In a case control study from the Roswell Park Memorial Institute in Buffalo, 490 incident cases of colon cancer and 340 incident cases of rectal cancer were compared to 1,138 controls that were healthy visitors to a cancer screening clinic and to another 524 controls who were hospitalized patients found not to have cancer. Aspirin use was recorded as the routine use over the previous one

year of <1, 1-2, or >2 tablets per day. Aspirin users had a relative risk for colon cancer of 0.6 and for rectal cancer of 0.3. In this study, as in the Cancer Prevention Study, there was a protective effect at all three levels of use, compared with non-users, and a dose-response effect was observed.[91]

The data from another large cohort, the National Health and Nutrition Examination Survey I (NHANES) have recently been analyzed. This survey was conducted between 1971 and 1975, in which a sample of a civilian non-institutionalized U.S. population between the ages of 1 and 74 years were to be followed over time. The survey did attempt to over-sample for individuals considered to be at risk for malnutrition. A subpopulation of 12,668 subjects who were age 25-74 at the time of the initial survey was analyzed. At the time of the survey aspirin use over the previous 30 days was assessed by questionnaire. At the time of analysis subjects had been followed for a median of 12.4 years. After the initial 2 years of follow-up (when prevalent colorectal cancers that might have been present at the time of initial survey should have presented) a total of 1,257 new incident cases of colorectal cancer were found. In this study, the relative risk for colorectal cancer for aspirin users was 0.9. Subgroup analysis suggested that for men under the age of 65, the relative risk for colorectal cancer in aspirin users was 0.4.[92] (Tables 18-19)

The effect of aspirin on the incidence of colorectal cancer was also examined as a post facto analysis of the Physicians Health Study.[93] This study randomized 22,071 U.S. male physicians to receive aspirin 325 mg every other day and beta carotene 50 mg every day using a 2 X 2 factorial design. Subjects received one of the following four treatments: i) aspirin and beta carotene, ii) aspirin and placebo, iii) beta carotene and placebo, or iv) both placebo. The

Table 18

ASPIRIN USE AND COLORECTAL CANCER
AGE EFFECT

	N.E. US (Ref. 85)		NHANES (Ref. 92)	
Age	<60	>60	<65	>65
RR	0.30	0.60	0.35	1.21

Table 19

ASPIRIN USE AND COLORECTAL CANCER

Study	vs. Dose	vs. Duration	vs. Age	Reversible	Ref.
Melbourne					84
N.E. US		+	+	+	85
California			(*)		86
CPS	+	+			90
Buffalo	+				91
NHANES			+		92
PHS		+			95

* Cohort much older than in other studies

primary hypotheses to be tested in the study were whether aspirin would reduce the risk of cardiovascular disease and whether beta carotene would reduce the risk of cancer. The trial was not designed to test the hypothesis that aspirin would reduce the risk of cancer, or more specifically colorectal cancer. In fact, the aspirin arm of the study was terminated after a mean follow-up of 5 years, when a statistically significant protective effect against cardiovascular disease was found.[94] Nevertheless, because the study was set up as a randomized clinical trial, it offered an opportunity to explore a potential effect of aspirin in an unbiased fashion. A total of 118 new incident cases of colorectal cancers were found between the time of study entry and study termination. Because of the small number of colorectal cancers which was primarily a result of the early stoppage of the aspirin arm, the study had inadequate power to determine if there was a protective effect of aspirin. The calculated relative risk was 1.2 but its accuracy was in doubt because of the inadequate power.[95] (Fig. 8) The power of the study was also influenced by the healthy outcome of the participants. At the start of the trial, the investigators had considered that volunteers for a prevention study might have only 80% of the general population's frequency of adverse cardiovascular and cancer events due to health-seeking behavior, and that physicians on a whole might have an additional 50% decrease in adverse events. They thus hypothesized that the standard mortality ratio for these physician volunteers would be 0.4 (0.8 X 0.5). The eventual standard mortality ratio for their physician participants was 0.15, showing that those physicians that volunteered for the study and were enrolled after demonstrating initial compliance were indeed very healthy. Parenthetically, this healthy physician volunteer effect appears not to have been considered in the currently debated nurses health trial and women health trials.

Figure 8. Effect of aspirin on colorectal cancer and polyps (Ref. 95)

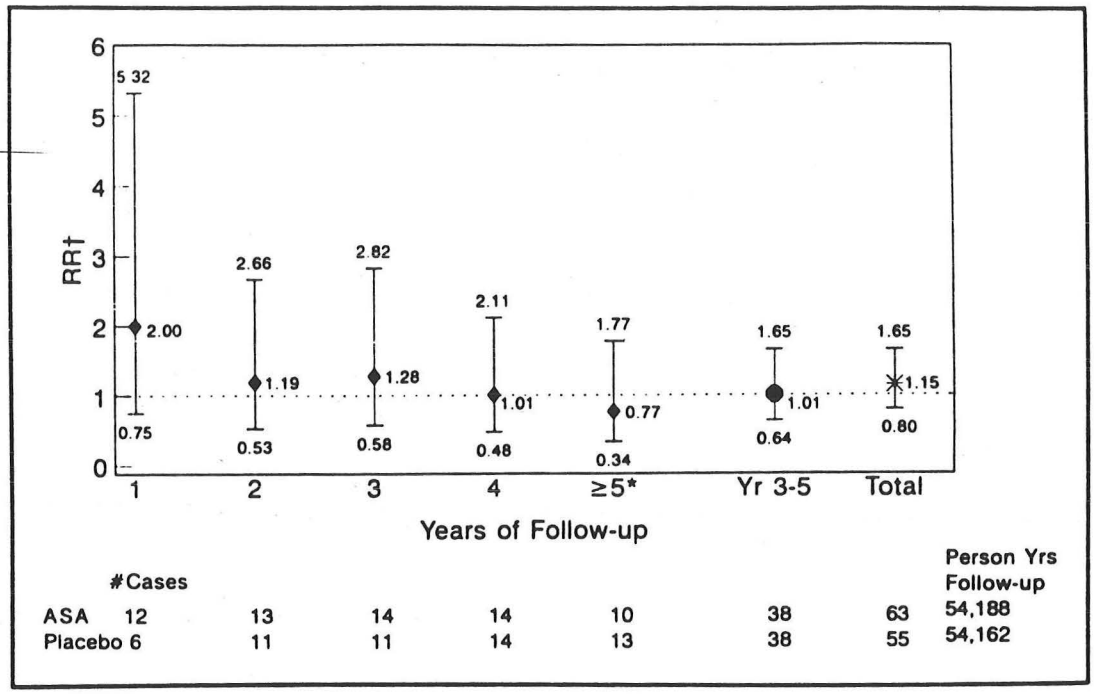


Fig. 1. Occurrence of invasive colorectal cancer in aspirin and placebo groups by year of follow-up. Data are from the Physicians' Health Study. ◆ = yearly, ● = years 3-5, and ✳ = total. †RR computed from a proportional hazards model including the variables of age, aspirin, and beta carotene assignment. *Includes approximately 1600 person-years of follow-up in Year 6 in each group.

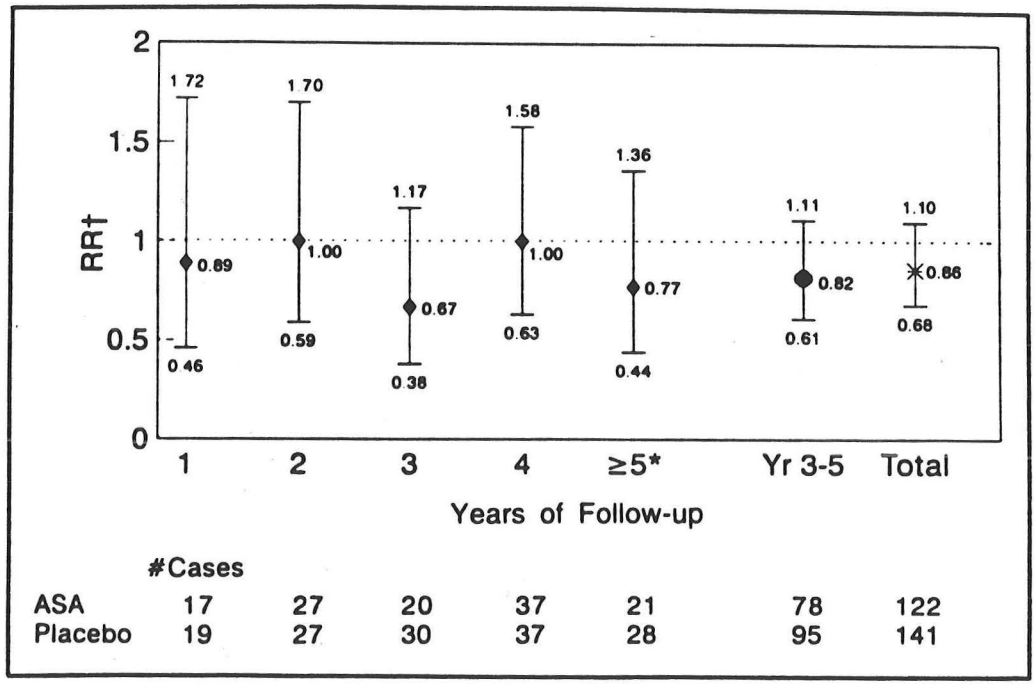


Fig. 2. Occurrence of colorectal polyps and cancer in situ in aspirin and placebo groups by year of follow-up. Data are from the Physicians' Health Study. ◆ = yearly, ● = years 3-5, and ✳ = total. †RR computed from a proportional hazards model including the variables of age, aspirin, and beta carotene assignment. *Includes approximately 1600 person-years of follow-up in Year 6 in each group.

Several other studies have also suggested that aspirin or other NSAIDs have a protective effect against colorectal cancer. In a cohort study of 11,683 patients with rheumatoid arthritis (who are presumably taking NSAIDs more frequently than the general population), there was a 30-40% decrease in the incidence of colorectal cancers.[96] It is also possible that other anti-inflammatory agents may play a role. A preliminary analysis has been completed in a case control study of 3,112 patients with ulcerative colitis, of whom 102 developed colorectal cancer. It was found that patients who took sulfasalazine regularly had a relative risk for colorectal cancer of 0.3 (Baron, J. - personal communication). (Table 20)

There are a few other ongoing epidemiologic surveys which suggest that NSAIDs, particularly aspirin, have a protective effect against colorectal cancer. Several of these have been presented in abstract form over the past year.[97-99]

Colorectal polyps (Table 14)

Three of the above studies also analyzed data for a potential protective effect of aspirin on colorectal polyps. The case control study from Buffalo also analyzed 212 incident cases of colorectal polyps (without any additional histologic classification). The relative risk for colorectal polyps in aspirin users was 0.4.[91] In a case control study in subjects participating in a randomized controlled clinical trial of fecal occult blood screening for colorectal cancer, a total of 147 new incident cases of colorectal adenomatous polyps who were found because of a positive fecal occult blood test were compared with 176 controls without colorectal adenomas who were also fecal occult blood positive and an additional 153 controls who had no colorectal adenomas and were negative for fecal occult blood. Aspirin use was determined by questioning

Table 20

SULFASALAZINE USE AND COLORECTAL CANCER

Case-control study in 3,112 UC pts in Uppsala
102 cancers vs. 196 controls

Sulfasalazine	RR
<3 mos	1.00
>3 mos	0.34

Table 21 (Ref. 90, 92)

ASPIRIN USE AND OTHER CANCERS

RR	Esophagus	Stomach	Pancreas	Lung	Breast	Other
CPS	0.59*	0.53*	-	1.07	0.88	1.05
NHANES	-	0.93	0.67	0.68*	0.70*	0.88

* Statistically significant difference from RR 1.00

whether any aspirin or aspirin containing drugs were taken on a regular basis for a period of 3 months or longer during the past 5 years. The relative risk for colorectal adenomas in aspirin users was 0.6 when compared to both control groups.[100] The protective effect was stronger when compared to the controls who were fecal occult blood negative than those who were fecal occult blood positive, thus suggesting that the protective effect of aspirin was probably not due to increased fecal occult blood loss or the resultant earlier serendipitous diagnosis of colorectal adenomas.

In a post facto cohort analysis of patients enrolled in a randomized controlled clinical trial of beta carotene, vitamin C and vitamin E, a total of 793 patients were asked for any ongoing use of aspirin at 6 months and 1 year after study entry. 593 did not report use of aspirin on either questionnaire and were recorded as non-users, 98 reported use on only 1 of the 2 questionnaires and were recorded as intermittent users, and 102 reported use on both questionnaires and were recorded as consistent users. For the consistent user, the relative risk for colorectal adenomas at 1 year after study entry was 0.52,[101] and the relative risk for the intermittent user was 0.95. Besides suggesting a dose response effect, the results also suggest that consistent use is required for a protective effect.

Lastly, the Physicians Health Study was also analyzed for colorectal polyps, and it was found that the relative risk for aspirin users for colorectal polyps was 0.9.[95] Several other epidemiologic surveys are also ongoing, with preliminary data showing a protective effect being presented in abstract form over the past year.[102-104]

Other cancers

In two of the above trials, relative risks for mortality from other cancers were also examined. The results suggested that there may also be a protective effect of aspirin, in esophageal and stomach cancer in the Cancer Prevention Study[90], and perhaps also in lung and breast cancer in the NHANES study.[92] (Table 21)

In summary, the results from the above epidemiologic surveys that aspirin use had a protective effect against colorectal cancers are consistent, with the exception of the Southern California study in elderly subjects, and the Physicians Health Study with inadequate power. The strength of the association is quite good, with a relative risk of about 0.5 for the positive studies. A dose response effect was seen in several studies. There was also a suggestion that the protective effect was lost once aspirin use stopped. Lastly, there is biological plausibility from cell culture and animal studies for the observed effect. Based on these epidemiologic results, as well as the extensive and uniformly positive animal results, many small scale human intervention trials of NSAIDs have been performed, and several others are ongoing.

HUMAN INTERVENTION STUDIES

Early uncontrolled reports suggested that the use of the NSAID sulindac was associated with regression of polyps in patients with familial adenomatous polyposis. Primarily in patients who have undergone total colectomy and ileo-rectal anastomosis, the number and size of polyps in the rectal stump appeared to decrease with sulindac administration.[105,106] Subsequently investigators reasoned that sulindac, which was a pro-drug that was activated by colonic microflora, might have its active metabolite sulindac sulfide concentrated in the colon and thus

Table 22

CHOICE OF NSAID

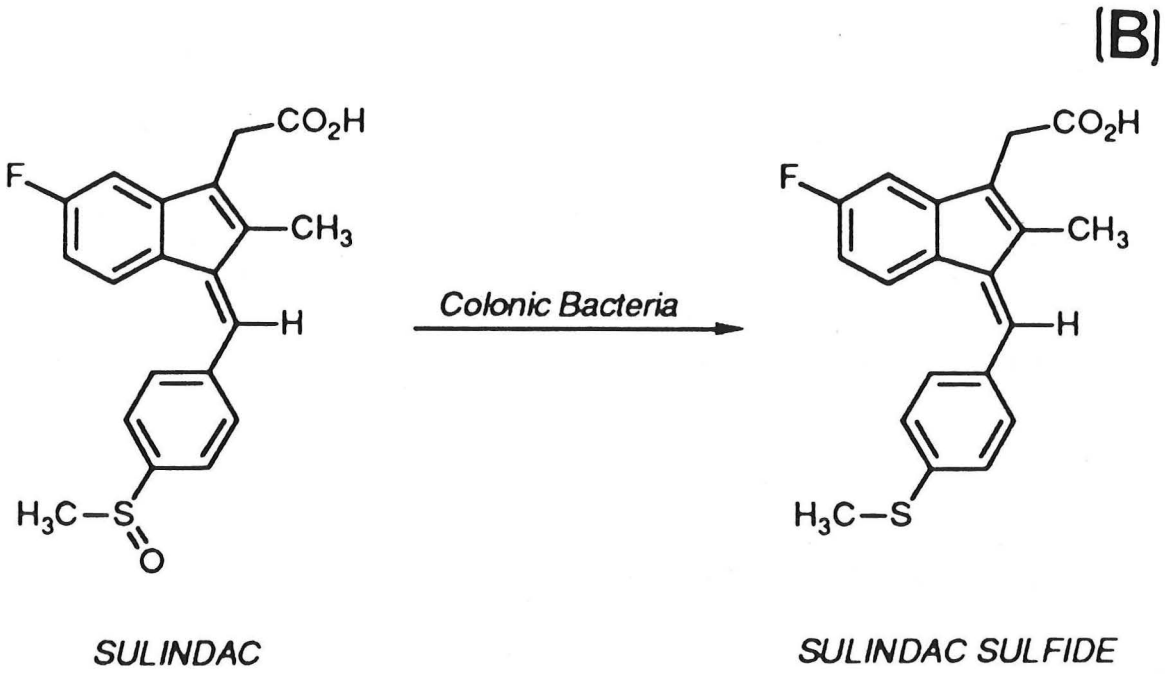
Indomethacin - high potency cyclo-oxygenase inhibitor
(Indocin)

Sulindac - activation by colonic microflora
(Clinoril)

Piroxicam - long half-life, qd dosing
(Feldene)

Aspirin - most widely used, cardiovascular Rx
(Bufferin)

Figure 9. Activation of sulindac by colonic bacteria (Ref. 14)



be highly effective in the colonic mucosa. (Table 22, Fig. 9) What is unexplained is that sulindac's effect has been seen primarily in those patients with total colectomies. Nevertheless, sulindac has been the NSAID of choice in studies involving FAP patients, and other NSAIDs, including aspirin, have been virtually ignored. (Tables 23-24) Several additional small trials have found a regression of polyps, although complete regression was not seen.[107-109] (Fig. 10-11) Two recent small controlled trials have been completed. In a double-blind crossover study completed in 9 FAP patients, 6 patients were found to have complete regression and the other 3 partial regression while on sulindac. After going off sulindac, 4 out of 5 patients had regrowth of polyps.[110] (Fig. 12) In another randomized placebo-controlled study, 11 FAP patients were randomized to receive sulindac 300 mg total daily dose for a total of 9 months and had follow-up for a total of 12 months. The number of polyps decreased by more than 50%, and the size of the polyps decreased by about one-third. However, complete regression was not observed in any patient.[111] One difference is that this study included 9 FAP patients who had not undergone colectomy and had a larger number of adenomas. (Fig. 13) Furthermore, this author and other investigators have found that rectal adenomas appear to be the most susceptible to regression, either spontaneously after initial colectomy and ileorectal anastomosis,[112] or with sulindac treatment.[111,113] A third randomized study found that 5 out of 7 FAP patients who had undergone colectomy and ileorectal anastomosis had significant but incomplete regression of polyps on sulindac.[114] A recent study found that rectal therapy with sulindac was also effective.[115]

Although most studies have suggested that duodenal adenomas in FAP patients were highly resistant to sulindac treatment and show little if any regression, one small randomized

Table 23

SULINDAC AND COLORECTAL ADENOMAS IN FAP

Study	n	Mg/day	Mos	F/U (mos)	CR/PR	Regrowth
Waddell '89	11	3-400	>6	12-85	6/5	All
Labayle '91	9	300	4	10	6/3	4/5
Rigau '91	5	400	6	4-36	0/5	4/5
Iwama '91	10	150	3	3	0/3	All
Giardiello '93	11	300	9	12	0/11	All
Winde '93	15	300	4	10	11/4	All
Tonelli '93	13	200	>6	28-64	0/8	All
Nugent '93	7	400	6	0	0/5	-
Spagnesi '94	20	200	2	0	0/20	-

Table 24

SULINDAC AND COLORECTAL ADENOMAS IN FAP

Study	Colectomy/-	Mg/d	CR/PR	Placebo	Refs.
Waddell '89	7/4	3-400	6/5		106
Labayle '91	9/0	300	6/3	0/2	110
Rigau '91	0/5	400	0/5		107
Iwama '91	5/5	150*	0/3		108
Giardiello '93	3/8	300	0/11	0/0	111
Winde '93	15/0	300	11/4		115
Tonelli '93	13/0	200*	0/8		109
Nugent '93	7/0	400	0/5	0/0	114
Spagnesi '94	14/6	200*	0/20		113

Figure 10. Adenoma regression on sulindac and regrowth off sulindac (Ref. 108)

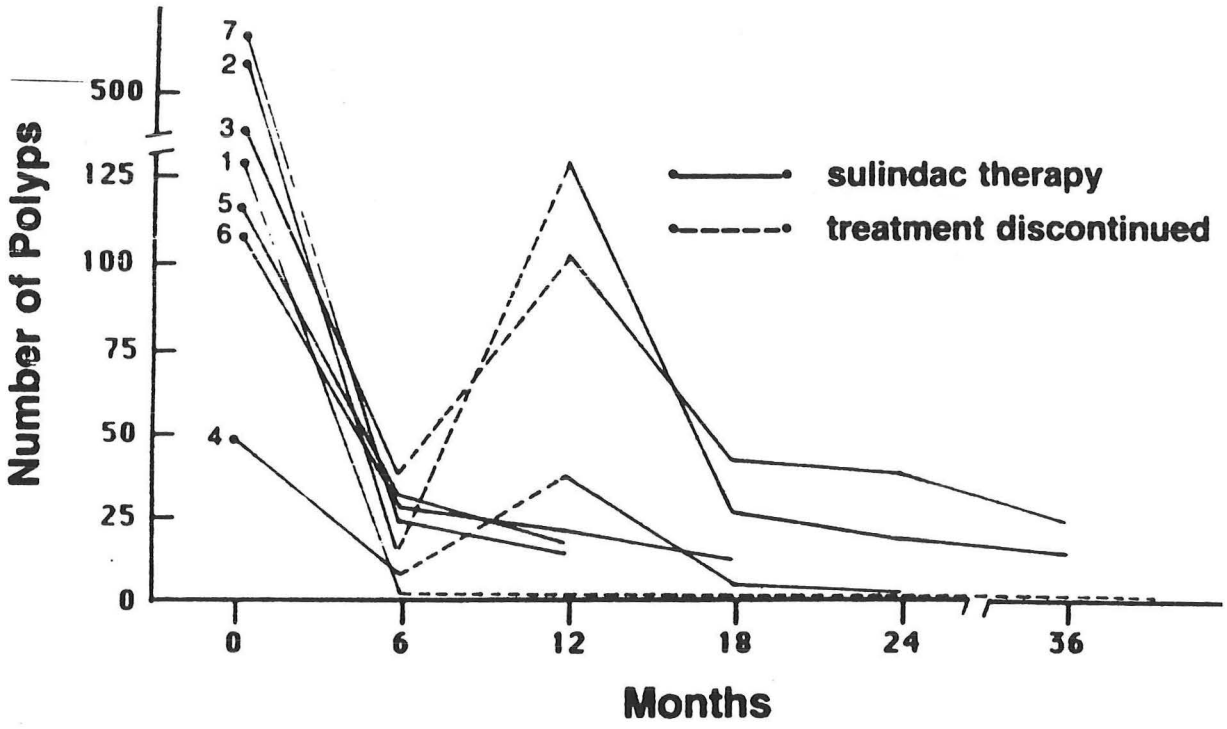


Figure 11. Sulindac decreases colonic mucosal prostaglandin levels (Ref. 108)

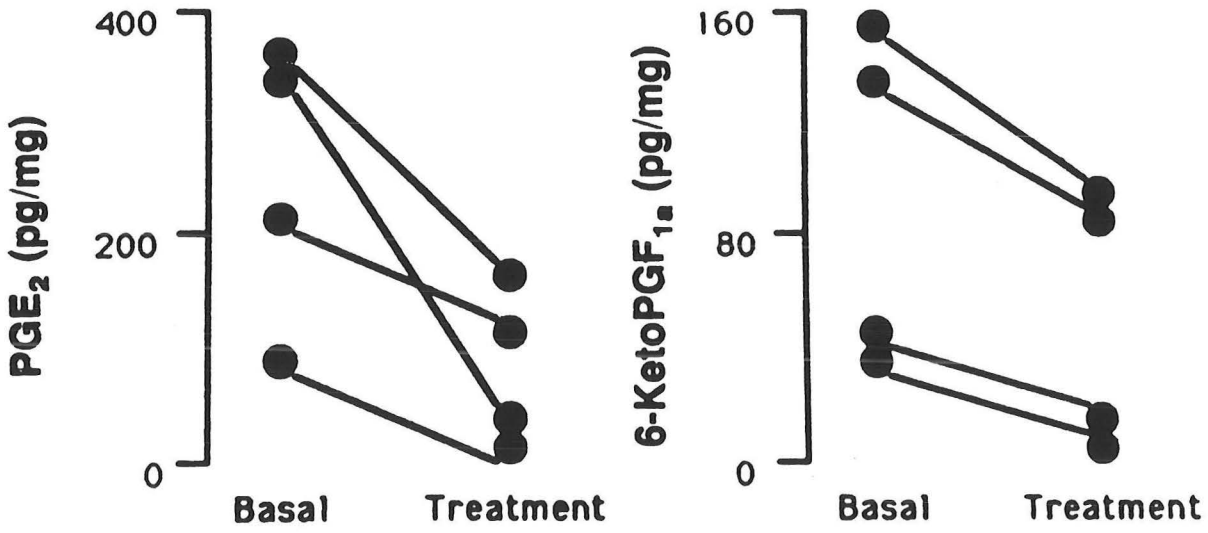
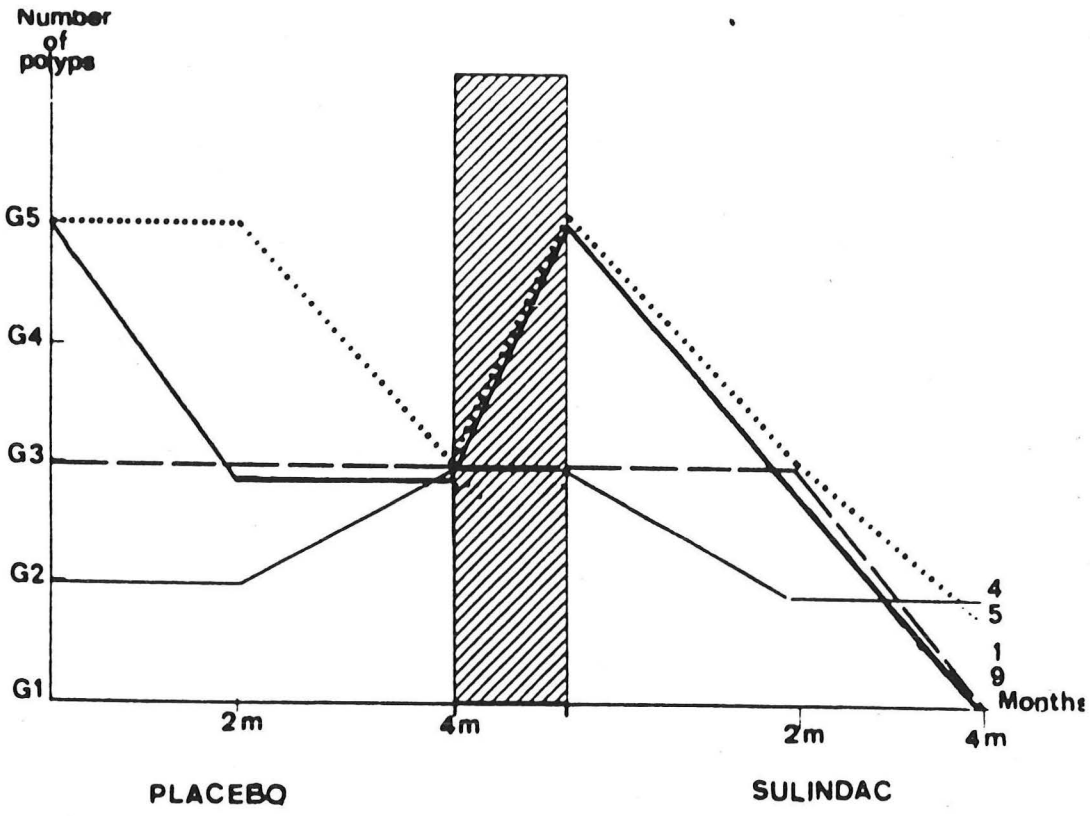


Figure 12. Top. Adenoma regression on sulindac. Bottom. Regrowth off sulindac (Ref. 110)



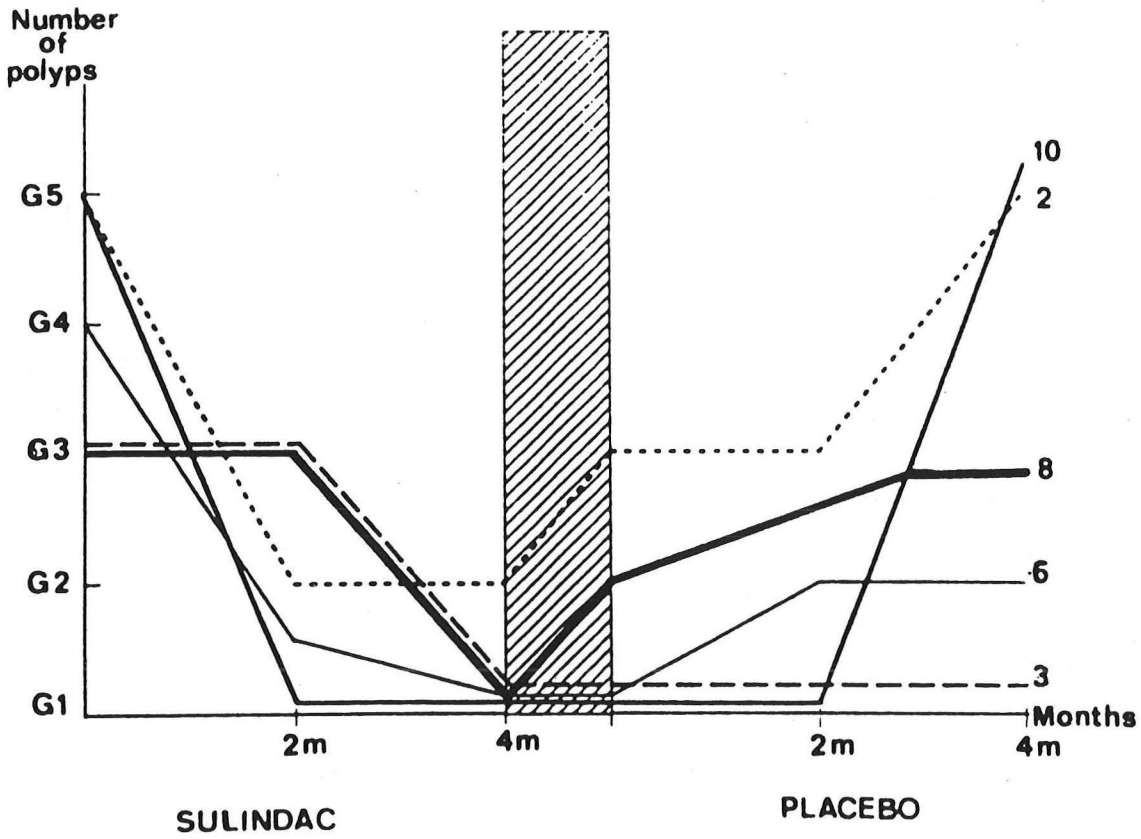


Figure 13. Adenoma regression with sulindac (Ref. 113)

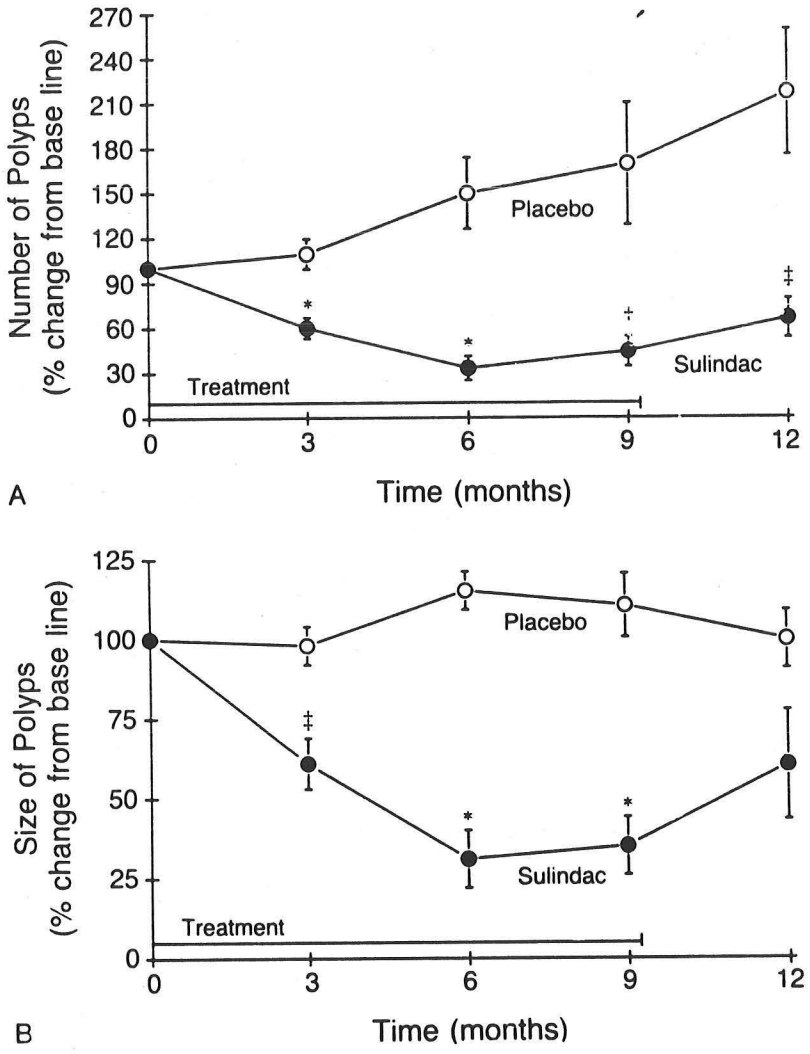


Table 25

SULINDAC AND DUODENAL ADENOMAS IN FAP

Study	n	Mg/d	Mos	CR/PR
Nugent '93	11	400	6	0/5
Parker '93	1	300	>36	1/0

(Gradual decrease over 24-36 mos)
(Electrofulguration also done)

trial[114] and a single case report[116] suggested a small effect. The single case report in an FAP patient with multiple recurrent duodenal adenomas for more than a decade described complete regression of duodenal adenomas with continuous sulindac, 300 mg daily, for 3 years. The patient was reported to be free of duodenal polyps for 6 months with continued administration of sulindac.[116] (Table 25)

In one small trial of sporadic colon polyps, sulindac 400 mg daily or piroxicam 20 mg daily was found to have no significant effect on adenomatous polyps ranging 3-12 mm in size, and numbering 1 in most and up to 3 in any individual patient.[117] One 6 mm polyp disappeared and 3 other small polyps appeared to have decreased in size. (Table 26) All remaining polyps were found to be adenomas on removal. Most importantly, of the 4 patients on piroxicam, 2 were withdrawn due to a bleeding gastric ulcer in one and rash in the other. (Table 27)

Therefore to date, all reported studies of sulindac have shown significant partial or complete regression of colorectal and perhaps also duodenal adenomas in FAP patients. Complete regressions have generally been seen in colorectal adenomas in patients who have undergone total colectomy and ileorectal anastomosis. Those with an intact colon generally had only partial regression. Furthermore, virtually all patients had regrowth of adenomas after discontinuation of sulindac. Therefore, sulindac cannot be recommended as definitive medical treatment, although it might have an adjunctive role in an experimental protocol in patients who have undergone ileorectal anastomosis and still have a rectal remnant with recurrent adenomas.

Table 26 (Ref. 117)

NSAIDS AND "SPORADIC" POLYPS

NSAID	n	Mos	CR/PR	
Sulindac 400 mg	5	6	0/1	(1 polyp disappeared)
Piroxicam 20 mg	2	6	0/1	(2 adverse events)

Table 27

SULINDAC ADVERSE EFFECTS IN FAP STUDIES (2-400 mg/d)

Dyspepsia	>5%
GI Bleed	>2%
Bleeding GU	>1%
Rash	>1%
Study Withdrawal	>4%

FUTURE DIRECTIONS

Although NSAIDs are widely used and widely prescribed, they are not totally free of side effects. NSAIDs have been shown to cause gastrointestinal lesions,[118,119] including colitis,[120] and to cause decrease in renal function[121] and worsening of blood pressure control. (Tables 28-30) The most commonly encountered adverse effect is probably gastrointestinal (GI) bleed. In fact, even at the lower doses used in cardiovascular prophylaxis trials, upper GI bleeding developed in 3% of patients on 1200 mg total daily dose of aspirin and about 1.5% of those on 300 mg daily aspirin.[122-124] (Table 31) Although the epidemiologic studies of a protective effect against colorectal cancer appear convincing they do not provide definitive proof of effect. In the only available large clinical trial, the study had inadequate power, and no protective effect of aspirin 300 mg every other day was detected.[95] Even in that trial, 1.5% of patients were hospitalized for GI bleeding during the 5-year aspirin study period.

Therefore, until there is more definitive proof of efficacy, in the form of positive results from randomized, placebo-controlled clinical trials, an endorsement of aspirin for prevention of colorectal adenomatous polyps or colorectal cancer cannot be provided. (Table 32) It would seem more prudent to endorse another intervention that has shown a similar protective effect in epidemiologic studies, with a relative risk of also about 0.5. Dietary changes to a low fat and high fiber diet has not been shown to have any adverse clinical effects (although life style changes can be traumatic), and can be endorsed with a bit more enthusiasm. (Table 33) Those dietary changes can also be beneficial for cardiovascular health. And of course tobacco use should be strongly discouraged.

Table 28 (Ref. 118)

NSAID TOXICITY IN SMALL BOWEL

Abdominal distress
Diarrhea
Increased mucosal permeability
Mucosal inflammation
Blood and protein loss
Ulceration
Perforation
Strictures

Table 29 (Ref. 118)

NSAID TOXICITY IN COLON

Rectal bleeding
Ulceration
Perforation (diverticula)
Strictures
Non-specific colitis/proctitis
Necrotizing enterocolitis
Ulcerative colitis
Crohn disease

Table 30

NSAID ADVERSE EFFECTS

Intracranial hemorrhage	↑22%	(0.3%)
GI bleeding	↑ 6-10X	(3%)
Gastric mucosal injury	↑10-25X	(20%)
Constipation	↑ 3X	(6%)
Upper GI symptoms	↑20-60%	(35%)
Worsen chronic renal disease		
Worsen hypertension		

Table 31 (Ref. 124)

NSAIDS AND GI TOXICITY

ASA/d	GI Bleed	UGI Sx	Constipation
0 mg	0.5%	26%	2%
300 mg	3%	31%	6%
1200 mg	5%	41%	7%

Table 32

NSAIDS AND COLORECTAL CANCER

- ASA for secondary cardiovascular prevention

- NSAIDs for colorectal cancer NOT proven
- Optimal agent or dose unknown
- Chronic daily NSAIDs have substantial adverse effects
- NSAIDs cannot be recommended for routine colorectal cancer
- Sulindac may be effective in SELECTED patients with FAP or recurrent adenomas with high grade dysplasia AFTER all surgical/endoscopic options exhausted
- Consider "healthy" life style changes

Table 33

PREVENTION OF COLORECTAL CANCER

	RR
No tobacco use (all cancers)	0.3
Low-fat, high fiber diet	0.5
NSAID use	0.5
Exercise	0.8
Ideal body weight	0.8

Aspirin, if shown to be effective for preventing or delaying the appearance of colorectal adenomas and cancers, would be almost an ideal drug. It has already been shown to be effective in the secondary prevention of cardiovascular and cerebrovascular events, and may be effective also for primary prevention.[1] Its significant GI toxicity remains a major obstacle to widespread use.[118,119] Currently active investigation is ongoing to search for other NSAIDs that may have less GI mucosal toxicity. Several studies suggested that liposome-encapsulated indomethacin may be targeted to tumor cells, with the possible benefit of decreased systemic toxicity.[125,126] It has further been suggested that COX-1 may be the constitutive enzyme that is responsible for maintaining mucosal integrity, whereas COX-2 is the inducible enzyme that may be responsible for inflammatory response and tumorigenesis.[17-19] (Tables 34-35) There are suggestions that some NSAIDs may preferentially inhibit COX-2 instead of COX-1.[127] (Fig. 14-15). Furthermore, there are no data available as to the exact mechanism for the tumor suppressive effect of NSAIDs, including aspirin. Several reports suggested that NSAIDs decrease the expression of myc and ras,[128] and that perhaps nitric oxide may have a modulatory role on COX.[129] (Fig. 16) The areas of NSAIDs, COX, and cancer appear to be fertile for additional research studies.

ONGOING UT SOUTHWESTERN ASPIRIN STUDY IN PATIENTS WITH ADENOMAS

Gastroenterology investigators at The University of Texas Southwestern Medical Center have had a long interest in aspirin and gastrointestinal mucosal injury.[130-133] My interest in colorectal cancer and adenomas and in chemoprevention prompted a natural collaboration to carry out a study on the potential pathophysiologic mechanism of the anti-tumor effects of

Table 34

CYCLOOXYGENASE 1 AND 2

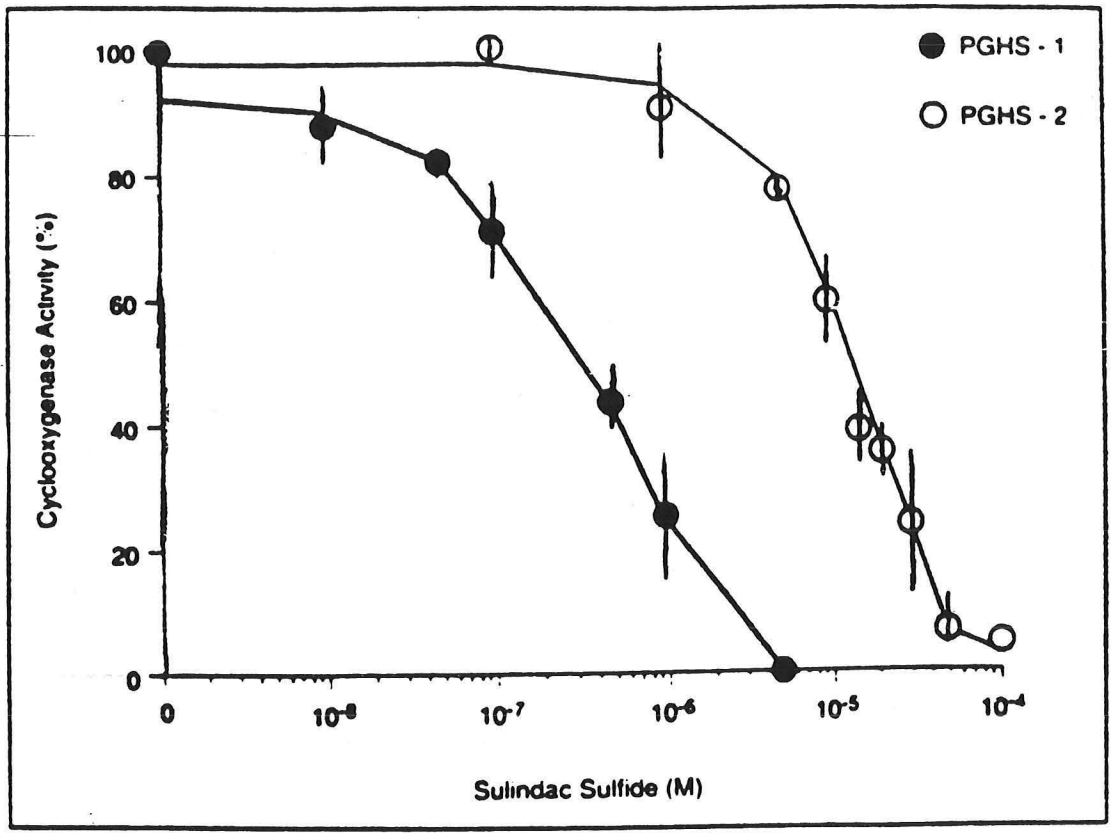
Cox-1	Cox-2
Platelets	Prostate
Endothelial cells	Brain
Stomach	Inflamed synovium
Kidney	Activated monocytes
Smooth muscle	Pre-ovulatory follicles
? Most tissues	? Inducible in most tissues

Table 35

CYCLOOXYGENASES 1 AND 2

Cox-1	Cox-2
Constitutive (2-4X)	Inducible (10-80X)
No effect	Glucocorticoids inhibit
? Housekeeping	? "Immediate early"
Platelet aggregation	Inflammation
Mucosal integrity	Mitogenesis

Figure 14. Top. Sulindac preferentially inhibits COX-1.
Bottom. 6 MNA preferentially inhibits COX-2. (Ref. 19)



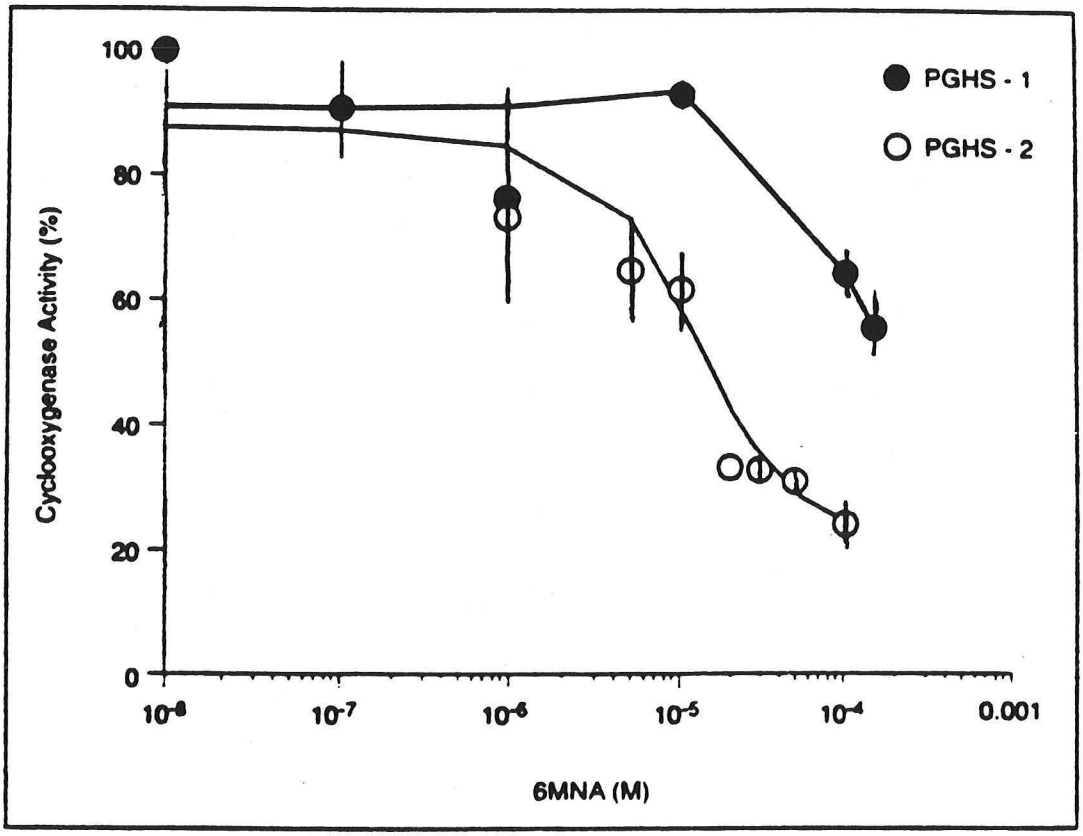


Figure 15. Ibuprofen inhibits COX-1 and COX-2 to a similar degree (Ref. 19)

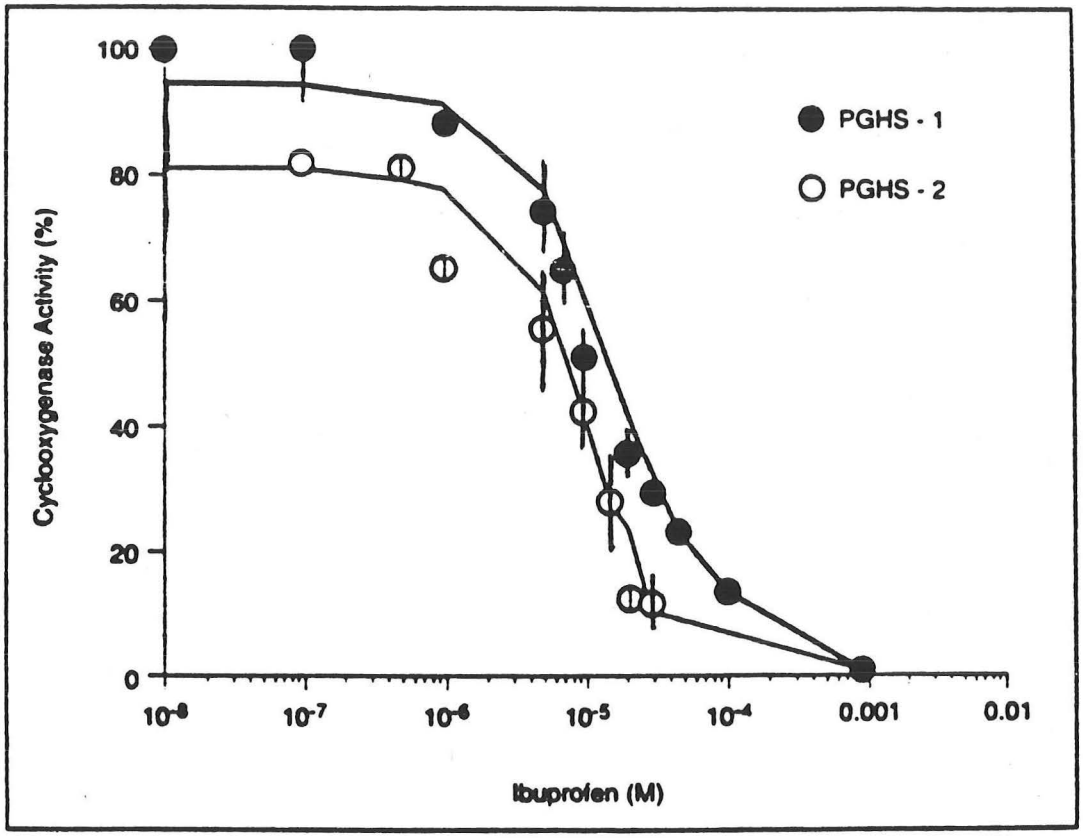
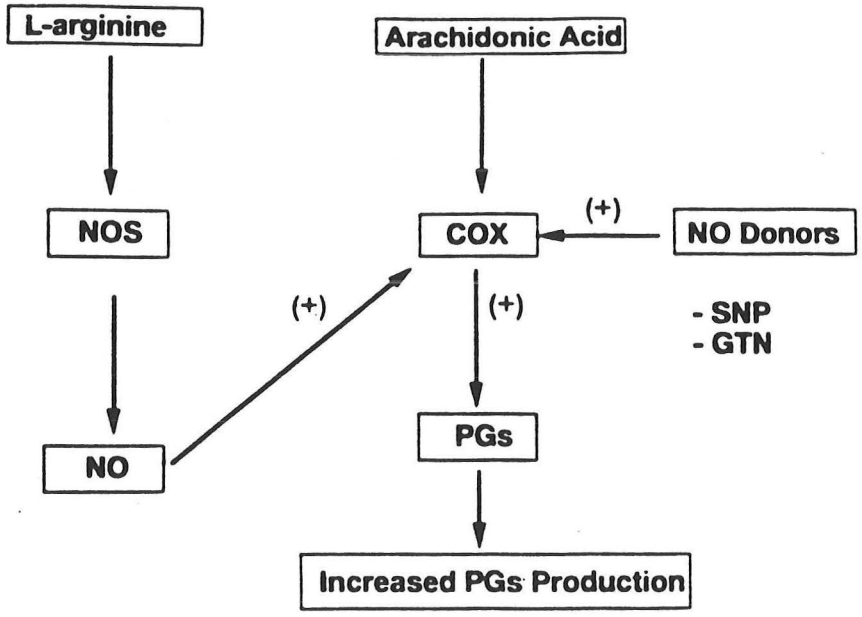


Figure 16. Proposed model for nitric oxide modulation of COX (Ref. 129)



aspirin. We now have a study funded by the National Cancer Institute to examine the effect of 2 doses of aspirin on colon mucosal prostaglandin levels, prostaglandin synthesis, COX expression, and mucosal proliferation.

We are studying patients who have had one or more colorectal adenomatous polyps diagnosed in the previous year, with no serious underlying medical illness. Exclusion criteria include those with active peptic ulcer disease or history of ulcer disease, GI bleeding, inflammatory bowel disease, as well as those who are currently on corticosteroids, anticoagulants, or antiplatelet drugs. Patients who are currently on NSAIDs or salicylates are eligible if they can be off the medication for longer than 3 months.

The investigators include myself, William Harford, Mark Feldman, Byron Cryer and may be contacted at 214-372-0467 or pager 214-822-7300. We would be most grateful for any patient referrals.

UT ASPIRIN STUDY

- Men and women over age 18
- One or more colorectal adenoma in past year
- No serious medical illness
- No active or past ulcer disease
- No GI bleeding or inflammatory bowel disease
- Must be off NSAIDs for >3 months
- No anticoagulants or antiplatelet drugs

- Patients reimbursed for their time

Patient referrals to:
Gordon D. Luk, M.D. 372-0467

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