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Effects of nonsteroidal anti-inflammatory drugs on glutathione S-transferases of the rat digestive tract

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Nonsteroidal anti-inflammatory drugs (NSAIDs) have been demonstrated to reduce cancer rates in oesophagus, stomach and colon of humans and animals. Earlier, we showed that high human gastrointestinal tissue levels of glutathione S-transferase (GST), a family of detoxification enzymes consisting of class α , μ , π and θ isoforms, were inversely correlated with cancer risk. We investigated whether the NSAIDs indomethacin, ibuprofen, piroxicam, acetyl salicylic acid (ASA), and sulindac, supplemented in the diet for 2 weeks at 25, 400, 400, 400, and 320 ppm, respectively, influenced gastrointestinal GSTs in male Wistar rats. In cytosolic fractions of oesophagus, stomach, intestine and liver, GST activity towards 1-chloro-2,4-dinitrobenzene was measured, GST isozyme levels were determined by densitometrical analysis of Western blots after immunodetection with monoclonal antibodies, and glutathione levels were determined by HPLC. GST activity and GST μ levels were increased (1.2–1.8 \times) in oesophagus and small intestine by indomethacin, ibuprofen, piroxicam and sulindac. GST α levels were induced (1.2–2.8 \times) in stomach by piroxicam, in small intestine by indomethacin, ibuprofen, piroxicam and sulindac, and in liver by piroxicam. GST π levels were raised (1.9–3.6 \times) in stomach by ibuprofen, ASA, and sulindac, and in small intestine by indomethacin, piroxicam, ASA, and sulindac. Glutathione levels were raised (1.2–2.3 \times) by indomethacin and ASA in small intestine and by piroxicam in oesophagus. Enhancement of GSTs in the upper part of the digestive tract, resulting in a more efficient detoxification, may explain in part the anticarcinogenic properties of NSAIDs.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs*) are among the most prescribed drugs worldwide. They have anti-inflammatory, analgesic, and antipyretic activities. They are used clinically for the treatment of patients with (e.g.) acute and chronic rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, gouty arthritis, bursitis, tendositis, and inflammatory arthritis (1).

In addition to their therapeutic use, there is strong epidemiological evidence that NSAIDs may have anticarcinogenic effects in humans. Sulindac caused regression of adenomatous polyps in patients with familial adenomatous polyposis (FAP) (2–4), whereas no effect on sporadic colonic polyps was found (5). Epidemiological studies suggest that regular, prolonged

use of aspirin-based NSAIDs may reduce the risk of development and mortality of oesophageal, gastric, colonic, or rectal cancer (6–9), although in one prospective study no support for such an association was found (10). Several NSAIDs are currently evaluated in clinical trials. Effects of NSAIDs on neoplastic growth in the colon of animals and humans, including possible mechanisms involved, were recently reviewed (11,12).

Many animal studies have revealed significant protection against development of chemically induced cancers by treatment with NSAIDs. Ibuprofen inhibited carcinogenesis in rat colon (13), mouse forestomach and lung (14). Indomethacin inhibited tumorigenesis in rat colon (15–19), stomach (16,20), mammary gland (21), urinary bladder (22,23), and liver (24,25), as well as in mouse oesophagus (26,27). Piroxicam reduced tumour incidence in the colon (13,15,17–19,28–31), small intestine (18), and liver (25) of the rat. Dietary acetyl salicylic acid inhibited carcinogenesis in rat colon (32–34) and bladder (35), whereas sulindac reduced tumour multiplicity in the rat colon (36) and mouse forestomach (14).

A generally accepted mechanism of action of NSAIDs is the inhibition of cyclooxygenases, the rate-limiting enzymes that catalyse the formation of prostaglandin precursors from arachidonic acid (12,37). Prostaglandins play a role in the control of cell proliferation and regulation of immune functions (38–41). However, doses of NSAIDs required to suppress inflammation may exceed substantially the doses necessary to inhibit prostaglandin synthesis, suggesting that the anticarcinogenic properties of these drugs may be achieved through additional unidentified mechanisms (42).

Inhibitors of carcinogenesis often have an enhancing effect on carcinogen detoxification systems such as glutathione S-transferases (GSTs; EC 2.5.1.18) (43–45). The soluble glutathione S-transferases are a gene family of dimeric enzymes comprised of four classes: α , μ , π and θ (43,44). They catalyse the binding of a large variety of electrophiles to the sulphhydryl group of glutathione (GSH). Since most reactive ultimate carcinogenic forms of chemical carcinogens are electrophiles, GSTs take considerable importance as a mechanism for carcinogen detoxification (43,44). Enhancement of the activity of this system may result in a more efficient elimination of carcinogens and may ultimately lead to the prevention of cancer.

The present study was designed to investigate the effects of dietary administration of indomethacin, ibuprofen, piroxicam, acetyl salicylic acid and sulindac on glutathione and glutathione S-transferases in the rat oesophagus, intestine, stomach and liver.

Materials and methods

Animal treatment

Forty-eight male Wistar rats (183 \pm 2 g; Central Laboratory Animal Centre, University of Nijmegen, The Netherlands) were housed in pairs on wooden shavings in macrolon cages, maintained at 20–25°C and 30–60% relative humidity. A ventilation rate of seven air cycles/h and a 12 h light/dark cycle

*Abbreviations: NSAIDs, nonsteroidal anti-inflammatory drugs; GST, glutathione S-transferase; ASA, acetyl salicylic acid; FAP, familial adenomatous polyposis.

were used. The rats were randomly assigned to one of the dietary treatment groups. All groups were fed powdered RMH-TM lab chow (Hope Farms, Woerden, The Netherlands) from the same batch. After acclimatization for 7 days the animals were fed either the basal diet (control group) or one of the five experimental diets. Food and water were available *ad libitum*. Food cups were replenished every 2–3 days. Food consumption and gain in body weight were recorded daily.

Diets

Selection of NSAIDs as well as feeding period and dose levels were based on studies by others, showing reduction of tumour incidence in humans and inhibition of chemically induced carcinogenesis in animal models, where NSAIDs were adjusted 2 weeks prior to carcinogen treatment (13,18,21,32,36). The following six diet groups (eight animals per group) were studied: (a) RMH-TM lab chow only or supplemented with (b) 25 ppm indomethacin, (c) 400 ppm ibuprofen, (d) 400 ppm piroxicam, (e) 400 ppm acetyl salicylic acid, or (f) 320 ppm sulindac. The NSAIDs were purchased from Sigma Chemical Company, St Louis, MO, USA. A food processor was used to obtain a homogenous mixture of test compound and powdered lab chow. After receiving the diets for 2 weeks the rats were killed by decapitation. The study protocol was approved by the local ethical committee for animal experiments of the University of Nijmegen.

Tissue preparation

All handlings were performed on ice. After decapitation, oesophagus, stomach, intestine (proximal, middle, and distal small intestine and colon) and liver were excised immediately. Intestine and stomach were slit longitudinally and the contents were removed by washing with cold buffer A (0.25 M saccharose, 20 mM Tris, 1 mM dithiothreitol, pH 7.4). The organs were directly frozen in liquid nitrogen and stored at -20°C until use. For preparation of the cytosolic fraction the tissue was thawed quickly using cold running water. The mucosal surface of stomach and intestine was collected by scraping with a scalpel and was homogenized in buffer A (4 ml/g tissue) in a glass/glass Potter-Elvehjem tube. The liver was homogenized in buffer A (4 ml/g tissue) with 10 strokes at 1000 rpm of a motor-driven glass/Teflon homogenizer (Braun, Germany). The homogenate was centrifuged at 9000 g (4°C) for 30 min. The resulting supernatant fraction was transferred to an ultracentrifuge tube and spun at 150000 g (4°C) for 60 min. The oesophagus was homogenized in 5 ml buffer A per gram tissue in a glass/glass Potter-Elvehjem tube. These homogenates were centrifuged at 150 000 g for 60 min (4°C). Aliquots of the 150 000 g supernatant, representing the cytosolic fraction, were frozen in liquid nitrogen and stored at -20°C .

Assays

Protein concentration was assayed in quadruplicate by the method of Lowry *et al.* (46) using bovine serum albumin as the standard. GST activity was determined in triplicate according to Habig *et al.* (47), using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. GST isoenzyme levels were determined as described before (45). In short, cytosolic fractions were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (11% acrylamide, w/v), and subsequently to Western blotting, using a semi-dry blotting system (Novablot II, Pharmacia, Uppsala, Sweden). Western blots were incubated with monoclonal antibodies against human GST class α (48), μ (49), and π (50). Class α antibodies react with rat GST subunit 1, class μ antibodies recognize rat GST subunits 3 and 4, and class π antibodies react with rat GST subunit 7 (45). The specific binding of the monoclonal antibodies to the isoenzymes was demonstrated by incubation with the secondary antibody peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dakopatts, Glostrup, Denmark) and subsequent development of the peroxidase label with 4-chloro-1-naphthol and hydrogen peroxide. Staining intensity on the immunoblots was quantified using a laser densitometer (Ultrosan XL, LKB, Bromma, Sweden). Known amounts of purified GSTs were run in parallel with the experimental samples and served as standards for the calculation of the absolute amounts of the isoenzymes in the cytosolic fractions. Total glutathione was quantified by high performance liquid chromatography after reaction with monobromobimane, as described before (45). In this assay, oxidized glutathione present is reduced by adding sodium borohydride to the reaction mixture.

Statistical analyses

The Wilcoxon rank sum test was used to assess statistical significance of differences between experimental and control groups: ^a $P < 0.05$, ^b $P < 0.01$ and ^c $P < 0.005$.

Results

Daily food consumption, intake of NSAIDs and gain in body weight are given in Table I. In the sulindac and piroxicam

groups food consumption was significantly reduced as compared to the control group, whereas in the ibuprofen group the food consumption was induced. In the piroxicam group the lower food consumption was paralleled by a reduced gain in body weight. During the course of the experiment no changes in behavioral pattern of the animals were observed. In addition, none of the organs studied showed any macroscopical sign of toxicity of the dietary additive at the end of the experiment.

Table II shows the effects of the NSAIDs on GST activity in the organs investigated. No change in activity was found with acetyl salicylic acid (ASA). In the oesophagus and proximal small intestine (PSI), GST activity was induced by indomethacin (both 1.3 \times), ibuprofen (1.3 \times and 1.2 \times , respectively), piroxicam (1.8 \times and 1.2 \times) and sulindac (both 1.3 \times). In addition, ibuprofen elevated GST activity in the colon (1.3 \times).

In Tables III, IV, and V the effects of the NSAIDs on GST class α , μ and π isoenzyme levels are given. In control animals GST α (Table III) was undetectable in oesophagus and colon, low in stomach (222 ± 20 ng/mg protein) and high in liver (12282 ± 1227 ng/mg protein) and small intestine. In middle small intestine (MSI), none of the diets significantly influenced GST α expression. ASA did not influence GST α levels. Indomethacin increased levels of GST α in PSI and DSI (1.6 \times and 1.9 \times , respectively) as compared to controls. Ibuprofen increased GST α levels in PSI (1.4 \times), piroxicam increased levels in stomach, DSI and liver (1.8 \times , 2.8 \times , and 1.2 \times) and sulindac had an inducing effect on GST α levels in DSI (2.1 \times). GST μ (Table IV) was expressed at high levels in all tissues examined. Parallel to GST activity, GST μ levels in oesophagus and DSI were modulated by the same NSAIDs: indomethacin (1.4 \times and 1.6 \times , respectively), ibuprofen (1.4 \times and 1.3 \times), piroxicam (1.6 \times and 1.4 \times) and sulindac (1.5 \times and 1.4 \times), whereas ASA did not change GST μ levels. GST π (Table V) was undetectable in oesophagus and liver, and low in all other organs studied, ranging from 114 ± 21 ng/mg protein in MSI to 568 ± 84 ng/mg protein in PSI in control animals. All NSAIDs tested increased GST π levels at one or more sites: indomethacin increased GST π levels in MSI (2.1 \times), ibuprofen in stomach (1.9 \times), piroxicam in both MSI and DSI (2.4 \times and 2.8 \times , respectively), ASA in stomach, MSI and DSI (1.9 \times , 3.2 \times and 1.9 \times , respectively) and sulindac in stomach and MSI (2.6 \times and 3.6 \times).

Table VI shows the effect of the NSAIDs on the GSH content in the organs studied. Gastric, colonic and hepatic GSH contents were not influenced by any of the NSAIDs tested. Elevation of the GSH content was seen by indomethacin in PSI, MSI and DSI (1.2 \times , 2.3 \times and 2.3 \times , respectively), by piroxicam in oesophagus (1.6 \times), and by ASA in MSI (1.6 \times).

Discussion

In the present study we have demonstrated that NSAIDs are able to induce glutathione S-transferases, especially in the upper part of the rat digestive tract.

During the last decade, many studies have shown significant protection against the development of cancer by NSAIDs. Compelling evidence is presented in several epidemiological studies, suggesting that NSAIDs have significant protective activity against human oesophageal, gastric, and colonic cancer (11,12). Regression of colon adenomas during treatment with NSAIDs, particularly sulindac, occurred in patients with familial adenomatous polyposis coli who are at high risk for

Table I. Daily food consumption, NSAID-intake and gain in body weight of male Wistar rats receiving diets supplemented with indomethacin, ibuprofen, piroxicam, ASA or sulindac

Treatment group (n = 8)	Dose (ppm)	Food consumption (g/day)	Total NSAID-intake (mg/day/kg b.	Gain in body weight (g/day)
Control	–	16.1±0.3	–	2.4±0.2
Indomethacin	25	15.9±0.5	2.0±0.1	2.5±0.3
Ibuprofen	400	17.0±0.2 ^a	34.0±0.5	2.3±0.2
Piroxicam	400	14.7±0.3 ^a	29.4±0.5	1.2±0.2 ^b
ASA	400	16.3±0.5	32.7±0.4	2.6±0.1
Sulindac	320	14.8±0.3 ^a	29.6±0.7	1.8±0.2

Values given are means ±SEM. The one-tailed Wilcoxon rank sum test was used to assess statistical significance of differences between control and treated groups. ^aP<0.05, and ^bP<0.01.

Table II. Effects of indomethacin, ibuprofen, piroxicam, ASA or sulindac on rat alimentary tract glutathione S-transferase activity

Treatment group (n = 8)	Glutathione S-transferase activity (nmol/min.mg protein)						
	oesophagus	stomach	PSI	MSI	DSI	colon	liver
Control	49±5	184±25	363±29	134±12	59±7	41±3	1817±152
Indomethacin	66±5 ^b	146±15	473±33 ^a	173±11	54±5	45±4	1708±155
Ibuprofen	63±6 ^a	164±2	446±34 ^a	134±7	49±4	52±4 ^a	1593±269
Piroxicam	88±7 ^c	157±16	452±33 ^a	152±13	59±4	47±4	2160±151
ASA	45±5	166±13	406±28	138±11	50±3	43±6	1593±87
Sulindac	63±4 ^a	152±17	472±35 ^a	147±12	50±4	43±4	1598±156

PSI, proximal small intestine; MSI, middle small intestine; DSI, distal small intestine; ^aP<0.05, ^bP<0.01, and ^cP<0.005.

Table III. Effects of indomethacin, ibuprofen, piroxicam, ASA or sulindac on rat alimentary tract glutathione S-transferase α levels

Treatment group (n = 8)	Glutathione S-transferase α level (ng/mg protein)						
	oesophagus	stomach	PSI	MSI	DSI	colon	liver
Control	ND	222±20	4705±753	2780±453	734±175	ND	12282±1227
Indomethacin	ND	308±79	7378±874 ^a	3322±509	1389±331 ^a	ND	11774±567
Ibuprofen	ND	252±15	6547±499 ^a	2849±643	974±131	ND	13614±2399
Piroxicam	ND	400±54 ^a	6413±662	2783±346	2028±447 ^b	ND	14817±1158 ^a
ASA	ND	288±36	5741±659	2682±386	951±165	ND	10267±676
Sulindac	ND	241±29	5694±300	3459±1043	1526±153 ^b	ND	11306±897

ND, Not detectable; ^aP<0.05, and ^bP<0.01.

Table IV. Effects of indomethacin, ibuprofen, piroxicam, ASA or sulindac on rat alimentary tract glutathione S-transferase μ levels

Treatment group (n=8)	Glutathione S-transferase μ level (ng/mg protein)						
	oesophagus	stomach	PSI	MSI	DSI	colon	liver
Control	3469±326	32771±1768	8112±1037	6839±774	4891±684	4567±291	31662 _ 5467
Indomethacin	4738±343 ^b	30755±2787	12918±1334 ^a	7581±873	5644±639	5178±744	37877 _ 3352
Ibuprofen	4792±268 ^a	39165±2641	10582±870 ^a	6313±993	5907±676	4598±637	33416 _ 5832
Piroxicam	5432±356 ^c	34470±1926	11740±1065 ^a	6859±789	6526±420	4691±240	32393 _ 2267
ASA	4008±399	37763±1268	9227±1338	6153±804	5432±676	5650±751	28760 _ 4384
Sulindac	5223±461 ^c	34591±813	11263±1027 ^a	6975±897	6289±785	5500±791	25555 _ 4719

^aP<0.05, ^bP<0.01 and ^cP<0.005.

development of colonic cancer (2–4). NSAIDs such as aspirin, indomethacin, piroxicam, and sulindac were repeatedly shown to inhibit chemically induced tumours of the colon (15,17–19,29–31,51–53), oesophagus (26,27), bladder (54), breast (21), and liver (24) in laboratory animals.

Several hypotheses have been proposed to explain the mechanism of chemoprevention by NSAIDs: (a) NSAIDs reduce the gastrointestinal permeability of carcinogens and

their metabolites (55), (b) NSAIDs are scavengers of reactive oxygen species involved in initiation and promotion of cancer (56), (c) NSAIDs can bind to cytochrome P450-monoxygenases, thereby inhibiting P450-mediated activation of procarcinogens to reactive electrophilic intermediates (57,58). On the other hand, ibuprofen and indomethacin are able to induce prokaryotic cytochrome P450_{BM-3} (CYP102) (59). (d) In parallel with the inhibition of tumour growth, aspirin, indomethacin,

Table V. Effects of indomethacin, ibuprofen, piroxicam, ASA or sulindac on rat alimentary tract glutathione S-transferase π levels

Treatment group (n=8)	Glutathione S-transferase π level (ng/mg protein)						
	oesophagus	stomach	PSI	MSI	DSI	colon	liver
Control	ND	240±39	568±84	114±21	125±50	238±51	ND
Indomethacin	ND	306±87	542±44	234±39 ^b	262±98	328±48	ND
Ibuprofen	ND	464±51 ^c	642±46	188±55	156±32	257±23	ND
Piroxicam	ND	397±89	590±59	278±67 ^b	356±89 ^b	247±24	ND
ASA	ND	447±95 ^a	599±59	368±105 ^b	236±54 ^a	318±30	ND
Sulindac	ND	616±27 ^c	554±73	415±72 ^c	249±58	268±47	ND

ND, Not detectable; ^a*P*<0.05, ^b*P*<0.01, and ^c*P*<0.005.

Table VI. Effects of indomethacin, ibuprofen, piroxicam, ASA or sulindac on rat alimentary tract glutathione levels

Treatment group (n=8)	Glutathione (ng/mg protein)						
	oesophagus	stomach	PSI	MSI	DSI	colon	liver
Control	18.1±1.6	24.8±2.1	12.2±1.9	3.8±0.8	2.4±0.5	8.8±2.9	39.2±2.9
Indomethacin	24.5±3.4	26.5±2.5	15.1±1.4 ^a	8.6±1.1 ^c	5.6±0.6 ^c	7.5±2.8	40.8±6.9
Ibuprofen	20.1±1.6	27.3±3.3	13.9±0.8	4.4±0.9	1.5±0.3	7.6±2.6	45.9±6.9
Piroxicam	28.2±3.1 ^b	27.0±2.9	15.2±1.8	5.4±1.4	2.3±0.3	6.5±1.7	51.2±6.0
ASA	16.6±1.4	25.3±2.0	12.3±1.5	6.0±0.9 ^a	1.5±0.2	6.7±2.4	37.0±3.5
Sulindac	17.8±1.2	19.0±1.9	13.9±1.7	5.9±1.1	1.3±0.1	4.8±1.6	46.1±3.7

^a*P*<0.05, ^b*P*<0.01, and ^c*P*<0.005.

and piroxicam, reduce the prostaglandin levels in the colon of rodents treated with carcinogens (15,17,60), by inhibition of cyclooxygenases, the rate-limiting enzymes in the synthesis of prostaglandins (61,62). On the other hand, GSTs are involved in the synthesis of prostaglandin D₂, E₂ and F_{2 α} (63). (e) NSAIDs can inhibit the induction of ornithine decarboxylase activity and tissue levels of putrescine, two markers of tumour promotion (64,65). (f) In addition, NSAIDs may inhibit the activity of enzymes such as phosphodiesterases or cyclic GMP-AMP protein kinases (66), which may be central to cancer initiation and promotion.

Much of the research on NSAIDs and cancer prevention at this moment is focused on the hypothesis that prostaglandins may play a key role in the regulation of neoplasia. However, there is no direct evidence that NSAIDs prevent tumour development solely through inhibition of cyclooxygenases (67), and prevention of cancer could be due to multiple mechanisms. Another way of action of NSAIDs, in addition to the possibilities cited above, may be the enhancement of carcinogen detoxification by GSTs, as shown in this study. A more efficient detoxification could lead to a reduction of biologically active compounds and thus prevent carcinogenesis. No information about the possible effects of NSAIDs on oesophageal, gastric, intestinal, and hepatic GST enzyme activity has been reported before. In our study, GST activity in the oesophagus and proximal small intestine was increased by indomethacin, ibuprofen, piroxicam, and sulindac. This may be of direct significance in the protection against cancer in these organs. However, organs such as the colon could also benefit from a more efficient detoxification in the upper part of the digestive tract, since lower levels of carcinogens may now reach the colon.

In human organs at high risk for cancer development low GST levels were measured, and vice versa (68). Recent data, mostly obtained from animal studies, have indicated that many naturally occurring dietary anticarcinogens are able to elevate

the levels of GSTs (44,45, and references therein). Enhancement of GSTs in humans was found after consumption of cruciferous vegetables with cancer preventing properties such as broccoli and Brussels sprouts (69,70) and a reduction of oxidative DNA damage in humans was measured after consumption of brussels sprouts (71). In this respect the chemopreventive properties of NSAIDs could very well be mediated in a similar way by the enhancement of GSTs.

In addition to the measurement of GST activity, it may be important to register changes in the levels of GST isoenzymes, which have different though partly overlapping substrate specificities. The isoenzymes showed a tissue specific distribution. Class α GSTs are abundant in liver and small intestine, whereas class Pi GSTs are present in stomach, small- and large intestine (43,45). In contrast, class μ enzymes seem to be less organ specific since they were detected at high levels in all tissues examined. GST α was not detectable in the oesophagus and colon, and GST π was undetectable in both oesophagus and liver and none of the NSAIDs had any effect on this. The increase in GST activity in oesophagus and PSI by indomethacin, ibuprofen, piroxicam and sulindac was paralleled by a rise in GST μ level in the same order of magnitude. This was expected, since GST μ is the most prominent of all GSTs in the rat (Tables III–V). In two of the sites in which both GST activity and GST μ levels were induced, GST α level was increased as well. The molecular basis for these inductions is not clear yet.

Indomethacin, ibuprofen, piroxicam, and sulindac each induced the GST enzyme activity as well as GST α , GST μ or GST π levels in at least one organ. Indomethacin, ibuprofen and sulindac were equally efficient in inducing glutathione S-transferases, in seven out of 28 possibilities (25%). Piroxicam appeared to be the most active, with inductions seen in 32% of all possibilities. Acetyl salicylic acid showed an increase of glutathione S-transferase enzyme activity or isoenzyme levels in only 11% of all possibilities, which makes it the least

active form of the NSAIDs tested. The amount of NSAIDs consumed by the rats in our study (2–35 mg/day/kg b.w.) matches very well with doses prescribed to patients suffering from rheumatic diseases (3–100 mg/day/kg b.w.; see Table I), suggesting that the effects of NSAIDs on GSTs as found in rats may be achieved in humans as well. NSAIDs have side-effects in both humans as well as animals. We observed a decreased food consumption in the piroxicam and sulindac groups. In these animals, however, none of the organs showed any macroscopical sign of toxicity at the end of the experiment, suggesting that the induction of GSTs are not the consequence of toxic effects of these NSAIDs. This is further supported by the observation that ibuprofen induced food consumption as well as GST activity and isoenzymes.

No data on the effects of the NSAIDs tested on GSH levels have been reported before. GSH is an important physiological nucleophile which is coupled with reactive electrophiles such as carcinogenic nitrosamines (45), catalysed by the glutathione S-transferases. Significantly increased levels of GSH, however, were found in only five out of 35 possibilities: in small intestine by indomethacin, in oesophagus by piroxicam and in MSI by ASA. In PSI and oesophagus, this increase in GSH level paralleled an induction of GST activity.

In conclusion, our data demonstrate that NSAIDs are able to elevate the detoxification potential of tissues from the gastrointestinal tract, by increasing the expression of GSTs. This seems to be a common working mechanism in the prevention of carcinogenesis.

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