

Mechanism of Action of Acetaminophen: Is There a Cyclooxygenase 3?

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Acetaminophen, also known as paracetamol, is a nonsteroidal anti-inflammatory drug with potent antipyretic and analgesic actions but with very weak anti-inflammatory activity. When administered to humans, it reduces levels of prostaglandin metabolites in urine but does not reduce synthesis of prostaglandins by blood platelets or by the stomach mucosa. Because acetaminophen is a weak inhibitor in vitro of both cyclooxygenase (COX)-1 and COX-2, the possibility exists that it inhibits a so far unidentified form of COX, perhaps COX-3. In animal studies, COX enzymes in homogenates of different tissues vary in sensitivity to the inhibitory action of acetaminophen. This may be evidence that there are >2 isoforms of the enzyme. Recently, a variant of COX-2 induced with high concentrations of nonsteroidal anti-inflammatory drugs was shown to be highly sensitive to inhibition by acetaminophen. Therefore COX-3 may be a product of the same gene that encodes COX-2, but have different molecular characteristics.

Acetaminophen, known as paracetamol in the United Kingdom, was introduced to medicine in 1893 [1]. It had only limited use, however, until 1949, when it was identified as the active metabolite of 2 older antipyretic drugs, acetanilide and phenacetin [2, 3]. Its popularity as an analgesic and antipyretic gradually increased, but it was not marketed in the United States until 1955, by McNeil Laboratories, and it is now the best-selling analgesic under the trade name of Tylenol. Recent clinical studies [4, 5] did not show any advantage of analgesic or anti-inflammatory doses of ibuprofen over acetaminophen as symptomatic treatment for patients with osteoarthritis [6]. In addition, the side effects of long-term administration of acetaminophen are less severe, without the gastrototoxicity of most nonsteroidal anti-inflammatory drugs (NSAIDs). Acetaminophen-induced liver damage is normally seen only with daily doses greater than 10 g, whereas the recommended therapeutic dose for adults is 4 g [7].

In spite of its wide use, the mechanism of action of acetaminophen has not been fully elucidated. It is only a weak inhibitor of prostaglandin (PG) synthesis in vitro and appears to have very little anti-inflammatory activity, although some reduction of tissue swelling after dental surgery has been reported [8, 9]. Because of its potent analgesic and antipyretic actions, it is generally regarded as an NSAID. However, it lacks the other typical actions of NSAIDs, such as antiplatelet activity and gastrototoxicity.

Anti-Inflammatory Activity

Although it is generally accepted that acetaminophen has only a weak anti-inflammatory action, reports of a reduction in tissue swelling after dental surgery have been recorded [8, 9]. In one study, acetaminophen was shown to be as potent as aspirin [8]. Also, in a double-blind crossover study, acetaminophen reduced postoperative soft tissue edema by 30% compared with placebo [10].

Although Flower et al. [11] were unable to measure any anti-inflammatory activity of acetaminophen on the carrageenin-induced edema in the rat paw, they may have been using doses too low to have a significant effect. Seegers et al. [12], who used 250 mg/kg of acetaminophen in the same model, found a moderate reduction of paw swelling, but not as much as with 250 mg/kg aspirin. Other groups recorded a 20% reduction of rat paw edema with 200 mg/kg acetaminophen [13] and a small non-dose-related inhibition with doses of 5, 15, and 45 mg/kg [14]. In a model of rat carrageenin pleurisy, the median effective doses ($ED_{50} \pm SEM$) against the 3-h pleural exudate volume were 210 ± 28.8 mg/kg for acetaminophen and 31 ± 7.0 mg/kg for aspirin, whereas these drugs administered daily in the diet for 14 days were active in rat adjuvant arthritis at ED_{50} doses of 460 ± 240.6 mg/kg for acetaminophen and 200 ± 88.5 mg/kg for aspirin [15].

By comparison, 200 mg/kg acetaminophen raised the threshold for stimulation 4-fold in the Randall and Selitto test for analgesia [13]. Moreover, in the rat carrageenin hyperalgesic assay, the ED_{50} for acetaminophen was 110 ± 23.4 mg/kg, whereas in the mouse writhing assay, it was 305 ± 218 mg/kg [15]. Thus, it is possible to demonstrate in animal models a weak anti-inflammatory effect with high doses of acetaminophen and some reduction of inflammation when this has been specifically measured in patients.

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Antipyresis

The antipyretic activity of acetaminophen has been demonstrated in a number of species. Among the earliest reports were those of Milton and Wendlandt [16, 17], who found that in the conscious cat, acetaminophen suppressed the fever caused by intracerebroventricular administration of endotoxin but did not affect fever induced by intracerebroventricular injection of PGE₁. This was soon explained by the work of Vane [18], who noted that antipyretic activity of NSAIDs was due to inhibition of synthesis of pyrogenic PG. In later work [19], it was shown that in unanesthetized cats, acetaminophen reduced fever induced by bacterial pyrogen in parallel with reduction in levels of a PG-like material in the cerebrospinal fluid. It is interesting that the fever and elevated PG levels in the cerebrospinal fluid in response to centrally or iv administered endogenous pyrogen (which has since been identified as IL-1) were also effectively lowered by acetaminophen [20]. In addition, fever produced by injection of sodium arachidonate into cerebral ventricles of conscious cats was reversed by iv administration of acetaminophen (figure 1) [21].

Intraperitoneal injections of indomethacin or acetaminophen reduced the rectal temperatures of unanesthetized rats; the animals' temperatures had been raised by intraventricular administration of endotoxin (figure 2). Indomethacin was more potent than acetaminophen [22]. Acetaminophen reduced en-

dotoxin-induced fever in the conscious goat [23], and also reduced fever in rabbits rendered febrile by IL-1 [24].

Acetaminophen has been frequently recommended as the drug of choice for treatment of febrile children [25–28] and used as a standard for comparison in trials of new antipyretic drugs such as ketorolac [29]. However, there is still speculation about the exact site for the antipyretic action of the NSAIDs. PGs that cause fever are necessarily produced in or close to the preoptic area of the hypothalamus where body temperature is regulated. Neither bacterial endotoxin (exogenous pyrogen) nor IL-1 (endogenous pyrogen) cross the blood-brain barrier to penetrate into the brain [30–32]. Because the organum vasculosum laminae terminalis (OVLT) lies outside the blood-brain barrier, it has been suggested that receptors in the region of the OVLT may be the site of action for IL-1 [32, 33].

Acetaminophen easily penetrates the brain [34], so it could inhibit PG synthesis whether these PGs are formed centrally or peripherally. Recent evidence in cyclooxygenase (COX)-2 knockout mice suggests that PGs synthesized by COX-2 are responsible for the febrile response [35]. Matsumura et al. [36] have postulated that circulating endotoxin induces this COX-2 in endothelial cells of hypothalamic blood vessels and PGs formed by this enzyme penetrate into the OVLT to produce fever.

A peripheral mechanism of antipyretic action has also been

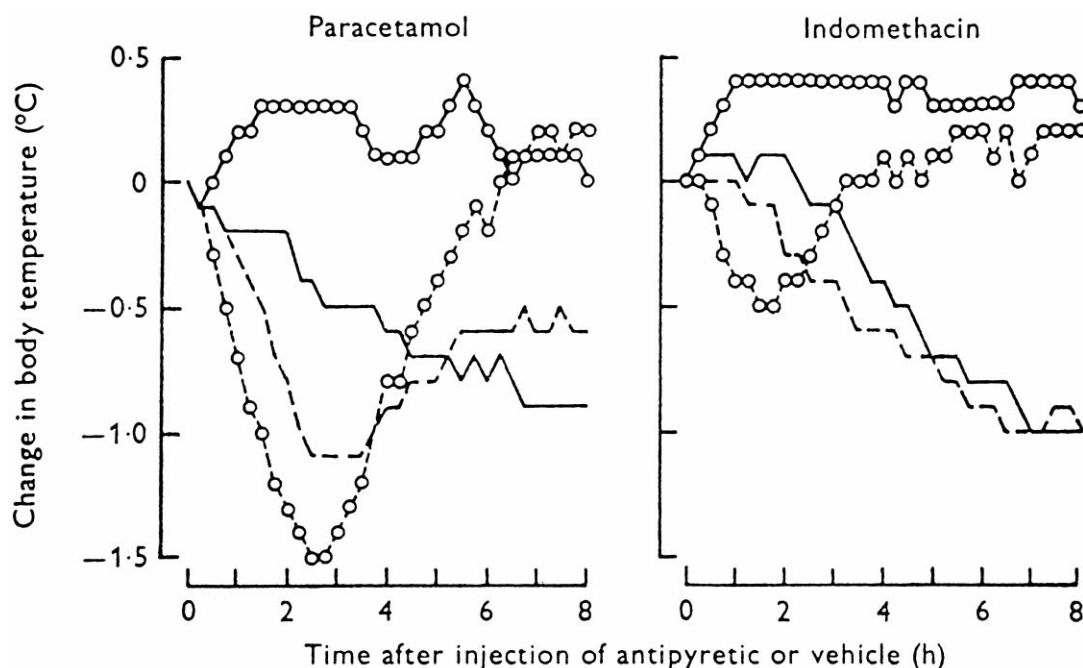


Figure 1. The effect of acetaminophen (paracetamol) on the body temperature of unanesthetized cats. Changes in hyperthermic responses to arachidonate and endotoxin after administration of acetaminophen (40 mg/kg) or indomethacin (40 µg/kg) were measured. *Open circles and continuous line*, pyrogen plus vehicle; *open circles and broken line*, pyrogen plus antipyretic; *continuous line*, arachidonate plus vehicle; *broken line*, arachidonate plus antipyretic. Reproduced with permission from [21].

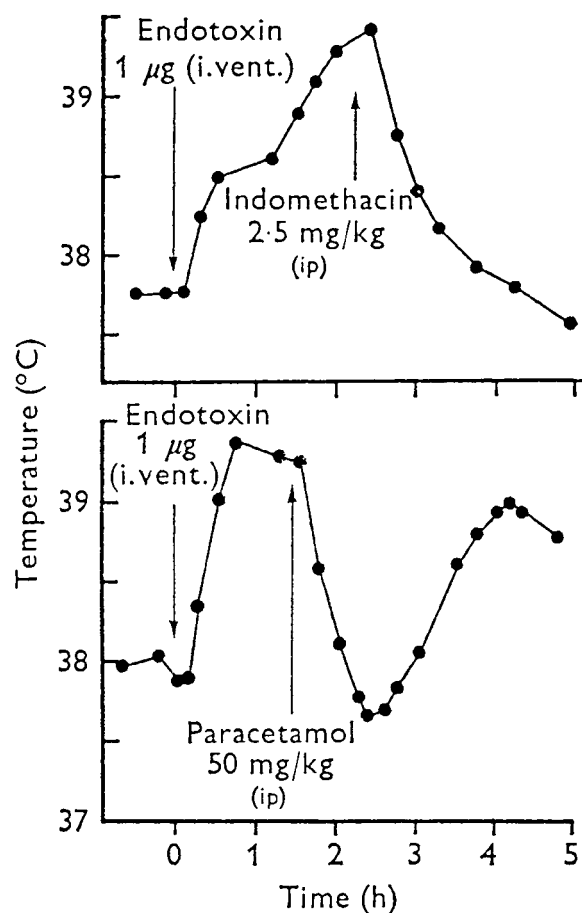


Figure 2. The effect of acetaminophen (paracetamol) on the rectal temperature of unanesthetized rats. *Left arrow*, injection into the cerebral ventricles of 1 µg endotoxin from *Salmonella abortus equi*. *Right arrow*, ip injection of indomethacin 2.5 mg/kg (*upper graph*) or acetaminophen 50 mg/kg (*lower graph*). I.vent., intraventricular. Reproduced with permission from [22].

proposed [37]. This postulates that endotoxin increases formation of PGs in the brain by stimulating receptors on sensory fibers of the vagus nerve [38]. Sectioning of the vagus nerve below the diaphragm in rats or guinea pigs prevents endotoxin-induced fever, induction of IL-1 β , and increase in preoptic PGE₂ levels [37, 39]. Perhaps this vagal sensory mechanism contributes to the genesis of fever but does not account entirely for the febrile response. The mechanism of acetaminophen-induced antipyresis must surely be explained by inhibition of COX-2 or a variant of this enzyme (see the article by Simmons et al. in this issue).

Analgesia

The early experiments of Lim et al. [40] on the cross-perfused dog spleen indicated a peripheral rather than a central site for the analgesic action of acetaminophen. Acetaminophen blocked the vocalization response to bradykinin injected into the spleen

of a recipient dog perfused with blood from a donor dog. Because the spleen of the bradykinin-treated dog was the only organ of the animal to receive acetaminophen, the conclusion was that in contrast to morphine, this drug had a peripheral site of action.

It was then generally accepted that the analgesic action of all NSAIDs was due to inhibition of PG formation at peripheral sites and that the only animal models that demonstrated their effect was the mouse or rat abdominal constriction tests. These tests involve the production of “writhing” or “stretching” along the abdominal wall by an ip injection of dilute acetic acid, acetylcholine, or phenylbenzoquinone [41]. Acetaminophen inhibited the abdominal constriction response to ip administration of acetic acid [42] or acetylcholine [43] in mice. Analgesic oral doses of acetaminophen also attenuated the ex vivo synthesis of brain PGE₂ in a dose-related manner [43], thus providing evidence for a central component of the analgesic action of this NSAID.

In a double-blind, placebo-controlled study in healthy volunteers, Piletta et al. [44] obtained evidence for a central analgesic action of acetaminophen. Application of a transcutaneous electrical stimulus to the sural nerve causes a flexion reflex and a subjective sensation of pain. In contrast to aspirin, acetaminophen raised the threshold to both types of pain, indicating an analgesic action both at the spinal cord level and in higher centers. Studies of Pini et al. [45] in the rat provided further evidence for a central analgesic action of acetaminophen. In the hot plate and formalin tests, high doses of acetaminophen produced an anti-nociceptive effect abolished by naloxone. Acetaminophen showed affinity for [³H] naloxone binding sites, increased brain 5-hydroxytryptamine concentrations, and reduced the number of 5HT₂ receptors in cortical membranes. These effects were similar to those of morphine.

Evidence for the involvement of a serotonergic mechanism in analgesia with high doses of acetaminophen has also accumulated from studies in rats. Chemical lesions of central serotonergic pathways reduced the antinociceptive effect in the hot plate and formalin tests [46, 47]. Pelissier et al. [48] concluded that acetaminophen indirectly activates spinal 5HT₃ receptors. Surprisingly, in these studies, the effects of acetaminophen were not blocked by naloxone.

Clinical trials have demonstrated that acetaminophen is a safe and effective analgesic for the relief of mild to moderate pain associated with oral surgery, episiotomy, postpartum pain, cancer, osteoarthritis, dysmenorrhea, and headache [49, 50]. It lacks the gastrointestinal side effects of aspirin but causes hepatotoxicity in overdose. As with all NSAIDs, the analgesic action of acetaminophen is limited by a ceiling effect, when an increase in dose produces only a minor increment in effect [50].

Hemostasis

Acetaminophen is a weak inhibitor of aggregation of human platelets [51] and does not reduce PG synthesis in isolated plate-

lets of the rat [52]. In contrast to aspirin, it has no effect on the hemostatic mechanism in children and can be used in clinical situations where the use of aspirin may cause dangerous bleeding.

A study that compared the effects of acetaminophen and aspirin on hemostasis in healthy volunteers and in patients with hemophilia found that acetaminophen did not alter template bleeding time or platelet function in either group [53]. Two h after ingestion of 975 mg of acetaminophen, the mean bleeding time was 5.5 min, compared with 12 min after the same dose of aspirin. The normal bleeding time was 5 min. Template bleeding time, platelet aggregation, and release of platelet factor 3 were not affected by a single administration of 1950 mg of acetaminophen or by 1950 mg given daily for 7 days. The same dosages of aspirin significantly prolonged bleeding time and impaired platelet aggregation. Aspirin greatly prolonged bleeding time in hemophiliac patients, whereas acetaminophen had no effect on this parameter. Comparison of acetaminophen with 10 nonsteroidal analgesics administered to normal volunteers established that only acetaminophen (1 g) and oxyphenbutazone (400 mg) failed to inhibit epinephrine-induced platelet aggregation [54].

The use of acetaminophen rather than aspirin is preferred where there is a likelihood of bleeding. For example, in a study of posttonsillectomy hemorrhage, there was a significantly lower incidence of secondary bleeding in acetaminophen-treated patients than in patients given aspirin [55]. It is clear that acetaminophen-induced analgesia, unlike that of other NSAIDs, need not be accompanied by impairment of platelet function.

Gastric Mucosa

The absence of gastrototoxicity of acetaminophen has been widely documented in human and animal studies. Ivey [56] describes how the use of aspirin declined in the 1960s and 1970s, while that of acetaminophen simultaneously increased. A study of 25,000 patients by Jick [57] from the Boston Collaborative Drug Surveillance Program found a significant association between gastrointestinal bleeding and regular heavy intake of aspirin but not of acetaminophen. Moreover, double-blind endoscopic studies comparing aspirin, acetaminophen, and placebo in normal volunteers showed that acetaminophen caused no significant gastric mucosal damage, whereas aspirin produced significantly more ($P < .005$) gastric erosions than placebo [58, 59]. Fecal occult blood loss, measured with the $^{51}\text{chromium}$ -tagged red cell technique, did not increase after administration of acetaminophen to normal subjects in doses of 2.6 g [60] or 4.0 g per day [61] for 5 to 7 days, or between 2.6 g and 5.2 g daily in patients with rheumatoid arthritis [62]. However, even 300 mg of aspirin 4 times a day [63] produced a mean blood loss of 4.5 mL per day compared with a normal blood loss of ~0.5 mL per day.

The effect of acetaminophen was also measured on PGE₂

synthesis by human gastric mucosa. In one study, indomethacin inhibited synthesis of PGE₂ by biopsy specimens of human gastric mucosa with a median inhibitory dose (IC₅₀) of 4.2 μg/mL, whereas acetaminophen in concentrations up to 310 μg/mL had no effect [64]. The study of Konturek et al. [65] compared the inhibition of PGE₂ generation by 2.5 g aspirin or acetaminophen given in divided doses during a single day. Although aspirin reduced PGE₂ formation by >60%, acetaminophen barely lowered the PGE₂ concentration. An interesting observation has been that acetaminophen protects the human stomach against the mucosal erosions caused by 1300 mg aspirin or 20% vol/vol ethanol [66]. Acetaminophen (2600 mg) given orally 1 h before aspirin or ethanol was administered significantly reduced the endoscopic damage caused by these agents. This protective effect of acetaminophen may be due to increased PG production because it was abolished by indomethacin in doses too low to damage the stomach mucosa.

Studies in rats have demonstrated that acetaminophen increases both PGE₂ and PGI₂ production by stomach mucosa. PGE₂ generation ex vivo in response to acetaminophen (250 mg/kg or 800 mg/kg) administration was significantly increased [67, 68], as was formation of PGI₂ by acetaminophen (250 mg/kg) [69]. Acetaminophen (50 μg/mL) added to homogenates of stomach mucosa in vitro also increased PGE₂ production [68]. PGI₂ formation in vitro was stimulated by low concentrations (<1 mM) and inhibited by higher concentrations (>1 mM) of acetaminophen [70].

The protective effect of acetaminophen against gastric mucosal damage caused by ethanol, aspirin, or indomethacin was also demonstrated in rats. Gastric lesions induced with 100% ethanol or 20 mg/kg aspirin were significantly reduced by acetaminophen (80 mg/kg) [71] and 200 mg/kg acetaminophen protected against an ulcerogenic dose of indomethacin (25 mg/kg) [72]. Surprisingly, these protective concentrations of acetaminophen did not raise PG production of the stomach mucosa above the low levels produced in the presence of aspirin or indomethacin [67, 71, 72]. However, low nonulcerogenic doses of indomethacin (5 mg/kg) reversed the protective action of acetaminophen against ethanol or aspirin damage, possibly by preventing the increase in PG production [71]. Thus acetaminophen protects the gastric mucosa against erosive damage not only by increasing synthesis of mucosal PGs but possibly also by other mechanisms, such as scavenging of free hydroxyl radicals.

PG Formation

Acetaminophen in low concentrations stimulates and in high concentrations inhibits the synthesis of PGs. This dual action was first demonstrated by Robak et al. [73] on COX enzyme of bull seminal vesicle microsomes. In concentrations of 67–667 μM and without addition of cofactors, acetaminophen increased the formation of PGE₂ more than 3-fold. However, above these concentrations or in the presence of the cofactors glutathione (165 μM) and hydroquinone (45 μM), acetaminophen (200–3330

μM) acted as an enzyme inhibitor, with an IC_{50} of $1500 \mu\text{M}$. McDonald-Gibson and Collier [74] confirmed the stimulation of PG production by acetaminophen ($0.07\text{--}1.98 \text{ mM}$) in homogenates of bull seminal vesicles and depression of PG synthesis by concentrations above this, with an IC_{50} value for inhibition of 5.56 mM . The double action of acetaminophen on PG synthesis by the rat stomach has already been mentioned [70].

Tissue-specific inhibition of PG production has also been described. Flower and Vane [75] found that acetaminophen was ~ 10 times more potent in inhibiting PG synthesis by the rabbit brain than by the dog or the rabbit spleen. Oral administration of $100\text{--}300 \text{ mg/kg}$ to guinea pigs stimulated PG production in cell-free preparations of stomach tissue *ex vivo*, whereas no effect was seen in preparations of kidney medulla or of lungs [76]. Rats receiving oral doses of acetaminophen (25 or 200 mg/kg) showed increased PG synthesis by the stomach *ex vivo*, no effect on the lungs, and, with a dose of 300 mg/kg , a significant decrease in PG formation by sections of cerebral cortex. Acetaminophen (10^{-6} to 10^{-4}) added to cell free preparations of guinea pig stomach *in vitro* stimulated PG release but inhibited release from sections of rat cerebral cortex. PGE_2 synthesis by rabbit inner medullary kidney microsomes was inhibited by incubation with acetaminophen with an IC_{50} of 0.1 mM [77]. However, Bruchhausen and Baumann [78] have presented evidence that there is no tissue-specific action; they found that PG synthesis by enzyme preparations from a neuronal cell line, a glial cell line, and rat renal medulla were all inhibited by 50% with a similar concentration of acetaminophen (1 mM).

In humans, acetaminophen inhibits the *in vivo* synthesis of PGs. Oral administration of 500 mg of acetaminophen reduced

the synthesis of prostacyclin, measured by the urinary excretion of the inactive metabolite, 2,3-dinor-6-keto- $\text{PGF}_{1\alpha}$, in healthy volunteers [79]. Urinary excretion of the thromboxane metabolite, 2,3-dinor- TXB_2 , was not affected. These results were confirmed in pregnant women by measuring excretion of 2,3-dinor-6-keto- $\text{PGF}_{1\alpha}$ after ingestion of acetaminophen (1000 mg). Urinary concentrations (mean \pm SEM) of the prostacyclin metabolite fell from 2233 ± 446 to $1246 \pm 199 \text{ pg/mg creatinine}$. No difference in the excretion of the metabolite of thromboxane could be measured. The inhibition of prostacyclin synthesis, perhaps formed by COX-2, may be taking place in endothelial cells because the COX enzyme of human umbilical vein endothelial cells is sensitive to inhibition with acetaminophen in doses as low as $10 \mu\text{g/mL}$ (equivalent to $70 \mu\text{M}$) [80]. The urinary excretion of the major urinary metabolite of PGE_2 , but not of PGE_2 itself, was reduced in volunteers who received 3 g daily of acetaminophen for 2 consecutive days [81]. This indicates that acetaminophen inhibits total body synthesis of PGE_2 but not PGE_2 synthesis by the kidney.

The reduction of PG synthesis by acetaminophen can be explained by its competitive inhibition with the substrate, arachidonic acid, for the active site on COX enzyme [77]. A possible explanation for its tissue selectivity may be the level of cellular peroxides present in various cells. Thus the weak anti-inflammatory activity of acetaminophen may be due to its inability to inhibit COX in the presence of the elevated cellular peroxides found in inflamed cells. Where cellular peroxide levels are low, acetaminophen can inhibit PG synthesis and produce analgesia and antipyresis [82, 83]. The stimulation of COX activity by acetaminophen is more difficult to understand. This

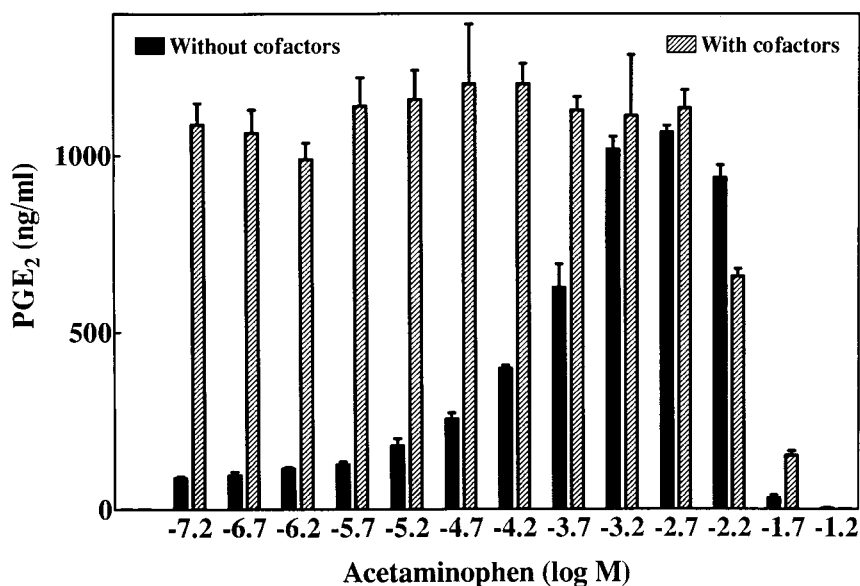


Figure 3. The effect of acetaminophen on homogenate of J774.2 mouse macrophages incubated with bacterial endotoxin with (hatched bars) and without (solid bars) added cofactors. PGE_2 , prostaglandin E_2 .

Table 1. Effect of acetaminophen on cyclooxygenase activity in mouse tissue homogenates, measured as levels of prostaglandin E₂ (PGE₂) or 6-keto-PGF_{1α}.

Activity	EC ₅₀ of acetaminophen, mean mM ± SEM, (n)			
	J774.2 cells	Gastric mucosa	Brain	Spleen
Stimulation ^a	0.12 (2)	0.08 (2)	ND	ND
Inhibition ^b	7.89 ± 0.52 (10)	12.00 ± 2.15 (4)	1.5 ± 0.23 (6)	1.00 ± 0.1 (6)

NOTE. EC₅₀, median effective concentration; ND, not done.

^a Means of 2 specimens.

^b Means of 4–10 specimens from 2–5 animals.

action, however, is shared by some other NSAIDs, including aminopyrine, dipyron, meclofenamic acid, mefenamic acid, and phenylbutazone [73, 82]. Low concentrations of weak COX inhibitors tend to stimulate PG synthesis in conditions of a limited supply of arachidonic acid [84].

Mechanism of Action

Therefore it is highly likely that acetaminophen inhibits the synthesis of PGs by competing with arachidonic acid for the active site on the COX enzyme. Why then does it potently reduce pain and fever while having very little effect on inflammation? Also, why is it able to prevent PG synthesis in some tissues, such as brain, spleen, kidney, and lung, and not in others, such as platelets and stomach mucosa? Because the properties of acetaminophen are obviously different from those of the typical NSAIDs, we have postulated the existence of a COX enzyme that can be selectively inhibited by this drug in preference to COX-1 or COX-2.

The elucidation of the structure and cloning of the gene for COX-1 [85–87] was followed by the discovery, in 1991, of the gene encoding for the second COX enzyme (COX-2), which could be induced with lipopolysaccharide, cytokines, or mitogens [88–90]. Investigations of the relative sensitivity of the

2 enzymes to acetaminophen found that although the drug was a weak inhibitor of both enzymes, COX-1 was marginally more sensitive than COX-2 (ratio of IC₅₀ for COX-2 to IC₅₀ for COX-1 = 7.4) [91]. In view of the low sensitivity to acetaminophen of both COX-1 and COX-2, the existence of a new, so far unknown isoform of COX has been postulated and provisionally named COX-3 [92].

Experiments were performed to confirm the report of Flower and Vane [75] that brain COX is more sensitive to inhibition by acetaminophen than spleen COX. The COX enzymes of homogenates of mouse brain, spleen, stomach mucosa, and macrophage cell line J774.2 were tested for their sensitivity to acetaminophen. For most measurements, the cofactors glutathione (0.1 mM) and epinephrine (5 mM) were added to the incubations, but some determinations were made in the absence of cofactors. The most striking difference of addition of cofactors was seen in the effect of acetaminophen on homogenates of cultured mouse J774.2 macrophages treated with lipopolysaccharide. Addition of cofactors dramatically elevated the activity of the induced COX-2; and acetaminophen, in concentrations above 1 mM, inhibited the activity of the enzyme. Without added cofactors, COX-2 activity was very low, but it increased to the same levels as those produced with cofactors when acetaminophen was added in concentrations from 10 μM to 1 mM (figure 3). In concentrations above 1 mM, acetaminophen again reduced PG synthesis [93]. Addition of cofactors raised the activity of stomach mucosal COX-1 only slightly. However, without added cofactors, low concentrations of acetaminophen caused a significant increase of PG synthesis by the stomach mucosa, and higher concentrations inhibited synthesis. Finally, similar concentrations of acetaminophen inhibited both mouse brain COX-1 and spleen COX-1. These concentrations were 10 times less than the concentrations needed to inhibit COX-1 in stomach mucosa or COX-2 in J774.2 macrophages.

Table 2. Classification of cyclooxygenase (COX) enzymes.

Animal	Enzyme sensitivity to acetaminophen inhibition in tissue			
	Insensitive		Sensitive	
	COX-1a	COX-2a	COX-1b	COX-2b
Dog	Spleen		Brain	
Rabbit	Spleen		Brain	
	Stomach (inhibits)		Lung	
Guinea pig	Kidney, lung		Stomach (stimulates)	
Rat	Lung		Stomach (stimulates); brain (inhibits)	
Rat			Stomach (stimulates, then inhibits)	
Rat			Kidney	
Mouse	Stomach (inhibits)	Macrophage J774.2 cells induced with LPS	Brain, spleen	Macrophage J774.2 cells induced with NSAIDs
Human	Platelets		HUVECS; endothe- lial cells in vivo	

NOTE. HUVECS, human umbilical vein endothelial cells; LPS, lipopolysaccharide; NSAIDs, nonsteroidal anti-inflammatory drugs. Based on data from [70, 75–77, 80, 93, 94].

A summary of the effects of acetaminophen on homogenates of mouse tissues is presented in table 1.

The effects of acetaminophen were also measured on the PG levels of rabbit brain, spleen, stomach mucosa and lungs after iv administration of 100 mg/kg of the drug. The COX enzymes of brain and spleen were almost equally inhibited by acetaminophen, whereas COX-1 in stomach mucosa was hardly reduced. An interesting observation was that COX-1 in homogenates of rabbit lungs was 10 times more sensitive to inhibition by acetaminophen than the enzymes of brain or spleen (author's unpublished observations). We are studying the possibility that lung cells may be a source of an acetaminophen-sensitive COX enzyme.

An NSAID-inducible COX was recently reported to be induced in cultured J774.2 macrophages after 48 h incubation with diclofenac. This enzyme was sensitive to inhibition by acetaminophen in concentrations similar to those found in plasma after administration of therapeutic doses of the drug. The induced enzyme appeared to be a variant of COX-2 that remained free in the cytosol rather than becoming attached to a cell membrane. It is possible that a similar induced enzyme is the target for the antipyretic and analgesic actions of acetaminophen [94].

In the rat pleurisy model of inflammation, COX-2 is not only induced in the initial stages of the inflammatory response but also during the resolution phase. This late-induced enzyme, appearing 48 h after the start of the inflammatory process, may produce PGs that are involved in the resolution of inflammation and that could have properties in common with the NSAID-induced COX-2 [95].

A statistically significant inverse association was found between the use of acetaminophen and ovarian cancer risk, whereas the apparent inverse association with aspirin use was not significant [96]. In a prospective study, women who reported taking acetaminophen daily had a 45% lower death rate from ovarian cancer than women reporting no use [97]. Human ovarian adenocarcinomas were found to overexpress COX-1, in contrast to most tumors, which overproduce COX-2 [98]. Thus, ovarian tumors are unusual in overexpressing COX-1 and in their susceptibility to inhibition by acetaminophen.

Conclusions

Both COX-1 and COX-2 enzymes localized in different tissues are variously sensitive to inhibition by acetaminophen. These different properties of the COX enzymes are summarized in table 2. Characterization of acetaminophen-sensitive enzymes from different sources may help to identify a COX enzyme with some properties in common with both COX-1 and COX-2, which is nevertheless unique and can be regarded as COX-3.

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