

Inhibition of phenolsulphotransferase by salicylic acid: a possible mechanism by which aspirin may reduce carcinogenesis

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Abstract

Background—Recent epidemiological evidence has shown that chronic use of aspirin decreases susceptibility to bowel cancer. Animal studies have shown that sulphotransferase inhibitors coadministered with sulphation activated carcinogens dramatically reduce the incidence of cancer.

Aims—To investigate the effect of the main aspirin breakdown product, salicylic acid, on the P and M isoforms of phenolsulphotransferase from human platelets and colonic mucosa.

Methods—Platelets were obtained from healthy blood donors and isolated within 24 hours after donation. Samples of colonic mucosa were obtained at resection for non-malignant disease. Phenolsulphotransferase activity was measured in cellular homogenates using a standard radiolabelling assay.

Results—Salicylic acid consistently and selectively inhibited the P form of phenolsulphotransferase at subtherapeutic concentrations in both tissue samples. A 50% inhibition of sulphation by the P phenolsulphotransferase occurred at salicylic acid concentrations of about 40 and 130 μ M in platelets and bowel mucosa respectively. M phenolsulphotransferase was virtually unaffected by salicylic acid up to a concentration of 1.5 mM (the therapeutic plasma concentration for salicylates when treating rheumatoid arthritis is about 1–2 mM).

Conclusion—The action of salicylic acid on P phenolsulphotransferase, by preventing the excessive activation of carcinogens, is a possible additional pathway by which aspirin can reduce cancer risk.

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Salicylic acid acts as a plant growth hormone and small quantities are present in most fruit and vegetables. Plant materials containing higher concentrations of salicylate have been used in herbal medicines since ancient times and aspirin (acetylsalicylic acid) is still widely used today.^{1,2} Following ingestion, aspirin is rapidly absorbed by passive diffusion from both the stomach and small intestine. The peak plasma concentration occurs about 25 minutes after taking the drug in soluble form.^{3,4} Once

absorbed aspirin has a half life of about 20 minutes regardless of the initial dose.⁴ Non-specific esterases found in the plasma, liver, and other tissues rapidly deacetylate aspirin to release salicylic acid. The latter may undergo hydroxylation, but is more commonly conjugated with either glucuronic acid or glycine.² These conjugation pathways are saturable and the half life of salicylic acid can vary from about 2.4 to 19 hours depending on the dose.¹ Although loss of the acetyl group lowers the negative logarithm of the acidic dissociation constant (pK_a) of the carboxyl group from 3.5 in aspirin to 3.0 in salicylic acid, neither compound is completely ionised at physiological pH.² Since aspirin is absorbed by passive diffusion from the alkaline small intestine^{5,6} it is likely that salicylic acid can cross cell membranes in a similar fashion. This would appear to be confirmed by experiments with cell cultures⁷ and would enable an equilibrium to be maintained between the plasma and cytosolic compartments.

There is a considerable body of evidence to suggest that regular use of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) may slow the development of malignancy in colorectal cancer.^{8–10} Current theories have concentrated on the effects of these drugs on prostaglandin synthesis and the inflammatory response,¹¹ but work by Rao and Duffel in 1991 showed that salicylic acid, the initial breakdown product of aspirin, is a potent inhibitor of aryl sulphotransferase IV (AST IV) in the rat.¹² Other studies by Boberg *et al*¹³ and Tsutsumi *et al*¹⁴ have shown that sulphotransferase inhibitors dramatically reduce the potency of sulphation activated carcinogens in both mice and hamsters. AST IV is the rat equivalent of the human phenolsulphotransferase (PST) enzymes which catalyse the sulphate conjugation of xenobiotic compounds, neurotransmitters, and drugs. PST is cytosolic and exists in two forms, P-PST which selectively sulphates micromolar concentrations of phenols and M-PST which is similarly selective for aromatic amines. This distinction is not absolute and the enzymes will metabolise substrates from either group at high (millimolar) concentrations. Sulphate conjugation occurs via the transfer of a sulphate group from 3'-phosphoadenosine-5'-phosphosulphate (PAPS) onto the target molecule.¹⁵ Phenolsulphotransferases are found throughout the body, but the bowel, liver, and platelets are known to contain particularly high activities. The total capacity of the body to sulphate compounds is not large as only small

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quantities of PAPS are available.¹⁶ However, in tissues containing high levels of PST, it may be possible for metabolites to achieve dangerous concentrations.

The aim of this study was to examine the effect of salicylic acid *in vitro* on human M-PST and P-PST. Platelets have been widely used as a representative source of both of these enzymes and could be obtained in the relatively large quantities necessary for the initial experiments. Once an effect had been established it was confirmed in a smaller number of samples of non-cancerous colonic mucosa.

Methods

TISSUE PREPARATION

Platelets were obtained from the remnant buffy coats of 12 individual anticoagulated whole blood donations produced by centrifugation and the separation of plasma and red cells. It was assumed that, as blood donors, these people would represent a fairly random sample of healthy members of the general population. Six age matched buffy coats were selected from each sex (mean age (SD): males, 47 years 4 months (11 years 11 months); females, 46 years 6 months (12 years)), centrifuged at 200 *g* for 15 minutes, and the platelet rich plasma collected. The platelets were then sedimented by centrifugation at 8000 *g* for five minutes at 4°C, washed three times in TES buffered saline (10 mM N-tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid (TES), 4 mM EDTA, 0.9% sodium chloride, pH 7.0) and resuspended in 4 ml storage buffer (10 mM TES, 0.25 M sucrose, 2 mM 2-mercaptoethanol, pH 7.0). The platelets were ruptured by ultrasonication (MSE 5–63 ultrasonic homogeniser, three 10 second bursts at 1.6A) and the homogenate was frozen in 1 ml aliquots at –20°C.

Normal mucosa was obtained from sigmoid colon resections taken from three patients with different disorders: a 45 year old female with

pelvic exenteration for advanced cervical cancer; a 47 year old female with constipation; and a 72 year old male with colitis. About 0.25 g tissue was homogenised in 1.5 ml ice cold storage buffer, and a 200 µl portion was removed for activity screening. The homogenates were stored at –20°C in 1.5 ml microcentrifuge tubes. Prior to use, the samples were thawed and the tubes suspended in a 250 ml beaker full of ice and water. The probe from an MSE 5–63 ultrasonic homogeniser was immersed in the water to a depth of about 25 mm and the samples subjected to five 15 second bursts of indirect ultrasonication at 1.6A.

PHENOLSULPHOTRANSFERASE ASSAY

Sulphotransferase activity was measured using a method based on that of Foldes and Meek¹⁷ as modified by Anderson and Weinshilboum.¹⁸ Tissue homogenate (20 µl) was incubated with 6.7 µM of the standard test substrates 4-nitrophenol (to measure P-PST activity) or dopamine (to measure M-PST activity), 6.7 µM ³⁵S labelled PAPS, and salicylic acid (as required) in a total volume of 150 µl of 20 mM TES buffer adjusted to pH 7.0 at 37°C. After 40 minutes incubation at 37°C, the reaction was stopped by adding 0.1 M barium acetate (200 µl). Any unreacted PAPS, free sulphate, or protein was precipitated by two additions of 0.1 M barium hydroxide (200 µl) followed by 0.1 M zinc sulphate (200 µl). Control assays showed that 99.5 (0.06)% (n=36 observations in triplicate, mean (SD)) of free PAPS was precipitated by this procedure. The loss of phenolic sulphates is very small¹⁷ and the assay has been found to be reliable and reproducible in this laboratory.¹⁹ After the precipitation steps, the radioactivity was measured in 500 µl supernatant following centrifugation at 11 500 *g* for three minutes, and all assays were performed in triplicate.

Protein concentration was determined by the method of Bradford using 1 mg/ml bovine serum albumin in storage buffer as the standard.²⁰

Results

PST activity, like that of most other drug metabolising enzymes, can vary by a factor of 10 in the general population. A portion of each tissue homogenate was therefore screened for activity and the results used to calculate a dilution factor for each sample such that less than 10% of the substrates would be metabolised in subsequent experiments. Although the numbers were limited, a particularly large variation in P-PST activity was seen among the platelet samples with no apparent correlation to M-PST activity (fig 1). However, despite the wide range in basal activities, the results from the inhibition studies (fig 2) clearly show the consistency and selectivity with which salicylic acid inhibits P-PST and not M-PST. It can be seen that 50% inhibition occurs at salicylic acid concentrations of about 40 µM and 130 µM for platelet and mucosal P-PST respectively. This difference may be due to protein binding as protein concentrations of the mucosal

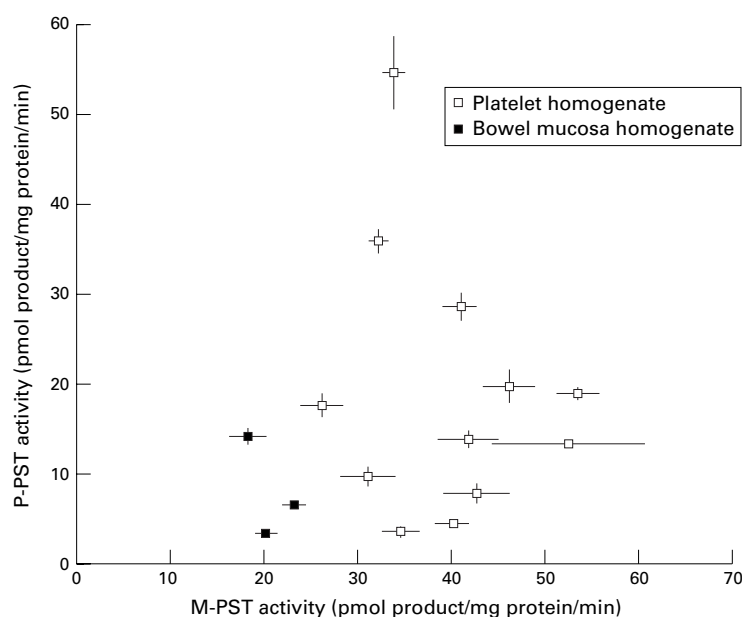


Figure 1 Basal activity of M-PST and P-PST in platelet and bowel mucosa homogenates. The points show mean (SD) of triplicate assays.

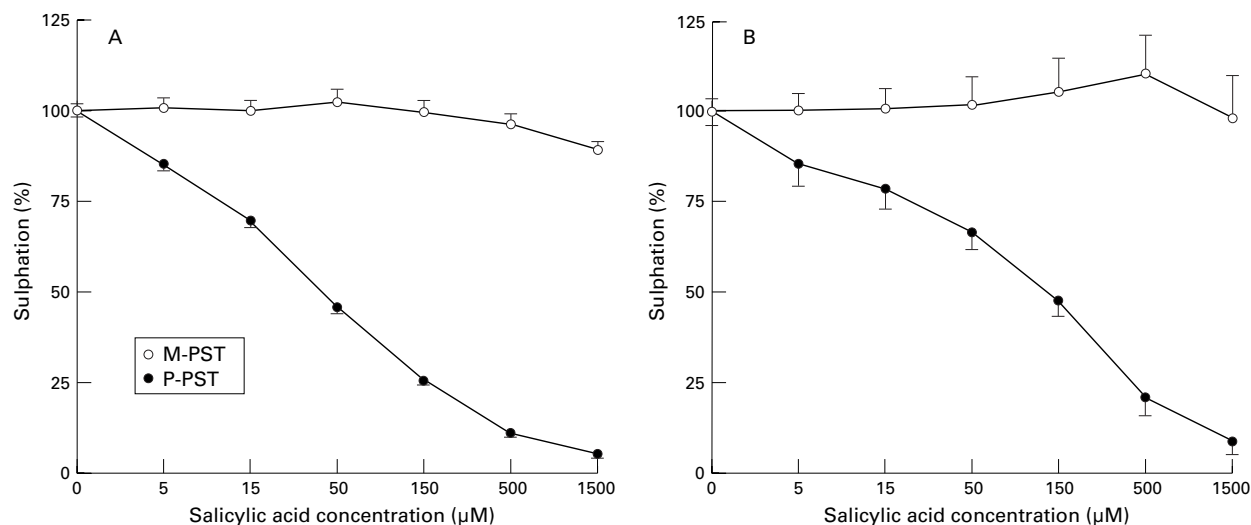


Figure 2 The effect of salicylic acid on M-PST and P-PST in (A) platelet and (B) bowel mucosa homogenates. The points show mean (SEM) values (platelet $n=12$, mucosa $n=3$); all samples were assayed in triplicate.

homogenates was about threefold greater than that of the platelet homogenate (12.3 (4.5) mg/ml versus 4.0 (1.1) mg/ml).

Discussion

With increasing longevity, the evolution of sulphation as a detoxification process has become a double edged sword. While it undoubtedly performs a vital function in scavenging low concentrations of endogenous and exogenous toxins from the body, the lability of the phenolic sulphate-ester bond means that it is liable to cause the formation of electrophilic free radicals. These react chemically with DNA which may cause mutations leading to neoplasia.¹⁶ PAPS has a limited availability in vivo which may be an evolutionary feature that minimises cytotoxic damage from sulphation.

The cooking process is known to form a variety of mutagenic compounds, including polyaromatic hydrocarbons and heterocyclic amines, particularly if the food becomes charred, for example, when grilled or barbecued. Several polyaromatic hydrocarbons have been shown to be activated by hydroxylation to phenols followed by sulphation via P-PST to the final mutagenic form.²¹ P-PST has also been found to be responsible for the activation of heterocyclic amines by N-sulphation, for example, the bladder carcinogen 2-naphthylamine,²² and a variety of carcinogenic N-hydroxy arylamines and N-hydroxy heterocyclic amines.²³ Potentially, therefore, the inhibition of P-PST would block one route of activation for both main groups of carcinogen found in food. Although the values for P-PST activity varied quite widely between individuals (fig 1), suggesting that different isoforms may have been present, inhibition was constant across the range. It is not clear why P-PST and not M-PST is affected by salicylate inhibition. Although the M and P isoforms have very similar primary sequences,²⁴ the differing substrate specificity¹⁶ suggests variations in tertiary structures. Flavonoids also inhibit P-PST activity, kinetic studies suggesting that non-

competitive inhibition may be involved,²⁵ and P-PST may therefore have a phenolic binding region at or near the active site.

Rainsford has shown that in rats the peak concentration of salicylate in intestinal mucosa is in excess of 2 mM following a single dose of aspirin of 200 mg/kg.²⁶ Given that a standard human dose for headache relief is 10–15 mg/kg it would not be unreasonable to assume a peak mucosal concentration of 100–150 μM. Our results suggest that this would inhibit P-PST by about 50%, and as salicylate has a half life of about 2.5 hours this effect would remain significant for a considerable period of time.¹ Pharmacokinetic studies in man suggest that even a single tablet will cause significant inhibition of platelet P-PST lasting for several hours.^{1,2} Epidemiological evidence suggests that aspirin may only be protective when taken in doses of 300 mg daily,²⁷ which are considerably higher than those now being increasingly used in cardiovascular prophylaxis and which are necessary to inhibit platelet cyclooxygenase. At this dosage salicylate derived from aspirin is likely to be present in sufficient concentrations to decrease sulphation significantly in the gut, so reducing the build up of metabolites and thus lessening the risk of triggering neoplastic growth.

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