Pharmacology of platelet inhibition in humans: implications of the salicylate-aspirin interaction

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ABSTRACT The current dispute over the effects of “low” vs “high” doses of aspirin should take into consideration the pharmacokinetics of this drug. In fact, different pharmaceutical formulations of aspirin may deliver little or no aspirin to the systemic blood. This was the case, for instance, in healthy volunteers taking 320 mg of aspirin or 800 mg of enteric-coated aspirin. In all instances thromboxane B2 generation in serum was fully inhibited. Platelet cyclooxygenase might therefore be effectively acetylated by exposure to aspirin in the portal circulation, whereas vascular cyclooxygenase could be spared. Thus aspirin formulations ensuring complete first-pass deacetylation should be sought rather than “low” or “high” doses of aspirin. Regardless of the type and dose of aspirin administered, salicylate is formed and accumulates in the circulation. It may antagonize the effects of aspirin on cyclooxygenase, at least in acute conditions. As an example, after administration of 1 g of salicylate to healthy volunteers, when plasma levels of the drug were about 75 µg/ml, the effect of 40 mg iv aspirin (given 40 min later) on platelet cyclooxygenase and aggregation was significantly diminished. In contrast, in patients undergoing saphenectomy, the same dose of salicylate (1 g) gave plasma drug levels of about 25 µg/ml; salicylate was unable to prevent the inhibitory effect on platelets of 40 mg iv aspirin (given 1 hr later) but did act on vascular prostacyclin. Thus the combination of salicylate with aspirin at an appropriate dose and blood level ratio may result in almost complete dissociation of the drug’s effect on platelets and vessels in man. Because the studies outlined in this review address only the short-term outcome of the salicylate-aspirin interaction, the possibility should be tested that inactivation of cyclooxygenase would occur after long-term administration of salicylate-aspirin. A pharmacokinetic approach to the “aspirin dilemma” should also consider the possibility that salicylate and/or its metabolites (e.g., gentisic acid) may interfere with lipoygenase activity, synthesis of the prothrombin complex coagulation factors, and fibrinolysis.

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Aspirin, salicylate, and inhibition of platelet aggregation and cyclooxygenase activity. Aspirin inhibits platelet aggregation and the concomitant release reaction.1-4 This exciting discovery, made more than 15 years ago, was characterized by three main facts: (1) aspirin was active in man at oral doses as low as 150 mg (about 2 mg/kg), (2) its effects were long lasting (at least 24 to 48 hr), and (3) salicylate, the main metabolite of aspirin, was virtually inactive. The last two observations suggested that the effect of aspirin on platelets was the result of irreversible acetylation. Consequently, the salicylate moiety of aspirin was considered irrelevant for the drug’s action on platelets.

In the early seventies, aspirin and other nonsteroidal anti-inflammatory drugs (NSAID) were shown to inhibit prostaglandin formation in human platelets.5-10 Again salicylate was inactive. Acetylation of platelet proteins by aspirin was subsequently reported.11 Because one of these proteins was a subunit of fatty acid cyclooxygenase, the role of this enzyme in the aspirin action was considered to be crucial. Although the nonspecific acetylation of other platelet proteins is currently of unknown biological significance, the inhibition of cyclooxygenase by aspirin does not appear to explain fully the antithrombotic effects of this drug.7-10 Aspirin is quickly deacetylated in vivo to salicylate. It has been suggested that in sites readily accessible to aspirin, such as circulating platelet cyclooxygenase, it might be expected to act in its own right as the acetylsalicylate ion and hence to be more potent than salicylate.11 It would also be expected that the salicylate ion, into which aspirin is converted, would contribute to the overall activity of aspirin, for instance in actions that

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need persistent presence of an anti-inflammatory drug or that are located at poorly accessible sites; this might apply for example in rheumatic joints. Additionally, salicylate may antagonize some effects of aspirin, such as its gastrointestinal ulcerogenicity.

Our aim in this article is to review the available evidence on (1) the occurrence and the possible clinical relevance of the interaction between salicylate (or other NSAID) and aspirin on platelet and vascular cyclooxygenase and (2) the existence of additional mechanism(s) related to salicylate and possibly involved in the antithrombotic effects of aspirin.

Salicylate may antagonize the effects of aspirin on cyclooxygenase

Salicylate-aspirin or other NSAID-aspirin interactions on animal platelet cyclooxygenase. Studies in vitro have demonstrated that salicylate, which is not inhibitory by itself, protects cyclooxygenase from aspirin inhibition in rat and rabbit platelets (figure 1). A similar pharmacologic interaction was apparent in vivo. In the rat, administration of salicylate resulted in dose-related prevention of aspirin's effect on platelet cyclooxygenase activity. This interaction appeared to be competitive and closely dose related for both drugs. Other NSAID structurally related or unrelated to salicylate, such as diflunisal and sulphipyrazone, respectively, are able in the rat in vivo to counter the effect of aspirin on platelets. This effect was apparent for diflunisal or sulphipyrazone at doses that did not inhibit platelet cyclooxygenase. The prevention by indomethacin of the effect of aspirin on platelets was apparent 24 hr after administration of aspirin. Actually the effect of indomethacin on platelet cyclooxygenase is short lasting, whereas that of aspirin lasts at least 24 hr. When the two drugs were given concurrently, the lasting effect of aspirin was no longer detectable and inhibition of cyclooxygenase lasted as if indomethacin alone had been administered. On the other hand, salicylate and diflunisal competitively blunted the inhibitory effect of indomethacin on platelet cyclooxygenase.

All these results could be explained by postulating a common site of interaction for the different NSAID on cyclooxygenase. The observation that salicylate, a virtually inactive compound or ineffective doses of diflunisal and sulphipyrazone counteract the effect of aspirin suggests that competition does not occur at the catalytic site but rather at a supplementary binding site. Interaction with this putative supplementary site is necessary but not sufficient for the efficacy of these drugs as cyclooxygenase inhibitors. Thus salicylate interacts with the binding site of cyclooxygenase but does not modify its enzymatic activity. However, neither aspirin nor indomethacin can exert their inhibitory activity on the enzyme when the binding site is occupied by salicylate.

Further experimental evidence of a supplementary binding site on cyclooxygenase was provided by the failure of salicylate to prevent enzyme inhibition by 5,8,11,14-eicosatetraynoic acid (ETYA), a competitive antagonist of arachidonic acid. Moreover, salicylate did not affect either ETYA or nor-dihydroguaiaretic acid inhibition of platelet lipoxygenase.

On the basis of these and other data presented in detail elsewhere, a functional model of pharmacologic inhibition of cyclooxygenase by aspirin and other NSAID can be proposed (figure 2). This model would explain the selectivity of NSAID for the cyclooxygenase.

**FIGURE 1.** Prevention by salicylate of aspirin inhibition of arachidonic acid–induced platelet aggregation. Rat platelet aggregation was induced by threshold concentrations of arachidonic acid (AA 0.5 mM). Aspirin (100 μM) preincubation (1 min) with PRP completely prevented aggregation, whereas sodium salicylate (1 mM) 1 min before was ineffective. The combination of both compounds resulted in an almost normal aggregation. ASA = aspirin; PRP = platelet-rich plasma.
Cyclooxygenase

**FIGURE 2.** Functional model of cyclooxygenase and lipoxygenase inhibition. Salicylate, aspirin, and the other NSAIDs bind to a binding site related to but distinct from the catalytic site of cyclooxygenase. This binding is necessary but not sufficient for enzyme inhibition. 5,8,11,14-Eicosatetraynoic acid (ETYA) competes with arachidonic acid for the catalytic sites of both cyclooxygenase and lipoxygenase. Thus salicylate does not interfere with the effect of ETYA.

ase pathway of arachidonic acid metabolism in relation to lipoxygenase.\(^\text{16, 17}\) The intensity of interaction with the supplementary site and the ensuing modifications of the catalytic site would determine these compounds’ potency as cyclooxygenase inhibitors. This model might also account for the finding that salicylate, aspirin, and indomethacin equipotently inhibit the lipoxygenase pathway.\(^\text{18}\) Recently, additional evidence for two distinct enzymatic sites on rabbit platelet cyclooxygenase has been presented.\(^\text{19}\)

**Salicylate-aspirin interaction on animal vascular cyclooxygenase.** The interaction between salicylate and aspirin is not restricted to platelet cyclooxygenase.\(^\text{12}\) In fact, salicylate administered to rats may prevent the inhibitory activity of aspirin on vascular cyclooxygenase.\(^\text{20}\) This effect was even more evident than that on platelets. Indeed the doses of salicylate needed to completely counteract the effect of the same dose of aspirin on vascular cyclooxygenase were lower than those on platelets. As a direct consequence of this observation, the administration of salicylate and aspirin at appropriate dose ratios could achieve complete inhibition of platelet cyclooxygenase without affecting the vascular enzyme.

Whether the different efficacy of salicylate in preventing aspirin effect derives from a different sensitivity to or access of the drug at various cellular levels remains to be established.

**Salicylate-aspirin interaction at other cellular levels in animals.** In the guinea pig, salicylate, inactive by itself, prevents the protective effect of aspirin in arachidonic acid–induced bronchoconstriction in vivo.\(^\text{21}\) Because this arachidonate effect was not mediated by platelet aggregation but most likely by endoperoxide and thromboxane \(A_2\) (TxA\(_2\)) production by the lung, the salicylate-aspirin interaction could occur in the lungs.

It has been reported that salicylate diminishes the gastric lesions induced by aspirin and other NSAID in rats.\(^\text{11}\) This effect seems to be secondary to a depression of prostaglandin synthesis in the stomach, and the counteracting effect of salicylate was correlated to its ability to protect gastric cyclooxygenase from NSAID inhibition.\(^\text{11, 22}\)

**Salicylate-aspirin interaction on human platelet cyclooxygenase.** All the above data have been obtained in vitro or in vivo in animal studies. Interference by salicylate with aspirin inhibition of human platelet cyclooxygenase activity has also been observed.\(^\text{23-25}\) The question remains as to whether a similar interaction occurs between salicylate and other NSAID on cyclooxygenase in vivo in man and as to its clinical relevance.

In a recent study in human volunteers we observed that orally administered indomethacin (50 mg) prevented the long-lasting effect of a subsequent dose of aspirin (500 mg) on platelet cyclooxygenase activity and on platelet aggregation.\(^\text{25}\) Similarly, ingestion of ibuprofen prevented the aspirin effect.\(^\text{26}\) More recently we investigated whether salicylate and aspirin interact on platelets in man at doses and plasma levels of clinical relevance.\(^\text{27}\) Six volunteers, given oral doses of sodium salicylate (250 or 1000 mg) presented peak plasma salicylate levels averaging 20 and 75 \(\mu\)g/ml, respectively. Neither platelet aggregation nor serum immunoreactive thromboxane \(B_2\) (TxB\(_2\)) formation was modified by either salicylate treatment. Forty minutes later, all volunteers received 40 mg iv aspirin, the lowest single dose that suppressed arachidonate-induced platelet aggregation and TxB\(_2\) generation. Aspirin plasma levels were not affected by previous salicylate ingestion. Inhibition by aspirin of both platelet
aggregation and TxB₂ generation was significantly prevented by the larger salicylate dose, giving plasma salicylate levels similar to those after an oral dose of 800 mg of enteric-coated aspirin. Pretreatment with 250 mg of salicylate, giving plasma salicylate levels similar to those obtained with an oral dose of 320 mg of compressed aspirin, resulted in proportionally diminished prevention of aspirin’s effect on platelet aggregation but did not significantly modify its effect on serum TxB₂ generation.

Salicylate-aspirin interaction in man as a possible pharmacologic solution to the “aspirin dilemma.” When it was shown that aspirin, while inhibiting TxA₂ in platelets, equally inhibited antiaggregatory prostacyclin (PGI₁) in vascular cells, the “aspirin dilemma” emerged. Simultaneous inhibition of PGI₁ and TxB₂ synthesis was considered a possible reason for the disappointing results of clinical trials on the drug’s antithrombotic effect.

In an effort to solve the “aspirin dilemma,” studies were devised to determine the aspirin dose that would inhibit TxA₂ production but would not interfere with PGI₁ synthesis in vascular cells. Platelet cyclooxygenase was believed to be more sensitive than vascular cyclooxygenase to aspirin inhibition. If it had been, the “aspirin dilemma” could have been solved easily by using low doses of the drug. Unfortunately, studies with endothelial cells in culture, laboratory animals, and man have not completely dissociated the inhibitory effect of aspirin on platelets from that on vascular cells (for review see refs. 8 and 30).

We have already mentioned that in the rat, salicylate was more effective in countering the inhibitory activity of aspirin on vascular cyclooxygenase than that on platelet cyclooxygenase. We have recently completed a study in patients undergoing saphenectomy, aimed at verifying a similar differential salicylate-aspirin interaction in man. Twenty-one female patients, admitted to the Milan University Institute of Vascular Surgery for removal of varicose veins, entered this study. Seven took no medications, and six were given 40 mg iv aspirin 1 hr before operation. The remaining eight patients took 1000 mg sodium salicylate orally 1 hr before aspirin. During surgery, segments of epigastric vein in close proximity to the saphenous vein were removed for measurements of prostacyclin production. At the same time, venous blood was collected for measurements of serum TxB₂ synthesis.

As reported in table 1, TxB₂ generation was completely inhibited in all patients given aspirin alone, whereas prostacyclin generation was reduced by more than 50%. In the salicylate-aspirin group, TxB₂ generation remained undetectable in three patients and reached about 10% of control values in the other five. In contrast, six patients generated prostacyclin levels within the control range and only two appeared to be partially inhibited. Mean salicylate blood levels at the moment of aspirin administration were 25.9 ± 5 μg/ml.

This observation confirms in man the results obtained in the rat. In this animal as well as in the present study in man, vascular cells showed lower sensitivity to aspirin than platelets. Salicylate resulted in both instances in an amplification of the difference in response to aspirin of platelets and vascular cells. The mechanism of this property of salicylate (e.g., different access of the drug at various cellular levels) remains to be defined. This observation, however, may constitute an original approach on which to base the clinical use of aspirin in a supposedly beneficial antithrombotic direction.

When analyzed with respect to the study in healthy male volunteers, this study in female patients undergoing saphenectomy merits some comments. As summarized in table 2, the same salicylate-aspirin dose ratio (1000 mg oral vs 40 mg iv) resulted in significant prevention of the aspirin effect on platelet TxB₂ generation in the group of volunteers but not in patients. The most likely explanation is that at the moment of aspirin administration, the plasma levels of salicylate, measured 1 hr after ingestion (about 25 μg/ml), were markedly lower than those measured 40 min after ingestion in volunteers (about 75 μg/ml). In fact, salicylate levels in patients were similar to those found in volunteers 40 min after 250 mg of salicylate. In the latter condition, as well as in patients, no significant prevention of aspirin’s effect on TxB₂ synthesis could be detected. Possibly, gastrointestinal absorption of salicylate was reduced in patients by anesthesia.
TABLE 2
Salicylate-aspirin interaction at platelet and vascular level and plasma salicylate levels in healthy volunteers and in patients undergoing saphenectomy

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Salicylate Interval</th>
<th>Salicylate levels</th>
<th>SAL/ASA interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volunteers</td>
<td>(mg, oral)</td>
<td>(min)</td>
<td>(µg/ml)</td>
</tr>
<tr>
<td>250</td>
<td>40</td>
<td>22.3 ± 1.7</td>
<td>No</td>
</tr>
<tr>
<td>1000</td>
<td>40</td>
<td>76.3 ± 5.1</td>
<td>Yes</td>
</tr>
<tr>
<td>Saphenectomy patients</td>
<td>1000</td>
<td>60</td>
<td>25.9 ± 5.0</td>
</tr>
</tbody>
</table>

SAL = salicylate; ASA = aspirin.
Plasma salicylate levels were measured immediately before aspirin (40 mg iv). For other details see table 1.

Independently of the reasons why the same dose of salicylate gave rise to different plasma levels of the drug in the two groups of subjects, it appears that the plasma levels of salicylate rather than the amount of the drug administered are relevant for an optimal interaction with aspirin.

In summary, we have discussed the hypothesis that platelet and/or vascular cyclooxygenase might be spared acetylation by aspirin if the plasma levels of salicylate are carefully balanced with the plasma levels of aspirin. This hypothesis has been tested experimentally both in vitro and ex vivo in short-term animal and human studies. Protection of cyclooxygenase with salicylate acutely prevented irreversible inactivation by aspirin; experimental evidence that salicylate protects cyclooxygenase from inactivation by aspirin after long-term administration of both compounds is still lacking. Cyclooxygenase protection by salicylate might slow the rate of its inactivation by aspirin but not prevent it completely; thus the cyclooxygenase population might be finally inactivated during long-term salicylate-aspirin therapy, despite a careful balance between salicylate and aspirin levels. On the basis of these considerations we addressed our attention to another possible approach to solve the “aspirin dilemma.”

Aspirin formulations that deliver only salicylate to the systemic circulation: a pharmacokinetic approach to the “aspirin dilemma.” The importance of salicylate plasma levels mentioned in the preceding section suggests that a better knowledge of the pharmacokinetics of aspirin might help solve the “aspirin dilemma.” Little attention has been paid to aspirin pharmacokinetics with regard to the different pharmaceutical formulations used in thrombosis prevention trials. This approach seemed of particular interest in view of observations in healthy volunteers that the peak plasma levels of aspirin after either 320 mg of compressed aspirin or 800 mg of enteric-coated aspirin were not statistically different (2.9 ± 0.06 and 3.3 ± 0.3 µg/ml, respectively). Serum TxB2 generation was almost completely inhibited 1 hr after either aspirin formulation.

Of even greater interest was the observation that similar full TxB2 inhibition was obtained in five volunteers in whose peripheral circulation aspirin levels were not measurable (figure 3). Three subjects had received a compressed aspirin and two an enteric-coated aspirin. No significant differences in salicylate area under the plasma concentration vs time curve could be found between subjects with detectable or undetectable aspirin plasma levels after either formulation (table 3). On account of the potential for erratic absorption pattern of aspirin, detectable plasma levels of the unchanged drug may depend on fortuitous blood collection times. In our study, however, this was unlikely because as many as eight blood samples were collected within the first hour after compressed aspirin and six

![320 mg COMPRESSED](image1)

![800 mg ENTERIC-COATED](image2)

FIGURE 3. Serum TxB2 measured at different times after ingestion of 320 mg of compressed (upper panel) or 800 mg of enteric-coated (lower panel) aspirin in subjects with detectable or undetectable aspirin levels. Figures are percent values of predrug serum TxB2 in each subject and SEM. Three subjects had detectable and three undetectable aspirin plasma levels after 320 mg aspirin, while four had detectable and two undetectable aspirin plasma levels after 800 mg enteric-coated aspirin. In this last group the mean and the two single values are reported, respectively. ASA = aspirin; ND = not detectable.
Vessels exposed to aspirin before the first-pass occurs. Thus aspirin formulations ensuring extensive first-pass deacetylation rather than merely “low” or “high” doses of unspecified aspirin formulations should be sought as a pharmacologic goal for solving the “aspirin dilemma.” The current dispute on the difference in sensitivity to “low” doses of aspirin between platelet and vascular cyclooxygenase may well arise from the wide variability of aspirin pharmacokinetics. Indeed, as in our experiments, the same dose or formulation may or may not deliver measurable amounts of aspirin to the systemic circulation; the inhibitory effect on platelet cyclooxygenase would be achieved in any case, but vascular PGI₂ synthesis would only be spared in the absence of detectable peripheral aspirin levels. Similar conclusions have recently been reached by other investigators.²⁴,³⁵

**Salicylate may contribute to the overall activity of aspirin in the prevention of vascular occlusion.** Although inhibition of prostanoid synthesis has become an attractive, unifying explanation of the anti-inflammatory activity of aspirin-like drugs, this mechanism does not explain all aspects of the anti-inflammatory action of salicylates, especially in conditions in which aspirin and salicylate are of approximately equal potency.⁷ It has therefore been suggested that the anti-inflammatory activity of aspirin-like drugs probably does not arise from a single mechanism but may be mediated through an additional or alternative mechanism, which becomes operative only at doses in excess of those required to suppress cyclooxygenase. The possibility has also been discussed that aspirin has additional components to its mechanism of action not possessed by other NSAIDs, such as flufenamic acid or indomethacin. The major lines of research into alternative mechanisms that might explain the therapeutic action of salicylate have been reviewed extensively.⁷

Because several pathways may be involved in the initiation and growth of thrombi, one would expect aspirin to be inhibitory only in conditions in which prostanoid biosynthesis (cyclic endoperoxides and TXA₂) plays a major role in thrombus formation.

**Effects of aspirin and salicylate on blood coagulation and fibrinolysis.** A recent study⁸ in rabbits with in-dwelling aortic catheters strongly suggests that aspirin at doses that inhibit only platelet cyclooxygenase activity is not a satisfactory inhibitor of arterial thrombosis when there is a significant fibrin component. At very high doses aspirin inhibited thrombosis, but this effect was not mediated through inhibition of platelet function. In fact salicylate was more effective than aspirin. The tendency for high doses of aspirin or salicylate to in-

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**TABLE 3**

Pharmacokinetics of aspirin after ingestion of two different drug formulations in six healthy volunteers

<table>
<thead>
<tr>
<th>Aspirin preparation (oral dose)</th>
<th>n</th>
<th>Aspirin</th>
<th>Salicylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compressed (320 mg)</td>
<td>3</td>
<td>17.3 ± 1.4</td>
<td>544 ± 42</td>
</tr>
<tr>
<td>Enteric-coated (800 mg)</td>
<td>4</td>
<td>31.9 ± 3.3</td>
<td>2680 ± 153</td>
</tr>
<tr>
<td>Enteric-coated (800 mg)</td>
<td>2</td>
<td>undetectable</td>
<td>2190-2851</td>
</tr>
</tbody>
</table>

AUC = area under the plasma concentrations between 0 and ∞. Detection limit of aspirin = 0.5 nmol/ml.

during the first 3 hr after enteric-coated aspirin. The time of peak aspirin concentration in subjects in whom it was detectable was 33 min for compressed and 83 min for enteric-coated aspirin.²⁷

Experiments in vitro showed that incubation of human whole blood from three different donors with 0.5 μM aspirin (corresponding to the detection limit of the high-performance liquid chromatographic assay method used) for 1 hr at 37°C did not result in any significant reduction of serum TxB₂ generation. As shown in figure 4, concentrations of aspirin at least 50 times this detection limit were required to prevent TxB₂ generation by more than 90%.

It has long been known that aspirin undergoes extensive first-pass deacetylation within the enterohepatic circulation.³³ Whether this first-pass effect is dose-dependent is not yet known. Thus inhibition of serum TxB₂ generation in the absence of measurable aspirin in systemic peripheral blood might result from acetylation of platelet cyclooxygenase by exposure of circulating cells to the drug in the portal circulation. Cyclooxygenase in the peripheral vasculature could thus come into contact only with inactive salicylate, with the possible exception of the mesenteric and portal circulation.

**FIGURE 4.** Effect of different aspirin concentrations on TxB₂ generation in human serum in vitro. Blood was collected without anticoagulant from the antecubital vein of healthy subjects and incubated in glass tube at 37°C for 1 hr in the presence of aspirin. TxB₂ generation was measured in serum. The values (mean from four experiments) are percent inhibition of control values.
hibit thrombus formation appears related to a prolongation of the one-stage prothrombin time and to activation of whole blood fibrinolytic activity; which of these pathways is most important in reducing thrombus formation remains to be established.

This experimental finding is in keeping with some observations in man. There are reports that doses of 2 to 3 g of aspirin per day in man prolong prothrombin time36,37 and increase whole blood fibrinolytic activity.38-40 Moroz39 reported that aspirin and sodium salicylate (1.8 g) were equipotent in stimulating fibrinolytic activity through the protease action of leukocytes.

The beneficial effect of high doses of aspirin in preventing venous thrombosis and pulmonary embolism after total knee replacement41 may be due not just to the effect of aspirin on platelets but also to its effect (and/or of the salicylate) on blood coagulation and fibrinolysis. However, in a recent study on healthy human volunteers, aspirin (650 mg given orally both 18 and 2 hr before blood collection) inhibited the rise in vascular plasminogen activity after venous occlusion.42 Similarly a decrease in fibrinolysis induced by both cellular and plasmatic factors after ingestion of 1 g of aspirin per day for 4 days had been reported.43

Recently Roncaglioni et al.44 have shown in rats that salicylate, like warfarin, with which it may have some common chemical features (figure 5), reduced the plasma concentration of the prothrombin complex clotting factors in vivo. It also caused an accumulation of microsomal substrates for vitamin K-dependent carboxylase in the liver and lung. These effects of salicylate were dose-dependent (between 4 × 50 and 4 × 200 mg/kg) and were counteracted by simultaneous administration of vitamin K (20 mg/kg). The mechanism of salicylate-induced hypoprothrombinemia in the rat and in the rabbit has been extensively investigated by several groups.45-48 Because the effect of salicylate was additive to that of warfarin,44 relatively low doses of aspirin or salicylate should interfere with oral anticoagulant therapy. Aspirin-induced fluctuation in clotting factor levels of as little as 5% to 10% of normal might have serious consequences in patients undergoing anticoagulant treatment, although it might not even be measurable in healthy volunteers.

These considerations should be borne in mind when analyzing the results of clinical trials combining aspirin and oral anticoagulants. On the one hand it is not surprising that this drug combination has been reported to be more effective than oral anticoagulants alone in preventing thrombotic complications in patients with aortic ball valves.49 On the other hand it could explain the high frequency of bleeding complications encountered in other trials.50 Two recent reports in rabbits51 and in rats52 have suggested that high doses of aspirin may prolong bleeding time independently of an effect on platelet cyclooxygenase and also of formation of salicylate.

**Effects of aspirin and salicylate on leukocytes.** The observation that aspirin and salicylate equipotently inhibit the generation of chemotactic 12-HETE from 12-HPETE in the lipoxygenase pathway of the arachidonic acid metabolism,58 although controversial, has been considered as diminishing the importance of the cyclooxygenase pathway in the anti-inflammatory activity of salicylate.7

A key event in any inflammatory reaction is the migration of leukocytes from the blood into the inflammatory lesion. Leukocytes metabolize arachidonic acid by lipoxygenase53 and the products of this enzyme are potent chemotactic agents54 that induce leukocyte aggregation.55,56 It has recently been proposed57 that migrating leukocytes contribute to the tissue injury accompanying myocardial ischemia, possibly by release of proinflammatory mediators and free radicals.
Aspirin and salicylate inhibit the production of superoxide anion by activated leukocytes but do not reduce leukocyte invasion into an inflammatory exudate. Thus, although an interaction with superoxide anion could be considered as a possible additional mechanism of the anti-inflammatory action of salicylates, the biological consequences of this pharmacologic effect are not obvious. A recent observation in our laboratory (A. Lorico and S. Villa, unpublished) may also be of interest in this context: gentisic acid, a hydroxylated metabolite of salicylate, not only prevented superoxide anion production by human leukocytes stimulated with either arachidonic acid or phorbol myristate acetate, but also prevented leukocyte aggregation. The concentrations of gentisic acid active in vitro (0.4 to 1.2 mM) are probably not reached in the human circulation after current therapeutic doses of aspirin. However, this finding merits further investigation.

Conclusions. Aspirin inhibits cyclooxygenase. This effect distinguishes it from salicylate, its major metabolite, with regard to inhibition of the platelet function induced by certain stimuli. Although inactive on cyclooxygenase, salicylate may interfere with the effect of aspirin on this enzyme both in platelets and (even more effectively) in the vasculature. Knowledge of the pharmacokinetics of aspirin and salicylate is essential for optimal use of aspirin as a selective inhibitor of platelet but not of vascular cyclooxygenase activity. The mechanism involving cyclooxygenase does not, however, appear to fully explain the antithrombotic action of aspirin. Therefore, it is best to assume that in preventing thrombosis aspirin acts by at least two mechanisms, one of which is the inhibition of platelet cyclooxygenase.

The best candidate mechanisms, most likely to be linked to the salicylate moiety of aspirin, are the inhibition of vitamin K-dependent clotting factor synthesis, cell-mediated activation of blood fibrinolysis, and interference with the platelet and/or leukocyte lipooxygenase pathway of arachidonic acid metabolism.

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