

Aspirin-like effects of selected food additives and industrial sensitizing agents

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Summary

A number of food additives and industrial chemicals, responsible for inducing symptoms of intolerance in some individuals, have been studied in tests measuring platelet activation by noradrenaline. All the investigated agents inhibited platelet aggregation and this was associated with inhibition of the cyclo-oxygenase-thromboxane pathway. Suboptimal inhibitory concentrations of the agents studied had additive inhibitory effects on platelet aggregation when they were tested in pairs, or when tested with salicylate or aspirin. The results support the theory that some food additives and industrial chemicals induce intolerance because of their aspirin-like properties.

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Introduction

Intolerance to food and drug additives has been recognized for at least 30 years [1]. In early studies, most patients reacting to tartrazine and benzoate were found to be aspirin-sensitive and intolerance to these chemicals was linked with aspirin sensitivity [2]. Since then other additives such as metabisulphite and monosodium glutamate have been recognized as agents associated with an intolerance syndrome [3,4]. The conclusions of recent clinical studies investigating the relationship between food additive intolerance and aspirin sensitivity have been far from unanimous [5].

The most important pharmacological property of aspirin is its capacity to prevent prostaglandin formation by inhibiting the cyclo-oxygenase enzyme. This property is common to other non-steroidal anti-inflammatory drugs (NSAIDs) and a close correlation exists between their cyclo-oxygenase inhibiting property and asthma-inducing potential [6]. Although NSAID intolerance is widely attributed to the capacity of these drugs to inhibit prostaglandin production, it is unclear if food and drug additives or industrial sensitizing agents are able to induce intolerance by the same mechanism.

Studies on the effects of these chemicals on platelets, in which the prostaglandin pathway is linked to cell functional changes, may help to clarify the mechanisms causing intolerance. In platelets, aspirin binds to the

substrate site of cyclo-oxygenase and irreversibly inactivates this enzyme [7]. Cyclo-oxygenase inhibition by NSAIDs prevents the formation of thromboxane species and inhibits *in-vitro* aggregation by agonists such as adrenaline and arachidonic acid, which activate platelets through the formation of thromboxane A_2 [8]. The effects of aspirin-like drugs on platelet prostaglandin production are therefore easy to observe *in vitro*. Despite this, previous studies using platelets to investigate one colouring agent (tartrazine) have produced contradictory results [9-11].

In the present study a number of food additives and industrial agents were investigated for their effects on platelet aggregation and thromboxane B_2 formation. The objective of this investigation was to determine if these substances had an aspirin-like effect on platelets and therefore a common mechanism for inducing intolerance.

Materials and methods

Chemicals

Sodium nitrite, sodium metabisulphite, sodium salicylate, sodium benzoate, ascorbic acid and phthalic anhydride were purchased from BDH (Poole, U.K.). Acetyl-salicylic acid, dimethyl sulphoxide (DMSO), maleic anhydride, monosodium glutamate, trisodium citrate and noradrenaline were from Sigma (Poole, U.K.). Tartrazine was obtained from the Aldrich Chemical Co (Gillingham, U.K.). All agents, with the exception of phthalic anhydride which was dissolved in DMSO, were used as aqueous solutions.

Platelet aggregation and thromboxane B_2 studies

Blood (9 ml) was obtained from 25 healthy volunteers (seven male and 18 female, age range 18–56 yr) who had not taken any medication during the previous 7 days. Platelet rich plasma (PRP) was immediately prepared by centrifuging ($250 \times g$ for 10 min) the blood, anti-coagulated with 1 ml 3.8% trisodium citrate. Platelet aggregation studies were carried out in cuvettes containing 250 μ l PRP using a Coulter aggregometer and Omniscribe chart recorder (Coulter Electronics, Luton, U.K.). Each subject gave a normal biphasic aggregation response to adrenaline ($4\text{--}20 \times 10^{-5}$ M). Phthalic anhydride (added in 1 μ l DMSO) and the other investigated agents (1–5 μ l) were tested over a concentration range of $1\text{--}40 \times 10^{-3}$ M and were mixed with PRP for 1 min before the addition of noradrenaline. The minimum concentrations necessary for the maximum inhibition of platelet aggregation responses by these agents were established from dose-response studies. Responses in the presence of these agents were compared with the inhibitory properties of aspirin ($5\text{--}10 \times 10^{-5}$ M) on platelet aggregation. The agents were also tested at suboptimal inhibitory concentrations, in pairs and separately with aspirin or salicylate, to study additive effects.

After measuring aggregation responses for 4–6 min, the cuvettes were centrifuged ($1500 \times g$ for 5 min) and samples of platelet poor plasma (PPP) were removed and stored at -20°C . Thromboxane B_2 measurements were carried out in duplicate on 20- μ l samples of PPP using a radioimmunoassay kit (Amersham plc, Aylesbury, U.K.).

Results

Platelets aggregated by noradrenaline or adrenaline, in citrated plasma, produced a characteristic biphasic curve when the aggregation was recorded. The second, steeper phase of this curve which results from activation of the platelet cyclo-oxygenase-thromboxane pathway is inhibited by aspirin and other NSAIDs [8]. These platelet responses are shown in Fig. 1. The second phase of aggregation by noradrenaline was inhibited by all the tested agents when they were added to PRP in higher concentrations than aspirin. Metabisulphite and phthalic anhydride were among the most potent substances tested, producing inhibition at 2 mM. Much higher concentrations of sodium glutamate (30 mM) were required to produce the same effect, while the other agents produced maximum inhibition at intermediate concentrations (4–15 mM). The higher potency of phthalic anhydride compared to maleic anhydride may have been due to an additive effect with the DMSO used as solvent. The same

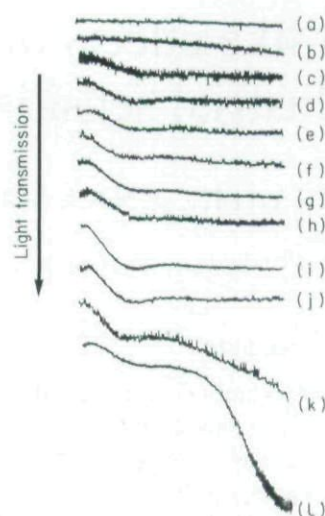


Fig. 1. Platelet aggregation responses induced by noradrenaline, in the presence of (a) maleic anhydride (8 mM), (b) ascorbic acid (10 mM), (c) tartrazine (10 mM), (d) glutamate (30 mM), (e) citrate (10 mM), (f) metabisulphite (2 mM), (g) benzoate (15 mM), (h) nitrite (4 mM), (i) salicylate (5 mM), (j) aspirin (0.1 mM), (k) phthalic anhydride (2 mM), and (l) noradrenaline (0.08 mM) alone. This figure is typical of aggregation responses obtained in experiments on at least 4 other subjects.

Table 1. Thromboxane B_2 formation by noradrenaline-activated platelets

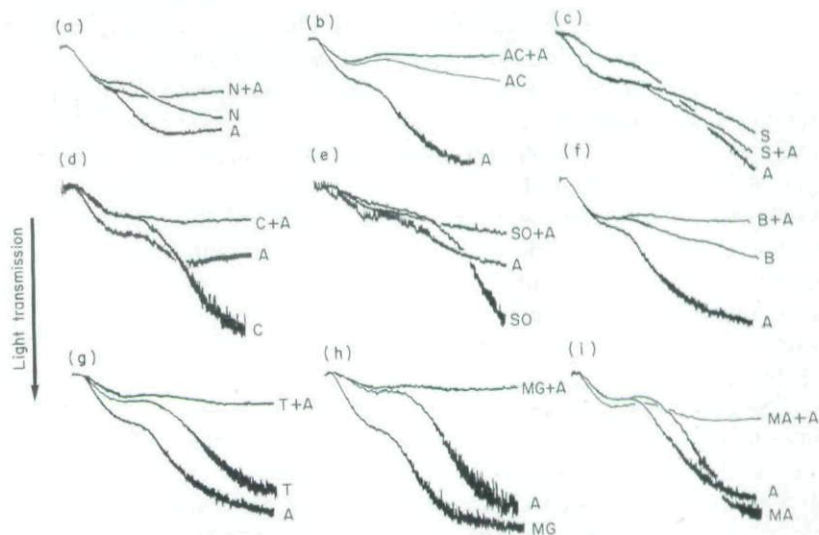
Added agent	Thromboxane B_2 (nmol/tube)*
None (i.e. noradrenaline 0.08 mM)	4.12 ± 0.41
Phthalic anhydride (2 mM)	1.37 ± 0.55
Maleic anhydride (8 mM)	1.09 ± 0.45
Ascorbic acid (10 mM)	1.04 ± 0.20
Trisodium citrate (10 mM)	0.98 ± 0.22
Monosodium glutamate (30 mM)	0.94 ± 0.23
Sodium nitrite (4 mM)	0.81 ± 0.21
Tartrazine (10 mM)	0.77 ± 0.16
Sodium benzoate (15 mM)	0.75 ± 0.24
Sodium metabisulphite (2 mM)	0.72 ± 0.22
Aspirin (0.1 mM)	0.39 ± 0.09
Sodium salicylate (5 mM)	0.31 ± 0.05

* Thromboxane B_2 levels, expressed as the mean ($\pm$ s.e.m.) concentration, were measured in at least 4 experiments.

concentration of DMSO alone did not affect aggregation.

Noradrenaline produced a sixfold increase in thromboxane B_2 during platelet aggregation. Mean thromboxane B_2 levels of 4.12 nmol/tube were found in PRP aggregated with noradrenaline, in comparison to 0.58 ± 0.1 nmol/tube ($n=6$) in controls. Table 1 lists the

Fig. 2. Platelet aggregation responses induced by noradrenaline in the presence of 0.05 mM aspirin (A) and aspirin in combination with (a) 1 mM nitrite (N), (b) 5 mM ascorbic acid (AC), (c) 2 mM salicylate (S), (d) 5 mM citrate (C), (e) 1 mM metabisulphite (SO), (f) 5 mM benzoate (B), (g) 5 mM tartrazine (T), (h) 20 mM glutamate (MG), and (i) 5 mM maleic anhydride (MA). The results are typical of aggregation responses obtained in experiments on at least 4 other subjects.



mean levels of thromboxane B_2 produced by platelets in aggregation tests carried out in the presence of the agents investigated in Fig. 1. Each agent which was tested significantly inhibited thromboxane B_2 production by noradrenaline-aggregated platelets ($P < 0.05$ – 0.01 , $n = 4$). The concentrations of salicylate and aspirin used inhibited thromboxane B_2 levels by over 90%. The other agents inhibited noradrenaline-induced thromboxane B_2 production by 67–83% and this was sufficient to inhibit the second phase of platelet aggregation completely. The addition of the same concentrations of tested agents to PPP containing a standard concentration of thromboxane B_2 did not affect its measurement by radioimmunoassay.

As the agents under investigation appeared to show weak aspirin-like properties, sub-optimal inhibitory concentrations of the agents were tested in aggregation experiments together with sub-optimal inhibitory concentrations of aspirin. These concentrations were selected by studying dose-response curves. For most agents, the sub-optimal concentrations were half of the maximum concentration used in previous experiments. Results typical of these experiments are shown in Fig. 2. Additive effects were noted for all the agents tested with aspirin, with the exception of salicylate. Moreover, these additive effects were not specific to aspirin and were also noted when sub-optimal inhibitory concentrations of the agents were tested in pairs, or when they were tested with a sub-optimal inhibitory concentration of salicylate (results not shown).

Discussion

This study shows that the investigated agents inhibit the noradrenaline-induced aggregation of platelets from nor-

mal subjects and this is dependent on a functional cyclo-oxygenase-thromboxane pathway. Inhibition of this enzyme system was confirmed by measuring the production of platelet thromboxane B_2 , a stable metabolite of thromboxane A_2 . This pharmacological action is characteristic of NSAIDs and the inhibitory effects of the investigated agents were found to be additive with those of aspirin. The results suggest that these chemicals may induce intolerance in the same way as aspirin, possibly by inhibiting prostaglandin production, as proposed by Szczeklik [12].

Although there is little data available to support the concept that additives act like aspirin, the evidence against these substances provoking intolerance through an immunological mechanism is probably even stronger. Specific IgE or precipitating antibody has only been detected for a few of the large number of low molecular weight additives or industrial chemicals associated with intolerance. Tartrazine, isocyanates [13], formalin [14] and sulphur dioxide [15] all belong to a group of substances that provoke intolerance by mechanisms that are contentious. Acid anhydrides (e.g. phthalic and maleic anhydride) are strong sensitizing agents and some of the problems caused by these substances in industry may be instigated by IgE antibody production [13].

Work by Gerber *et al.* [10] is frequently cited as evidence against tartrazine acting like aspirin. In their study on a variety of tissues, including human platelets, 30 mM tartrazine failed to inhibit the cyclo-oxygenase enzyme system and did not inhibit platelet aggregation. However, Cesarani *et al.* have reported that tartrazine inhibits thromboxane A_2 production, in guinea-pig lung tissue [11]. The paper by Gerber *et al.* also contradicts work by Gallagher [9], who found that tartrazine (17.4–34.8 mM) inhibited platelet aggregation, as well as

contradicting the results presented here. The type of aggregating agent used in these platelet studies probably accounts for the conflicting results. Gerber used extremely high arachidonic acid concentrations to activate cyclo-oxygenase (0.165 mM) and to initiate platelet aggregation (0.5 mM). In both the present study and that of Gallagher, physiological concentrations of arachidonic acid were generated by the addition of noradrenaline and collagen respectively. It is not surprising that in the assay system used by Gerber tartrazine failed to compete with such a high concentration of the natural substrate for cyclo-oxygenase.

The inhibitory tartrazine concentrations used in this investigation are lower than those used in previous studies. With the exception of monosodium glutamate, 1–5 mM concentrations of the tested agents were all strongly inhibitory in combination with low concentrations of aspirin or salicylate. While there is little, if any, information relating to the plasma levels attained by food additives, the lowest additive concentrations tested in this study are known to be similar to plasma aspirin levels achieved during therapy.

One other criticism against an aspirin-like action for tartrazine and other agents producing intolerance has been their dissimilarity in molecular structure and their lack of resemblance to aspirin [5]. The perpetuation of this criticism is surprising. Over 20 years ago, Chafee and Settiple pointed out the similarity in the chemical structures of aspirin and certain azo dyes causing asthma [1]. These substances all contained acidic sulphonate (SO_3H) or carboxyl (COOH) groups; carboxyl groups are the characteristic molecular feature of the largest class of NSAIDs [16].

The molecular structures of the agents investigated in this study are depicted in Fig. 3. All possess double-bonded oxygen atoms, mainly attached to carbon and sulphur atoms in carboxyl, sulphonate, carbonyl ($\text{C}=\text{O}$) or sulphonyl ($\text{O}=\text{S}=\text{O}$) groups. The apparent differences in the chemical structures of these agents are superficial and primarily confined to the planar cyclic rings of the aromatic molecules. In terms of their functional groups these molecules are quite similar. The essential groups for cyclo-oxygenase inhibition are probably carbonyl and sulphonyl groups, as in the other major class of NSAID, enolic acids, a carbonyl group replaces the carboxyl group. The molecular structure of isocyanates (Fig. 3), which were not investigated in this study because of their insolubility, and that of formaldehyde ($\text{H}_2\text{C}=\text{O}$) suggest that they could also cause intolerance in the same way as the agents already discussed.

The lack of an additive effect for aspirin and salicylate in platelet aggregation studies were not unexpected. Salicylate inhibition of the antiplatelet effect of aspirin

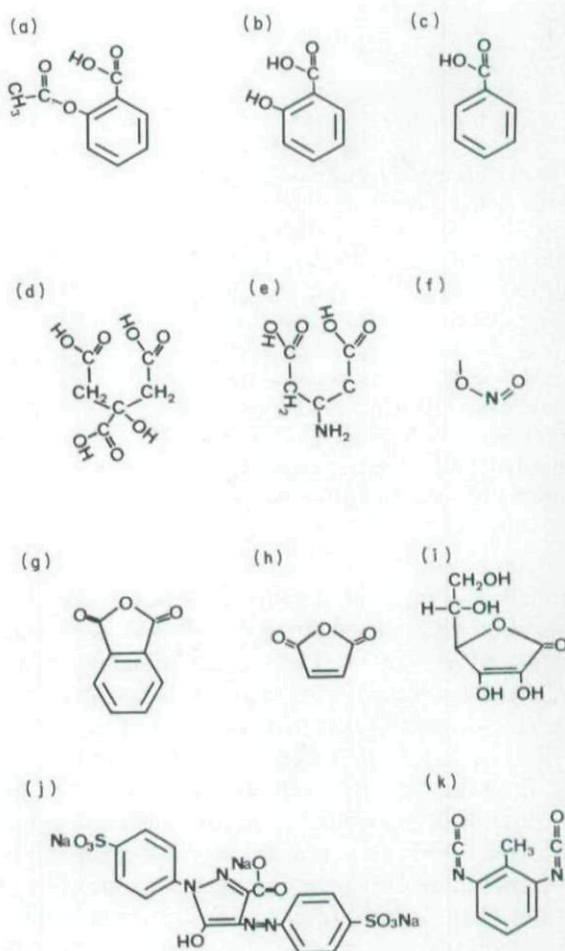


Fig. 3. Molecular structures of the agents investigated. (a) aspirin, (b) salicylic acid, (c) benzoic acid, (d) citric acid, (e) glutamic acid, (f) nitrite, (g) phthalic anhydride, (h) maleic anhydride, (i) ascorbic acid, (j) tartrazine, (k) tolulene 2,6 diisocyanate.

has been observed before and has implications for aspirin therapy [17]. These experimental and clinical effects of salicylate may both be a consequence of the two drugs binding at the same supplementary site on the cyclo-oxygenase enzyme, resulting in displacement of aspirin by a less effective drug [18].

The results obtained in this study with ascorbic acid are interesting in view of the liberal addition of this substance to food and drink. The inhibitory effect of ascorbic acid on platelet aggregation is well known [19]. Previous studies have also reported synergistic effects with aspirin in rat gastric tissue and an interaction with indomethacin in experimentally induced bronchospasm in human subjects [20,21]. These results all suggest that ascorbic acid acts by altering the production of prostaglandins. The

effect of this substance on aspirin sensitive asthmatics should be investigated.

In conclusion, the results presented here show that some food additives and industrial chemicals are able to inhibit the platelet cyclo-oxygenase-thromboxane system *in vitro*. These molecules possess weak aspirin-like properties and may induce intolerance in susceptible subjects for this reason. Food additives are seldomly encountered singularly in processed foods, highly seasoned meals, or beverages. In combination, these additives may work at even lower concentrations than those shown to be inhibitory in this study. A common mechanism of action may have implications for some methods of challenge testing in current use. Challenge tests using a series of these agents, over a short period of time, may desensitize patients and be responsible for the sometimes poor correlation between test result and patient history. The additive effects of these substances also suggest that they are likely to be more injurious to health than was previously thought and this aspect should continue to be investigated in clinical studies.

Acknowledgments

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