

Inhibition of platelet aggregation by tartrazine and a pyrazolone analogue in normal and allergic individuals

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Summary

The effect of tartrazine (T) (yellow dye No. 5) and one of its metabolites an aminopyrazolone analogue (1-sulphophenyl-3-carboxy-5-hydroxypyrazole, SCHP) upon collagen-induced platelet aggregation (C-PA) was investigated in fourteen atopic patients and fourteen normal subjects. Both T and SCHP inhibited C-PA in atopic patients at significantly lower doses than in normal volunteers. The mean inhibitory concentrations of SCHP were similar to aspirin in both atopic and normal individuals. Although the precise mechanism by which these chemicals block C-PA has not been elucidated, this *in vitro* system may be a useful method of assessing non-immune mechanisms involved in reactions to tartrazine.

Introduction

Adverse reactions to the food and drug colour additive, tartrazine, have been reported in approximately 15% of individuals with the aspirin intolerance syndrome (Samter & Beers, 1968; Juhlin, Michaelson & Zetterström, 1972). These responses are similar to those induced by aspirin and other non-steroidal anti-inflammatory agents and can be confirmed by oral provocation tests (Juhlin *et al.*, 1972; Settipan & Pubupakkam 1975; Bernstein *et al.*, 1980). Symptoms and signs usually mimic clinical manifestations associated with the classical IgE-mediated diseases. However, two independent groups of investigators could not confirm the presence of tartrazine-specific IgE by either direct skin or radioallergosorbent tests (Weltman, Szaro & Settipane, 1978; Bernstein *et al.*, 1980). Since it has been previously demonstrated that platelets of asthmatic patients were more susceptible to the effects of aspirin in collagen-induced platelet aggregation (Schwartz & Bennett, 1973), we postulated that tartrazine could have comparable effects which might help to elucidate alternative non-immunological mechanisms responsible for tartrazine reactivity. The current experiments were per-

formed in atopic patients because it had been previously determined in our laboratory that the secondary (release) phase of platelet aggregation was abnormal in patients with allergic rhinitis (Gallagher *et al.*, 1978) and extrinsic asthma (Maccia *et al.*, 1977).

Materials and methods

Patients

Fourteen atopic patients were selected on the basis of well documented pollinosis, confirmed by positive histories and skin tests. A control population of fourteen normal volunteers had negative allergic histories and direct skin tests.

Reagents

Tartrazine was donated by the Allied Chemical Company. We also studied the effect of 1-sulphophenyl-3-carboxy-5-hydroxypyrazole (SCHP), an intermediate in the synthesis of tartrazine (Freeman, Jones & Graichen, 1950) and a close analogue to one of the intestinal metabolites of tartrazine, 1-sulphophenyl-3-carboxy-4-amino-5-oxy pyrazole (SCAOP) (Fig. 1). Although SCAOP is known to be a minor intestinal metabolite of tartrazine by virtue of its urinary excretion of $\leq 10\%$ in rats after ingestion of a known amount of the parent compound (Ryan, 1972; Honohan *et al.*, 1977), we were particularly interested in studying its effects on human platelet aggregation because it has the pyrazoline ring. However, SCAOP itself is a relatively unstable metabolic compound and was unavailable in sufficient quantities. Therefore, the analogue compound, SCHP, which is identical to SCAOP except for the amino group at pyrazole position four and the oxygen group at position five, was used in these studies. This chemical was kindly supplied by Drs Howard Johnson and Douglas

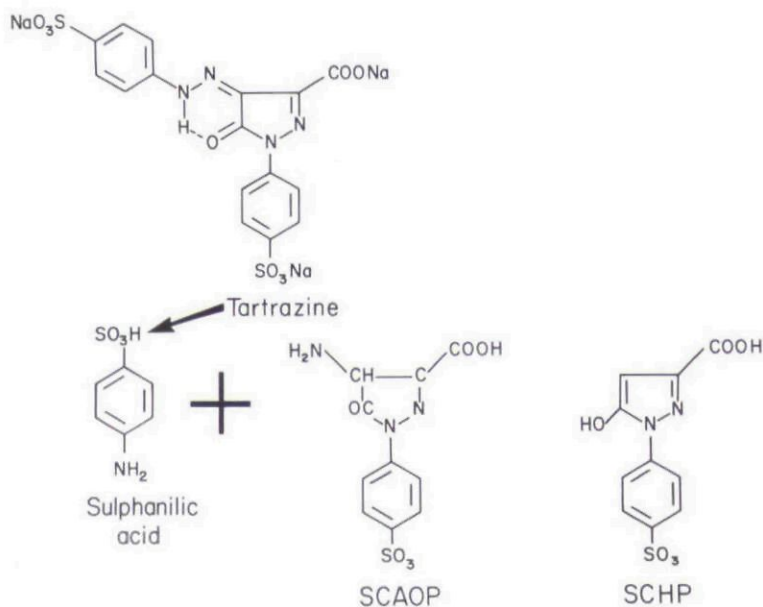


Fig. 1. Chemical structures of tartrazine, sulphanilic acid, a tartrazine metabolite, 1-sulphophenyl-3-carboxy-4-amino-5-oxy pyrazole (SCAOP) and an analogue of the tartrazine metabolite, 1-sulphophenyl-3-carboxy-5-hydroxypyrazole (SCHP).

Archer (Food and Drug Administration, Cincinnati, Ohio). Aspirin anhydride-free aspirin powder, U.S.P. (Lot No. 45, 191) was purchased from the Baker Chemical Company.

Fresh solutions of aspirin, tartrazine and SCHP were prepared daily. Since full dose-response effects of each reagent were required in each individual platelet aggregation assay, standard solutions of tartrazine (60, 30, 15, 5, 2.5 and 1 mM) and SCHP (30, 15, 5, 2.5 and 1 mM) were prepared with 0.9% saline. Standard solutions of aspirin (33, 16.50, 8.25, 4.13, 2.06 and 1.03 mM) were prepared in barbital saline buffer (pH 7.6).

Platelet aggregation studies

Platelet tests were conducted 3 months after the end of respective pollen seasons and all individuals were monitored closely for avoidance of drugs known to affect platelet aggregation for at least 7 days prior to experimental studies by a strict drug elimination regimen (Maccia *et al.*, 1977). Sufficient blood from each patient was drawn for full dosage-response assays with control diluent and all three reagent solutions (aspirin, tartrazine and SCHP). Since it was essential that each aggregation experiment was performed with 300,000 platelets per mm³, platelet-rich plasma (PRP) preparations were diluted with platelet-poor plasma (PPP), control diluent or various standard reagent solutions until this final concentration was attained. To determine whether baseline collagen-induced aggregation was normal, a preliminary platelet aggregation test was performed with PPP as the diluent. Upon satisfying this prerequisite, dose response assays with standard solutions of each of the test chemicals were incubated with PRP for 5 min, after which 0.5 ml of these reactant mixtures were transferred to sampling cuvettes. All platelet aggregation experiments were induced with 0.05 ml of a 2.3 µg/ml collagen solution diluted in 1.7 mM acetic acid and the platelet profile was recorded for 10 min, as previously reported (Maccia *et al.*, 1977). The inhibitory dose of platelet aggregation was defined as the lowest concentration of chemical which caused 50% inhibition of platelet aggregation. This value was equivalent to 40% on the light transmittance scale setting of 10% before addition of the aggregating agent. Maximum platelet aggregation rarely exceeded 90% on this scale. This level of inhibition is at least 2 standard deviations below that of normal mean aggregation data previously determined in our laboratory in twenty-five normal volunteers (Maccia *et al.*, 1977). It was also necessary to determine the critical concentration of test chemicals which caused inhibition in as much as the volume of standard reagent solutions required to adjust the final platelet count to a uniform value of 300,000 per mm³ varied from patient to patient.

Statistical analysis

An unpaired Student's *t* test was used to compare mean inhibitory concentrations between atopic and normal populations.

Results

Both tartrazine and SCHP inhibited collagen-induced platelet aggregation in normal and atopic subjects. The inhibitory effect of tartrazine upon normal platelets is illustrated in Fig. 2. The preliminary control profile shows a normal brisk platelet aggregation response reaching 90% on the light transmittance scale within 5 min after addition of the aggregating agent. In sharp contrast to the control profile, the tartrazine dose response assay reveals almost complete absence of aggregation (70%

inhibition) or partial inhibition (40%) at doses of 34.8 and 17.4 mM, respectively. The aggregation response is normal at a tartrazine dose of 8.7 mM. Thus, in this experiment the tartrazine concentration of 34.8 mM was considered to be the critical inhibitory dose because inhibition of platelet aggregation was $\geq 50\%$. Similar inhibitory effects upon atopic platelets were also observed, but usually at smaller concentrations of tartrazine (Fig. 3). While the tartrazine dose response assay of atopic platelets was quantitatively comparable to normal platelets, the critical inhibitory concentration was less than half (16.2 mM) that of the example of a normal platelet study shown in Fig. 2.

The pyrazolone compound, SCHP, also inhibited collagen-induced platelet aggregation in both normal and atopic platelets (Fig. 4) at much lower concentrations. In this paired experiment the critical inhibitory concentrations of SCHP upon normal and atopic platelets were 7.05 and 3.53 mM, respectively.

Mean critical inhibitory concentrations of tartrazine, SCHP and aspirin causing inhibition of collagen-induced platelet aggregation in normal and atopic individuals are compared in Fig. 5. In the normal population, 50% or greater inhibition of collagen-induced platelet aggregation was produced by tartrazine, SCHP and aspirin at mean doses of 27.2, 7.9 and 7.8 mM, as compared to critical inhibitory concentrations of 16.3, 5.4 and 3.5 mM in the atopic patients. Both tartrazine and SCHP exhibited more potent *in vitro* inhibitory effects on allergic patients as compared to

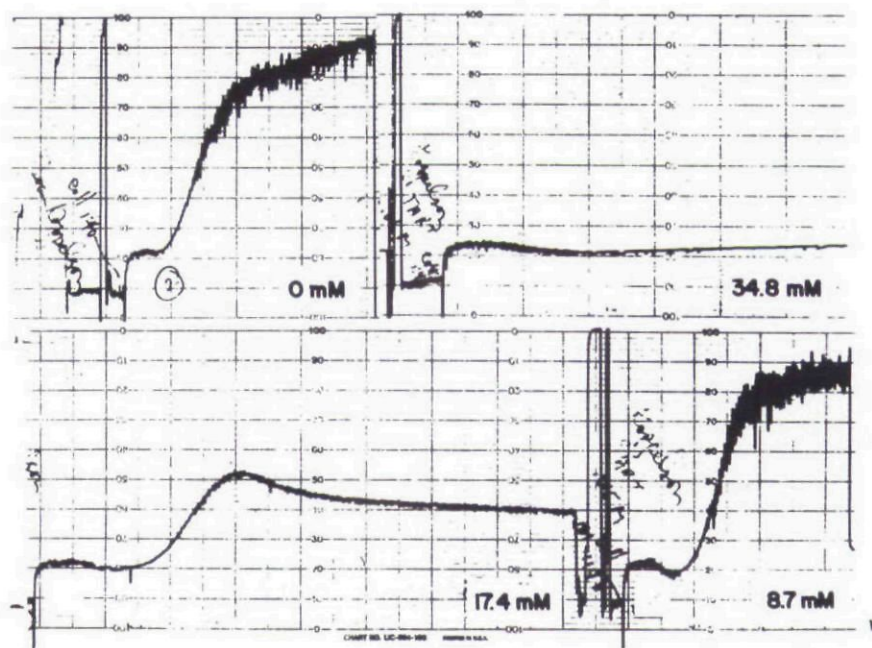


Fig. 2. Platelet aggregation profiles showing inhibition of normal platelets by tartrazine. The preliminary control profile shows a normal aggregation (upper left panel). The tartrazine dose response assay reveals almost complete absence of aggregation and partial inhibition at doses of 34.8 and 17.4 mM respectively (upper right and lower left panels). The aggregation response is not inhibited at a tartrazine dose of 8.7 mM. Thus, in this experiment the tartrazine concentration of 34.8 mM was considered to be the critical inhibitory dose because inhibition of platelet aggregation was $\geq 50\%$.

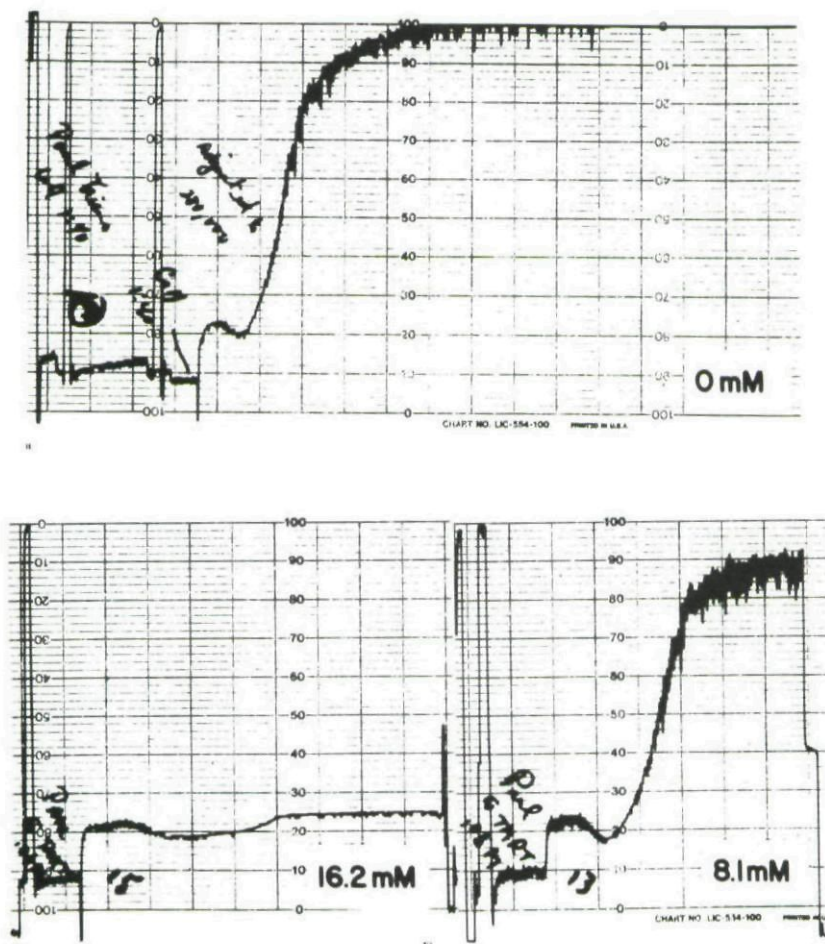


Fig. 3. Platelet aggregation profiles showing inhibition of atopic platelets by tartrazine. The preliminary control profile shows a normal aggregation (upper panel). The tartrazine dose response assay reveals almost complete absence of aggregation (85% inhibition) at a dose of 16.2 mM (lower left panel). The aggregation response is only slightly inhibited (10%) at a tartrazine concentration of 8.1 mM (lower right panel). Thus in this experiment the tartrazine concentration of 16.2 mM was considered to be the critical inhibitory dose because inhibition of aggregation was > 50%.

normal platelets. Larger doses of tartrazine were consistently required to cause inhibition of platelet aggregation than either SCHP or aspirin. Although mean aspirin inhibition results in the current study tended to be similar to data previously reported by Schwartz & Bennett (1973), a significant difference between normal and atopic groups in our study was not noted. The most likely explanation for this finding is that different subsets of patients were evaluated. However, it is noteworthy that the mean inhibitory concentrations of SCHP and aspirin in the current group of normal subjects was identical (7.8 mM) to aspirin data obtained by Schwartz and his co-workers. Moreover, the mean inhibitory concentration of SCHP in atopic patients (5.3 mM) was comparable to inhibitory data for aspirin observed in asthmatic patients (5.2 mM) by these investigators.

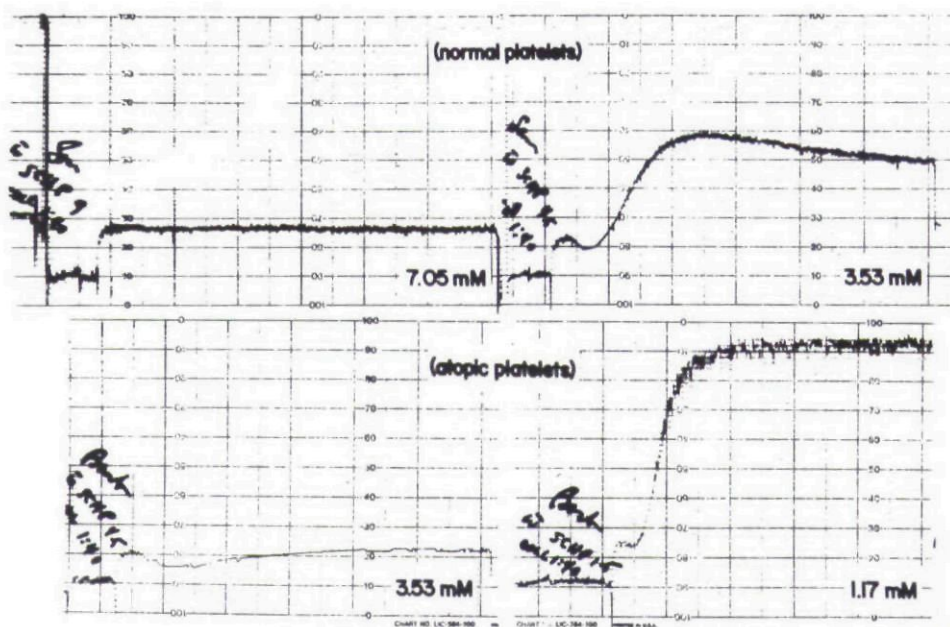


Fig. 4. Platelet aggregation profiles showing critical concentrations of SCHP necessary to inhibit platelet aggregation in representative normal and atopic patients. With representative normal platelets, 7.05 mM SCHP was considered to be the critical inhibitory dose because inhibition of platelet aggregation was $> 50\%$ (upper left panel). With representative atopic platelets 3.53 mM SCHP was considered to be the critical inhibitory dose because inhibition of platelet aggregation was $> 50\%$ (lower left panel).

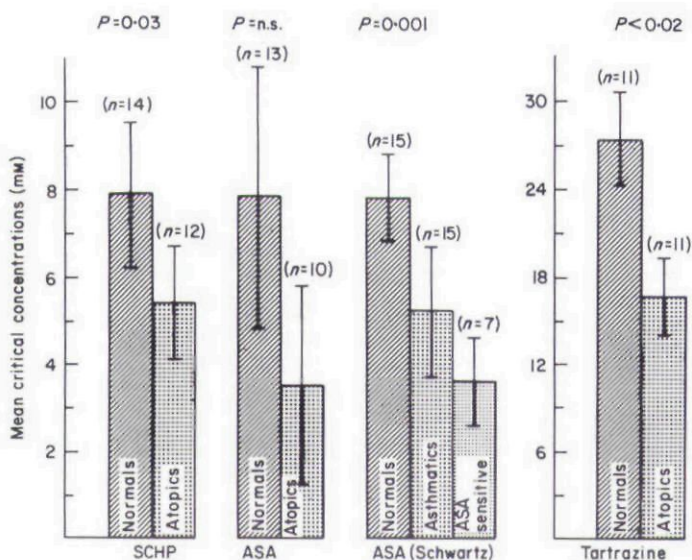


Fig. 5. Mean critical inhibitory concentration of tartrazine, SCHP and aspirin (ASA) causing inhibition of collagen-induced platelet aggregation in normal and atopic individuals. $\square$, Normal population; $\square$, patient population. The number of subjects is represented in brackets above the notation and the standard error of the mean is indicated. Due to experimental design, dose-response assays to all drugs were not done in some instances. The ASA data of Schwartz & Bennett (1973) have been converted from mg/ml to mM for ease of comparison with current SCHP and tartrazine data.

Discussion

These studies reveal that tartrazine and SCHP inhibit *in vitro* collagen-induced platelet aggregation in both normal and atopic subjects. Moreover, the quantitative effects of SCHP and aspirin on *in vitro* platelet aggregation in both normal and atopic subjects were almost equimolar. Thus, diminished platelet aggregation might be encountered in individuals who ingest large quantities of tartrazine on a habitual basis, in those persons whose intestinal catabolism of tartrazine might favour increased production of SCAOP in the gastrointestinal tract, or in patients with renal failure which would cause increased accumulation of tartrazine and/or its metabolites (Brettell *et al.*, 1979). The current data also indicate that platelets of atopic patients are more susceptible to the effects of tartrazine or SCHP than normal subjects and they are consistent with past results of Schwartz & Bennet (1973) who observed increased susceptibility of asthmatic platelets to aspirin. Decreased platelet aggregatory function may also have been partially responsible for some cases of tartrazine-induced non-thrombocytopenic purpura previously described in two other clinical reports (Criepp, 1979; Michaelsson, Petterson & Juhlin, 1974).

The precise mechanism by which these chemical compounds block one or several stages of the platelet aggregation process cannot be gleaned from these experiments because of the complexity of collagen-induced aggregation itself and possible non-specific membrane stabilizing effects of one or both chemicals. Interference with collagen-induced aggregation could occur either by inhibiting the ADP release stage or by interrupting the arachidonic acid cascade leading to production of intermediary prostaglandin endoperoxides and thromboxane A_2 (Packham, Kinlough-Rathbone & Mustard, 1978).

The effect of tartrazine on prostaglandin metabolism in two different mammalian systems has been reported with somewhat conflicting results. In one study production of prostaglandins and thromboxane A_2 in guinea pigs was inhibited by preincubation of lung tissue with tartrazine (Casarani, *et al.*, 1978). In contrast the other report revealed that tartrazine did not affect aggregation of human platelets induced by arachidonic acid (Gerber, *et al.*, 1979). This fatty acid, a substrate for cyclo-oxygenase, is a precursor of labile endoperoxides and ultimately, thromboxane A_2 , both of which stimulate platelet aggregation. The effect of T upon collagen-induced aggregation, which, as previously cited, also causes release of ADP, was not evaluated in the latter investigation. Moreover, the possible inhibitory function of the aminopyrazolone metabolite (SCAOP) of T upon arachidonate-induced aggregation was not determined because the gastrointestinal absorption of this compound was thought to be nil by these investigators. However, in view of the fact that several reliable metabolic studies have demonstrated measurable urinary and lung excretion of the ^{14}C labelled pyrazolone ring after oral administration of a ^{14}C pyrazolone analogue of T (Ryan, 1972; Honohan *et al.*, 1977) further investigation of aminopyrazolone or its analogue, SCHP, was warranted. While recent experiments in our laboratory confirmed that pharmacological levels of tartrazine did not affect arachidonate-induced aggregation, we found that the effect of a low dose of SCHP was the same in platelet aggregation induced by both arachidonate and collagen. Clearly, further research is needed to resolve these discrepancies, but these results indicate that the effect of tartrazine or SCHP on collagen-induced platelet aggregation may be a useful *in vitro* method of assessing possible non-immune mechanisms involved in reactions to tartrazine and/or its metabolites.

Acknowledgments

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