Tartrazine and the prostaglandin system

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The effect of tartrazine on prostaglandin production was evaluated in several in vitro systems in order to elucidate the interrelationship between aspirin-sensitive asthma and tartrazine. Unlike the nonsteroidal anti-inflammatory drugs, tartrazine did not inhibit cyclooxygenase activity in sheep seminal vesicles, guinea pig lung microsomes, and human platelets. Tartrazine had no effect on the activation of acyl hydrolase, which is the rate-limiting step in prostaglandin production. The major metabolite of tartrazine, sulfanilic acid, also had no inhibitory effect on the sheep seminal vesicle cyclooxygenase. In view of these findings, if there is a cross-sensitivity between tartrazine and aspirin in aspirin-sensitive asthmatics, it is unlikely to be on the basis of prostaglandin inhibition.

Intolerance to aspirin was described over half a century ago, but the pathophysiology of this common illness is not known. A major breakthrough in the understanding of the pathogenesis of this illness occurred when Vane and co-workers[1] discovered that aspirin inhibits prostaglandin synthesis. Szczeklik, Gryglewski, and Czerniawski-Mysik[2] further showed that in patients with aspirin-sensitive asthma, other nonsteroidal anti-inflammatory drugs provoke an asthmatic attack, and that the most potent inhibitors of the cyclooxygenase activity in vitro were also the most potent drugs to alter pulmonary function in vivo. These findings agreed with an early observation by Cooke[3] that the acetyl group of aspirin is necessary in the untoward reaction to aspirin, as the acetyl group is important in the inhibition of cyclooxygenase. Thus a close correlation between the prostaglandin system and aspirin-induced asthma has been established.

Where tartrazine (FD&C yellow dye No. 5) fits into this schema is unresolved. Samter and Beers[4] noticed that 14 of 182 patients intolerant to aspirin also manifested an adverse reaction to tartrazine. One drawback of this epidemiologic study was that aspirin-sensitive patients were lumped into one group while the possibility that aspirin intolerance is a heterogenous disease has been inferred by other researchers. In a small group of asthmatics, Vedanthan and co-workers[5] could not find any cross-reactivity to tartrazine in children with aspirin-induced bronchospasm. Since tartrazine is an FD&C-approved dye that is included as a coloring agent in numerous drugs,[6] as well as in many common foods, we felt it was of importance to determine the dye's effect on the prostaglandin system. In this way we could determine if tartrazine fits into the aspirin-sensitive asthmatic schema the way the other nonsteroidal anti-inflammatory drugs do. In addition, we studied the effect of sulfanilic acid, the major metabolite of tartrazine,[7] on the cyclooxygenase activity to preclude the possibility that this organic acid metabolite is responsible for the dye's adverse reaction.

MATERIALS AND METHODS

1. Effect of tartrazine on ram seminal vesicle microsomes: The classic system to test for an effect on cyclooxygenase activity. Microsomes from 2 gm of sheep seminal vesicles (a gift from The Upjohn Co.) were prepared by centrifugation (600 × g for 10 min; supernatant, 8,000 × g for 20 min; supernatant, 100,000 × g for 90 min; pellet contains microsomal fraction) in 0.1 M ethylenediaminetetraacetic acid (EDTA) buffer at pH 8. The microsomes were diluted to 5 ml and divided into five 1-ml incubations. The first incubation medium contained only the buffer and was the control. The second contained 30 μM indomethacin and was used as a positive control for cyclooxygenase inhibition. The third contained 30 μM tartrazine, the fourth, 300 μm tartrazine, and the fifth, 30 mM tartrazine. Each of the five incubation media contained 165 μM arachidonic acid (Nu Check Co., Inc.) as the substrate, as well as 2 mM phenol, 3 μM hemoglobin,

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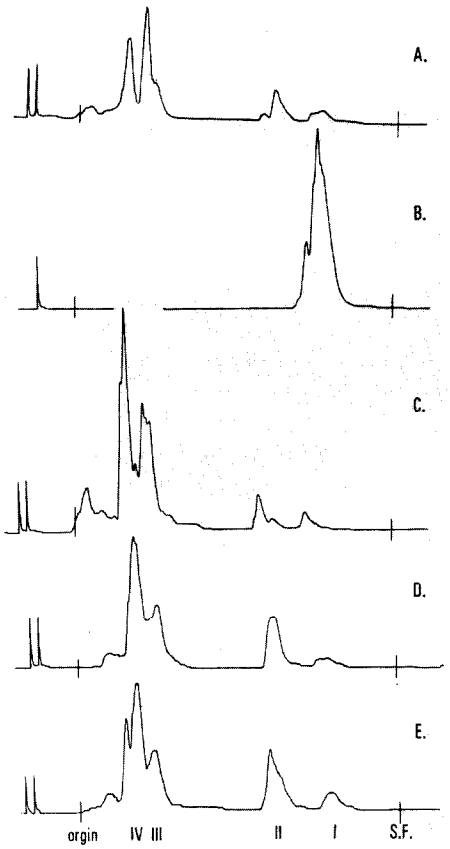

FIG. 1. The effect of increasing concentrations of tartrazine on the cyclooxygenase activity in sheep seminal vesicles. The products were separated on silica gel G thin layer chromatography using the solvent system, chloroform (90): methanol (8): acetic acid (1): H_2O (0.65). Incubation *A* is the control; *B* contains 30 μM indomethacin; *C* contains 30 μM tartrazine; *D* contains 300 μM tartrazine; *E* contains 30 mM tartrazine. Peak I is the unchanged arachidonic acid; peak II is hydroxy-fatty acids; peak III travels as PGE_2; and peak IV travels as $PGF_{2\alpha}$.

and 1 mM tryptophan. A tracer amount of ^{14}C arachidonic acid (New England Nuclear) was added to each medium so that the prostaglandin conversion could be followed by radioactive scanning of the extracted medium. The incubations took place at 37° C for 10 min. The medium was then acidified to pH 3.5, extracted in 3 volumes of diethyl ether. The organic phase was evaporated to dryness, resuspended in ethanol, and spotted on a 250 μ thick silica gel thin layer plate with the prostaglandins separated using the solvent system chloroform (90): methanol (9): acetic acid (1): water (0.65).

2. *Effect of tartrazine on thromboxane B_2 production in vitro.* We used guinea pig lung microsomes to test this enzyme system as guinea pig lung microsomes produce mainly thromboxane B_2 and 12-hydroxy-5,8,10 heptadecatrienoic acid (HHT) from arachidonic acid.[8] Microsomes from 15 gm of guinea pig lungs were prepared in 0.1 M EDTA buffer as described in section 1. The microsomes were divided evenly into six incubations containing (1) buffer alone, (2) imidazole 6 mM, (3) indomethacin 100 μM, (4) tartrazine 30 μM, (5) tartrazine 300 μM, and (6) tartrazine 30 mM. All of the incubations contained 165 μM of arachidonic acid as a substrate, as well as a 2 mM phenol, 3 μM hemoglobin, and 1 mM tryptophan. A tracer amount of ^3H-arachidonic acid (New England Nuclear) was added so that prostaglandin conversion could be followed by radioactive peaks. The incubations were at 37° C for 25 min, after which the media were acidified to pH 3.5, and extracted in 3 volumes of ethyl acetate. The organic phase was evaporated to dryness, resuspended in ethanol, and spotted on a 250 μ thick silica gel G thin layer plate with the prostaglandins separated using the solvent system benzene (60): dioxane (30): acetic acid (2). This solvent system separates thromboxane B_2 from the other prostaglandins. An authentic thromboxane B_2 and arachidonic acid standard was run with the separation to determine their Rf values. On the plates these two spots were scraped and counted for radioactivity. A ratio of thromboxane B_2 to arachidonic acid radioactivity was calculated to determine a relative conversion rate.

3. *Effect of tartrazine on human platelet cyclooxygenase-thromboxane synthetase system.* Human blood was collected in a syringe containing 0.1 M trisodium citrate. Platelet-rich plasma was obtained by centrifugation at 200 × g for 15 min. Platelet aggregation was monitored with a Payton dual-channel aggregation module, with an Omniscribe recorder. Platelet-rich plasma, 0.5 ml, was preincubated for 2 min, then 0.5 mM sodium arachidonate was added to the plasma to induce platelet aggregation. Arachidonic acid–induced platelet aggregation was repeated in the presence of 30 μM, 300 μM, and 30 mM of tartrazine.

4. *Effect of tartrazine on acyl hydrolase activity.* To test the ability of tartrazine to interfere with acyl hydrolase activity, the rate-limiting step in the prostaglandin cascade, we used papillae from 10 rat kidneys and preincubated them in 20 μCi of tritiated arachidonic acid in Krebs-Henseleit medium under 95% O_2-5% CO_2 for 30 min. This enriches mainly the tissue phospholipids with the radiolabeled arachidonic acid.[9] The papillae were then washed twice in Krebs-Henseleit solution and divided into 7 incubations as outlined below. All of the incubations were in Krebs-Henseleit medium containing 1% bovine albumin and were continuously oxygenated under 95% O_2-5% CO_2.

Drugs in the incubation medium were: (1) mepacrine hydrochloride, 9.2 mM; (2) tartrazine, 30 μM; (3) tartrazine, 300 μM; (4) tartrazine, 30 mM; (5) meclofenamic acid, 200 μM; (6) and (7), none.

The papillae were preincubated for 10 min; then the acyl hydrolases were stimulated by adding 4 nM of angiotensin II. After 30 min the papillae were separated from the medium. The medium was then acidified to pH 3 and extracted in three volumes of ethyl acetate. The organic phase was evaporated; the residue was resuspended in 1 ml of ethanol and counted for radioactivity. The papillae from the various incubations were homogenized in 10% tri-

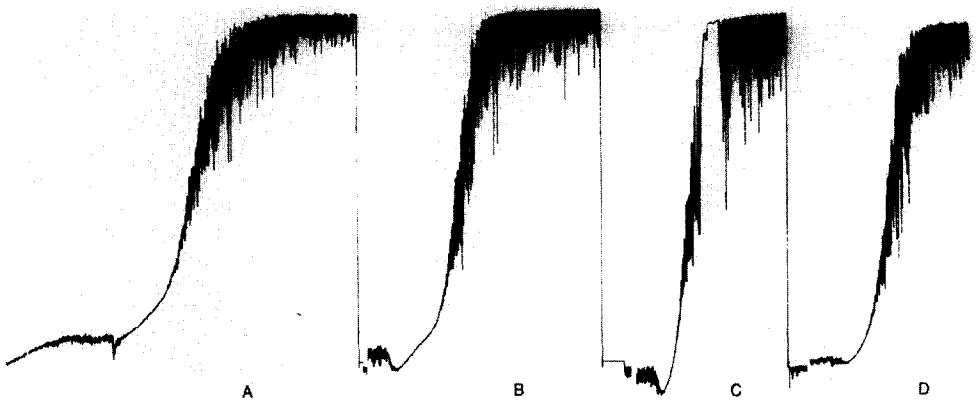

FIG. 2. The effect of increasing concentrations of tartrazine on sodium arachidonate–induced platelet aggregation. Incubation *A* is control; *B* contains 30 μM tartrazine; *C* contains 300 μM tartrazine; *D* contains 30 mM tartrazine. Tartrazine had no effect on platelet aggregation induced by sodium arachidonate.

TABLE I. Effect of tartrazine on thromboxane B_2 generation in guinea pig lung microsomes

Incubation	Arachidonic acid (cpm)	Thromboxane B_2 (cpm)	Ratio
Control	68,710	13,874	5:1
Imidazole 6 mM	88,462	4,408	20:1
Tartrazine 30 μM	40,782	6,422	6.5:1
Tartrazine 300 μM	55,048	8,552	6.4:1
Tartrazine 30 mM	46,536	11,076	4.2:1
Indomethacin 100 μM	95,945	1,705	56:1

TABLE II. Effect of tartrazine on acyl hydrolase activity in rat kidney papilla

Incubation	Total lipid radioactivity (cpm)	Media radioactivity (cpm)	% release
Control	187,294	4,480	2.4
Angio II	124,863	12,837	12.4
Angio II + mepacrine	145,610	7,420	5.5
Angio II + tartrazine 30μM	115,977	11,960	12.3
Angio II + tartrazine 300μM	187,989	18,720	11.9
Angio II + tartrazine 30 mM	177,483	24,536	12.1
Angio II + meclofenemate 200 μM	121,817	12,422	12.2

chloroacetic acid to disrupt the cells and precipitate the proteins. The supernatant was collected; the precipitate was washed twice with chloroform and twice with methanol to extract the polar and nonpolar lipids. The supernatant along and organic washes were evaporated, resuspended in methanol, and a fraction counted for radioactivity. A ratio of medium radioactivity to total extractable tissue radioactivity gives us an estimate of the stimulation of arachidonic acid turnover by angiotensin II.

5. *Effect of sulfanilic acid on cyclooxygenase activity.* The preparation and medium were identical to group incubations discussed in the first section under "Materials and Methods" except that increasing concentrations of sulfanilic acid were used instead of tartrazine. The concentrations were 30 μM, 300 μM, and 30 mM. The medium pH was adjusted to be identical in all incubations.

RESULTS

1. Tartrazine even at the highest concentration (30 mM), a concentration that is probably never achieved in vivo, had no effect on the cyclooxygenase system (Fig. 1). In contrast, 30 μM indomethacin, a known inhibitor of cyclooxygenase, completely inhibited the enzymatic transformation of arachidonic acid to the various prostaglandins. Since there is a good correlation between in vivo and in vitro inhibition of cyclooxygenase, we can conclude that tartrazine does not inhibit the cyclooxygenase as the other nonsteroidal antiinflammatory drugs do.

2. Since tartrazine was inactive at inhibiting cyclooxygenase in the sheep seminal vesicle microsomes, we studied to see if this lack of inhibition was reproducible in another in vitro system, as well as to evaluate tartrazine's ability to inhibit another enzyme in the prostaglandin cascade. However, tartrazine was again inactive in inhibiting either the cyclooxygenase or the thromboxane synthetase. In Table I the ratio of arachidonic acid to thromboxane B_2 showed that both indomethacin and imidazole effectively inhibited the production of thromboxane B_2 (ratios 56:1 and 20:1,

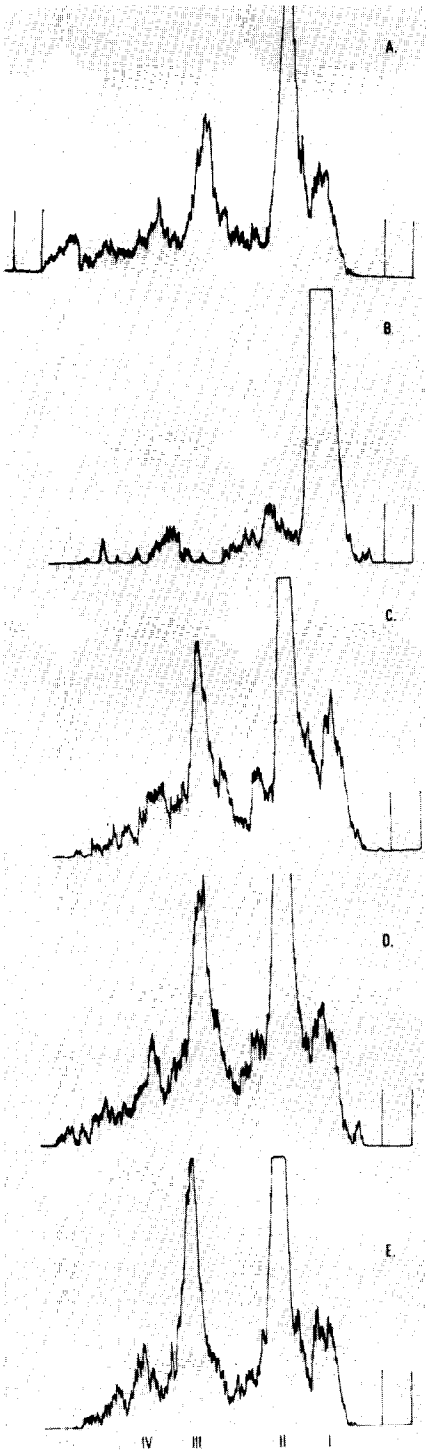

FIG. 3. The effect of increasing concentrations of sulfanilic acid on the cyclooxygenase activity in sheep seminal vesicles. The products were separated on silica gel G thin layer chromatography using the solvent system, chloroform (90): methanol (8): acetic acid (1): H_2O (0.65). Incubation *A* is the control; *B* contains 30 μM indomethacin; *C* contains 30 μM sulfanilic acid; *D* contains 300 μM sulfanilic acid; *E* contains 30 mM sulfanilic acid. Peak I is the unchanged arachidonic acid; peak II is hydroxy-fatty acids; peak III travels as PGE_2; peak IV travels as $PGF_{2\alpha}$.

respectively), but tartrazine in all concentrations was ineffective in inhibiting the formation of thromboxane B_2. Since indomethacin works only on the cyclooxygenase and imidazole only on the thromboxane synthetase, we can conclude that tartrazine did not inhibit either enzymatic step in this in vitro system.

3. The effect of tartrazine on a human cyclooxygenase system was evaluated in human platelets. Arachidonic acid is a strong stimulant of platelet aggregation that is completely inhibited by indomethacin in micromolar concentrations. However, tartrazine even at the highest concentration was unable to alter platelet aggregation induced by arachidonic acid (Fig. 2).

4. Since the first step, and probably the rate-limiting step, in prostaglandin generation is the release of arachidonic acid from the lipid stores, we investigated tartrazine's ability to alter acyl hydrolase activity. Angiotensin II has been shown to activate the acyl hydrolases in renal papillae.[10] Indeed, angiotensin II stimulated the release of radioactivity from the rat papillae fivefold over control (Table II). This stimulation was significantly inhibited by mepacrine hydrochloride, a known inhibitor of the acyl hydrolase. Meclofenamate did not inhibit the release of radioactivity by angiotensin, confirming previously reported data that nonsteroidal anti-inflammatory drugs do not interfere with the hydrolysis of arachidonic acid from lipids in the kidney.[11] Tartrazine at all concentrations was inactive in inhibiting the angiotensin II–stimulated release of radioactivity from the rat papillae.

5. The major urinary metabolite of tartrazine is sulfanilic acid. In fact, in experimental animals tartrazine is very quickly metabolized to sulfanilic acid possibly by the intestinal bacteria.[7] Since sulfanilic acid, in common with other nonsteroidal anti-inflammatory drugs, is an organic acid, we looked at this compound's ability to inhibit cyclooxygenase in the sheep seminal vesicles. At all the concentrations tested, sulfanilic acid was inactive in inhibiting the cyclooxygenase pathway (Fig. 3). Thus, this organic acid is unlike the nonsteroidal anti-inflammatory drugs in respect to its action on the prostaglandin pathway.

DISCUSSION

The role of prostaglandins in the tracheobronchial tree is essentially undefined. Data in both the non–aspirin-sensitive asthmatic and in normal population would indicate that prostaglandins do not have a pivotal role since nonsteroidal anti-inflammatory drugs do not affect airway resistance.[12] However, in those asthmatics who develop bronchospasm after aspirin

ingestion and also react adversely to other nonsteroidal anti-inflammatory drugs, inhibition of cyclooxygenase seems to alter the balance of bronchial smooth muscle activity toward constriction. The exact mechanism of the aspirin-induced bronchoconstriction is unclear. It could be related to the inhibition of bronchodilatory PGE_2, but likely the inhibition of prostaglandins is associated with the release of bronchoconstrictors from the mast cells.

Tartrazine is an FD&C-approved food additive that has been implicated in numerous adverse reactions. Chaffee and Settipane[13] reported a case of severe asthmatic attacks related to the intake of tartrazine and aspirin, but, on immunologic testing, the patient had peripheral eosinophilia to tartrazine as well as an eosinophilotactic response to tartrazine by the skin window technique. They felt that the adverse reaction to the dye was on an immunologic basis. Samter and Beers[4] estimated less than 10% cross-reactivity to tartrazine in aspirin-sensitive patients. Stenius and Lemola[14] found that 20% of the asthmatic population reacted adversely to tartrazine but could not see any differential response between the atopic and nonatopic groups. They also found that 44% of the aspirin-sensitive patients reacted adversely to tartrazine. Since aspirin sensitivity may be mediated through both an allergic and a nonallergic mechanism, the cross-reactivity to tartrazine has to be defined more closely.[15] Szczeklik, Gryglewski, and Czerniawska-Mysik found a 100% cross-reactivity between aspirin and other nonsteroidal drugs in affecting bronchoconstriction, which they were able to relate to an in vitro inhibition of prostaglandin synthesis. Their studies were confined to asthmatic patients while in other reported studies aspirin sensitivity included patients with bronchoconstriction, urticaria, anaphylaxis, and angioedema which were all lumped together.

In our extensive search for an effect of tartrazine on the prostaglandin cascade, we could not find an interaction at any step. The drug does not inhibit the acyl hydrolase activity, cyclooxygenase activity, or thromboxane synthetase activity even in very high concentrations. Tartrazine's major urinary metabolite is also inactive at the important cyclooxygenase step. We chose not to study the amino pyrazoline metabolite of tartrazine on the prostaglandin system because evidence indicates that this compound is either not absorbed or is quickly metabolized by intestinal microflora as this metabolite does not appear in the urine but only in the feces after oral tartrazine administration.[17] If tartrazine were to cause reaction similar to the nonsteroidal anti-inflammatory drugs, the cyclooxygenase pathway should have been inhibited and the cross-reaction between tartrazine and aspirin should be seen much more commonly than is reported. It is possible that tartrazine sensitivity has an allergic basis, and patients sensitive to tartrazine are sensitive to many other drugs including aspirin, thus making it not a cross-sensitivity but a coexisting sensitivity. Nonetheless, from our data it seems quite unlikely that the adverse reaction to tartrazine is mediated through an inhibition of the prostaglandin pathway as it seems to be with the nonsteroidal anti-inflammatory drugs.

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