Sulphinpyrazone and the platelet serotonergic mechanism in ischaemic heart disease

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Abstract

A double blind study in 25 patients with ischaemic heart disease and 20 matched healthy controls examined the effect of sulphinpyrazone on the uptake of serotonin by platelets and the basal concentrations of serotonin in platelets. Uptake was measured using tritium labelled serotonin and basal concentrations estimated spectrophotofluorometrically. Serotonin uptake was significantly increased both in the patients with chronic stable angina of effort and in those with a history of myocardial infarction six months or more previously. Sulphinpyrazone reduced serotonin uptake from 94.25 (SE 8.65) to 57.86 (5.37) cpm/10⁸ platelets after 24 weeks of treatment in the group with stable angina and from 137.45 (16.26) to 68.08 (8.38) cpm/10⁸ platelets in the myocardial infarction group. Raised basal concentrations in the two groups were also reduced by sulphinpyrazone. Placebo had no effect on serotonin uptake or basal concentrations in either group of patients.

The ability of sulphinpyrazone to inhibit uptake and reduce basal concentrations of serotonin in patients with ischaemic heart disease may be yet another mechanism through which this drug exerts its beneficial antiplatelet effect.

Introduction

The results of the Anturane (sulphinpyrazone) reinfarction trial showed the beneficial effects of this drug in preventing sudden death after myocardial infarction, though its exact mechanism of action was not clear.^{1,2} Various antiplatelet agents act in different ways—for example, sulphinpyrazone and aspirin inhibit the platelet arachidonic pathway and therefore the synthesis of thromboxane A₂ and platelet aggregation,³ whereas dipyridamole increases platelet cyclic adenosine monophosphate concentrations, thus inhibiting platelet calcium activation, leading to inhibition of platelet aggregation and adhesion.³

Other mechanisms through which sulphinpyrazone may act are by reducing thrombus formation⁴ and preventing ultrastructural changes in platelet morphology.⁵ In addition, in vitro studies show that sulphinpyrazone inhibits the release of serotonin by platelets,⁶ affects platelet survival time,⁷ and reduces platelet aggregation.⁸

Platelets are the main reservoir of serotonin in man and show definite uptake and efflux of this amine.⁹ We have reported finding a significant increase in serotonin uptake by platelets from patients

with ischaemic heart disease,¹⁰ and the present study examines the effect of sulphinpyrazone on this mechanism.

Subjects and methods

Twenty five patients with ischaemic heart disease and 20 controls matched for age and sex (healthy volunteers) were selected for the study. The patients comprised 22 men and three women with a mean age of 50.1 years (range 33–60). Eleven were suffering from chronic stable angina of effort and 14 had effort angina as a legacy of myocardial infarction six months or more previously. In all cases myocardial infarction had been diagnosed according to established criteria.¹¹ All patients and controls were subjected to a routine clinical check, electrocardiography, and a treadmill stress test. Ischaemic heart disease in the chronic stable angina group was established with the help of positive treadmill stress test findings according to Chung's criteria¹² and by coronary angiography when indicated.

No subject had any history of depression or psychosis or had taken any antidepressant or neuroleptic drugs. Foods and drugs influencing biogenic amine metabolism were avoided from three days before the study.

After allowing the patients a washout period of two weeks during which all previous medications were stopped and only nitroglycerin permitted all subjects had initial estimations of their serotonin uptakes and basal concentrations in platelets. The patients were then given alternately and on a double blind basis either sulphinpyrazone (400 mg twice daily) or identical looking capsules of placebo. Decoding was done at the end of the study.

Serotonin uptake was re-estimated in all subjects at 12 weekly intervals and basal concentrations re-estimated in the patients after 24 weeks of treatment. The entire study was conducted over one year. Statistical analysis was by Student's *t* test (unpaired).

Collection of blood—Platelet rich plasma was prepared by the method of Born and Gilson.¹³ Glassware used in the experiments was coated with silicon. Blood samples were collected from cubital veins. Nine ml blood was immediately transferred into a centrifuge tube of cellulose acetate containing 1 ml sodium citrate buffer (3.8%; 129 mmol/l) to give a final concentration of 12.9 mmol/l and placed in an ice bath. Blood was centrifuged at 200 *g* for 15 minutes at 0°C. The supernatant platelet rich plasma was carefully taken out. Platelet counts were done in a Naebourhaemocytometer at a dilution of 1/20.

Measurement of serotonin uptake by platelets—Serotonin uptake was measured by determining the concentration of radioactivity in platelets incubated with tritium labelled serotonin binoxalate (see below). Platelet rich plasma samples (1 ml) were preincubated in duplicate for five minutes at

TABLE I—Mean (SE) platelet counts in patients with ischaemic heart disease and controls

Group	No	Platelets ($\times 10^6/\text{ml}$)	
		Before sulphinpyrazone	After sulphinpyrazone
Controls	20	1.36 (0.25)	—
Angina	11	1.32 (0.73)	1.47 (0.52)
Myocardial infarction	14	1.56 (0.32)	1.38 (0.36)

TABLE II—Mean (SE) serotonin uptake in patients with ischaemic heart disease and controls

Group	No	Platelet serotonin uptake (cpm/10 ⁸ platelets)
Controls	20	28.14 (2.76)
Angina	11	89.85 (7.68)*
Myocardial infarction	14	141.80 (11.41)**

Compared with control value: **p* < 0.01; ***p* < 0.001.

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37°C. Serotonin was then added (1 pmol/ml; 25 µl) and the mixture incubated for 10 minutes with constant stirring. Uptake was terminated by adding 2 ml ice cold 4% (wt/vol) formaldehyde solution. Tubes were centrifuged at 10 000 rpm at 0°C for 15 minutes to separate the platelets from plasma. The supernatant was decanted and the inner side of the centrifuge tube wiped with filter paper. The pellet was digested in 0.2 ml formic acid (21 mol/l) and 0.3 ml distilled water for one hour at room temperature and transferred to counting phials containing 10 ml scintillant. Radioactivity was measured in an automatic Packard scintillation counter having 33% efficiency for tritium. Values depicted in the tables were obtained after

myocardial infarction). There was no significant difference in counts among the groups and counts were not altered by sulphinpyrazone.

Of the 11 patients with chronic stable angina, six were allocated to receive sulphinpyrazone and five to receive placebo. Of the 14 patients in the myocardial infarction group, seven were given sulphinpyrazone and seven placebo. Serotonin uptake was significantly higher in both groups of patients compared with controls (table II).

In neither group of patients was the raised serotonin uptake altered significantly by placebo (table III). By contrast, those given sulphinpyrazone showed pronounced falls in uptake (table III; fig 1). Fall in uptake increased

TABLE III—Effect of sulphinpyrazone and placebo on platelet serotonin uptake in ischaemic heart disease. Values are means (SE in parentheses)

Group	No	Treatment	Platelet serotonin uptake (cpm/10 ⁸ platelets)		
			Initial	12 Weeks	24 Weeks
Angina	5	Placebo	84.37 (13.95)	80.07 (15.90) [5.09]†	83.95 (16.90) [0.49]
	6	Sulphinpyrazone	94.25 (8.65)	70.48 (5.05)* [25.2]	57.86 (5.37)** [38.6]
Myocardial infarction	7	Placebo	145.60 (17.16)	139.01 (15.45) [4.51]	136.54 (15.79) [6.22]
	7	Sulphinpyrazone	137.45 (16.26)	89.30 (12.05)* [35.03]	68.08 (8.38)** [50.46]

Compared with initial value: *p<0.01; **p<0.001.

†Figures in square brackets are percentage reductions from initial values.

TABLE IV—Effect of sulphinpyrazone and placebo on basal platelet serotonin concentrations in ischaemic heart disease. Values are means (SE in parentheses)

Group	Basal platelet serotonin concentration (ng/10 ⁸ platelets)					
	Sulphinpyrazone group			Placebo group		
	No	Initial	After 24 weeks	No	Initial	After 24 weeks
Controls	10	24.51 (4.72)	—	10	24.51 (4.72)	—
Angina	6	50.97 (3.34)	36.80 (7.13)* [27.8]†	5	51.85 (4.62)	49.84 (7.59) [3.8]
Myocardial infarction	6	70.10 (7.82)	48.46 (6.20)* [38.7]	5	75.43 (5.72)	71.33 (2.04) [5.4]

Compared with initial value: *p<0.01.

†Figures in square brackets are percentage reductions from initial values.

subtracting the blank group counts. Samples of platelet rich plasma from patients and controls were analysed simultaneously under similar conditions.

Estimation of basal platelet serotonin concentration—Basal serotonin concentration was determined using 1.0 ml platelet rich plasma. Samples were spun at roughly 10 000 rpm for 10 minutes at 0°C and the supernatant carefully decanted. The platelet pellet was resuspended in 1 ml physiological saline and used for the determination of serotonin. Platelet suspension (1 ml) was placed in a glass stoppered tube containing borate buffer (0.5 ml, 0.5 mol/l, pH 10.0). pH was adjusted to between 9.5 and 10.0 and sodium chloride (1.0 g) and *n*-butanol (10 ml) added. Tubes were shaken for 10 minutes and then centrifuged at 3 000 rpm for 10 minutes. The butanol layer was aspirated out and 8 ml transferred into another glass stoppered tube containing *n*-heptane (10 ml) and phosphate buffer (2 ml, 0.05 mol/l, pH 7.0). The tubes were shaken by rapid inversion for 10 minutes. A 1.2 ml sample of the aqueous phase was taken out and assayed for serotonin using the spectrofluorometric method of Snyder *et al.*¹⁴ Solution of the appropriate concentration of serotonin (standard) and blanks (reagent) were run throughout. Recovery of serotonin by this method was 60%.

Drugs and chemicals—Tritiated serotonin binosalate solutions (specific activity 0.98 TBq (26.4 Ci)/mmol; New England Nuclear Corporation) were made up in physiological saline stored in the cold and dark. Trisodium citrate was used for the preparation of citrate buffer (129 mmol/l). The composition of scintillant was naphthalene 21 g; (1,4-bis-2,5-phenyloxazolyl)-benzene (POPOP) 0.024 g; 2,5-diphenyloxazole (PPO) 1.3 g; toluene 100 ml; methanol 60 ml; and dioxane 100 ml. Borate buffer (0.5 mol/l, pH 10.0) was made by dissolving 6.183 g boric acid in 200 ml double distilled water, pH being adjusted with sodium hydroxide (10 nmol/l), saturated with salt.

Results

Table I shows the mean platelet counts recorded from the three study groups (controls, patients with chronic angina, and patients with a history of

FIG 1—Individual falls in uptake of serotonin by platelets in patients treated with sulphinpyrazone.

with the duration of treatment. With angina pectoris the mean uptake fell from 94.25 (SE 8.65) to 70.48 (5.05) cpm/10⁸ platelets (25.2% decrease) after 12 weeks of treatment and to 57.86 (5.37) cpm/10⁸ platelets (38.6% decrease) after 24 weeks. In the myocardial infarction group serotonin uptake fell from 137.45 (SE 16.26) to 89.30 (12.05) cpm/10⁸ platelets (35.03% decrease) after 12 weeks and to 68.08 (8.38) cpm/10⁸ platelets (50.46% decrease) after 24 weeks (table III).

Basal platelet serotonin concentrations were also substantially higher in the two groups of patients compared with controls and fell significantly after 24 weeks of sulphapyrazone (table IV; fig 2). In the group with angina the concentration fell close to the control value. Placebo had no effect on basal platelet serotonin concentration in either group of patients.

FIG 2—Individual falls in basal platelet serotonin concentrations in patients treated with sulphapyrazone.

Discussion

The Anturane Reinfarction Trial Research Group found a reduction in sudden deaths after 24 months of treatment with sulphapyrazone.^{1,2} The precise mechanism of the beneficial effect of the drug, however, is still not known. Endothelial damage, adhesion to subendothelium, aggregation, formation of plaques, and local release of vasoactive substances from platelets leading to spasm of coronary arteries^{15,20} superadded with embolic phenomenon²¹ are considered to be the sequence of events in the various stages of ischaemic heart disease. Interestingly, sulphapyrazone has been shown to inhibit several platelet functions—namely, adhesion to subendothelial cells, prostaglandin synthesis, and release of serotonin.^{6,22} Inhibition of aggregation and the release reaction appear to be important in the beneficial effects of sulphapyrazone. We have reported finding yet another mechanism (serotonergic) that is altered in ischaemic heart disease. Serotonin uptake by platelets was increased in patients with ischaemic heart disease,¹⁰ and this observation led us to study the effect of sulphapyrazone on uptake and basal concentrations of serotonin in platelets in patients with chronic stable angina of effort and angina after myocardial infarction.

Serotonin is an important biogenic amine widely distributed in the body and is of considerable importance in ischaemic heart disease for three main reasons: it is stored in large amounts in

platelets⁹; platelets possess active uptake and efflux processes to control concentrations of the amine⁹; and it induces aggregation both in vitro and in vivo.²³ Active uptake of serotonin by platelets appears to be the most important step and probably explains the major portion of the pathological alteration in platelet function.

We found that serotonin uptake was significantly increased ($p < 0.01$) both in patients with stable angina and in those with a history of myocardial infarction. These observations confirm our earlier work.¹⁰ Furthermore, significantly higher basal concentrations of serotonin were found in these patients compared with controls.

Sulphapyrazone significantly reduced ($p < 0.01$) uptake and the raised basal concentrations of serotonin in both groups of patients. Inhibition of serotonin uptake increased with the duration of treatment. Placebo, however, failed to have any significant effect on serotonin uptake. These results clearly show a tendency of sulphapyrazone to return serotonin uptake towards normal, and it may be that the drug exerts its antiplatelet effect through this serotonergic mechanism.

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