Antiplatelet effects of oral diltiazem, propranolol, and their combination

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- 1 The antiplatelet effects of a single oral dose of the calcium entry blocker diltiazem (60 mg), the β -adrenoceptor blocker propranolol (40 mg), and their combination were studied in five healthy subjects.
- 2 Platelet aggregation and ATP release induced by adrenaline and ADP and ADP induced platelet thromboxane A_2 generation were significantly inhibited ($P < 0.05$) by either diltiazem or propranolol, although propranolol tended to have greater inhibitory effects on platelet function than diltiazem that did not reach statistical significance.
- 3 Combination therapy resulted in additive antiplatelet effects that were significantly ($P < 0.05$) greater than either drug alone.
- 4 These data indicate that combined administration of a calcium entry blocker and a β -adrenoceptor blocker results in additive inhibitory effects on platelet function. These effects may mediate part of the therapeutic efficacy of combination therapy in patients with coronary artery disease.

Keywords platelets diltiazem propranolol thromboxane A_2

Introduction

Calcium entry blockers and β -adrenoceptor blockers are frequently used in the management of coronary artery disease. Substantial evidence suggests that platelets mediate an important role in the pathogenesis of myocardial ischaemia (Mehta, 1983; Fuster *et al.*, 1985). Platelet activation may lead to formation of platelet plugs that impede coronary flow as well as cause thromboxane A_2 release. Thromboxane A_2 induces vasoconstriction and further promotes platelet activation.

Propranolol, a frequently used β -adrenoceptor blocker, has been shown to have antiplatelet effects (Weksler *et al.*, 1977; Vlachakis & Aledort, 1980; Campbell *et al.*, 1981) which may mediate part of its beneficial effect in patients with coronary artery disease. In patients with angina, propranolol inhibits platelet aggregation

at rest and during exercise (Frishman *et al.*, 1974) and during tachycardia stress (Mehta *et al.*, 1978). Propranolol also inhibits platelet thromboxane A_2 production in patients with coronary artery disease (Mehta & Mehta, 1982).

Calcium entry blockers inhibit platelet aggregation *in vitro*, but at concentrations several orders of magnitude higher than those measured in patients during clinical use (Addonizio *et al.*, 1982; Kiyomoto *et al.*, 1983; Han *et al.*, 1983). Interestingly, inhibition of platelet aggregation following oral doses of these agents has been reported (Dale *et al.*, 1983; Cremer *et al.*, 1984; Jones *et al.*, 1985; Ring *et al.*, 1986).

The antiplatelet effects of combined oral administration of a calcium entry blocker and a β -adrenoceptor blocker have not been previously reported. Combination therapy is fre-

quently employed in the management of patients with coronary artery disease whose symptoms do not respond to treatment with a single agent. Several studies have documented improved therapeutic efficacy with combined use of calcium entry and β -adrenoceptor blockers that was superior to either agent alone (Ekelund & Oro, 1979; Leon *et al.*, 1981; Subrahanian *et al.*, 1982; Kenny *et al.*, 1984). It is generally believed that the improved efficacy of combination therapy is primarily mediated by haemodynamic effects (i.e. decreased pressure-rate product). In view of the role of platelets during coronary ischaemia, and the antiplatelet effects of calcium entry and β -adrenoceptor blockers, it is conceivable that combination therapy results in enhanced antiplatelet effects that may account, at least partially, for the improved therapeutic efficacy of combination therapy. In this study, the effects on platelet aggregation, ATP release and thromboxane A_2 generation following oral administration of diltiazem (a calcium entry blocker), propranolol and their combination are reported.

Methods

Protocol

Five healthy male non-smokers (aged 28–32 years) who had not taken any drugs for at least 2 weeks were studied. The control values for each subject were obtained at the beginning of study, thereafter platelet function was assessed in each subject 2 h after each of the following randomized treatments: diltiazem 60 mg p.o.; propranolol 40 mg p.o.; the combination of diltiazem 60 mg p.o. and propranolol 40 mg p.o. There was a minimum 1 week washout period between treatments. All studies were performed in the fasting state at the same time of day for each subject. The study protocol was approved by the Human Subjects Committee of the University of Arizona and informed consent was obtained from each subject.

Sample processing

Blood was collected by atraumatic venepuncture of an antecubital vein using a 19G needle and minimal or no tourniquet pressure. Blood was immediately anticoagulated with 3.8% sodium citrate at a ratio of 9 volume blood: 1 volume citrate. Platelet rich plasma (PRP) was prepared by centrifugation at 180 g for 12 min. Platelet poor plasma (PPP) was obtained by centrifuging the remaining sample at 5,000 g for 15 min. PRP was adjusted to a final platelet concentration of 300,000/mm³ with autologous PPP.

Platelet aggregation studies

Platelet aggregation was performed using a Chrono-log Lumi-Aggregometer (Haverstown, PA) according to the method of Born (1962). All studies were performed 45 to 90 min after sample collection. PRP was preincubated at 37°C and stirred at 1000 rev min⁻¹. Adrenaline and adenosine diphosphate (ADP) (Sigma Chemical Co., St Louis, MO) were used as aggregating agents. The final concentration of adrenaline was 10 μ M. Aggregation to ADP was studied using a threshold concentration of ADP that was determined for each subject during the control study. Threshold concentration was defined as the minimum concentration of aggregant required to induce complete secondary aggregation. Values for platelet aggregation studies are expressed as the maximum percent change in light transmission occurring within 5 min after addition of aggregant (PRP = 0%, PPP = 100%).

ATP release

ATP release was measured in the Lumi-aggregometer by a modification of the Chrono-log luciferin/luciferase technique. Luciferase was added to the PRP sample 5 min after addition of aggregant to avoid potentiation of platelet aggregation (Mehta *et al.*, 1983a). Luminescence was calibrated to the light response produced by the addition of freshly prepared stock ATP at a final concentration of 1.8 μ M.

Thromboxane generation

Platelet thromboxane A_2 generation during aggregation to threshold ADP was assessed by measurement of its stable metabolic product thromboxane B_2 . Indomethacin, at a final concentration of 10^{-4} M, was added to PRP 5 min after the addition of threshold ADP. Samples were immediately frozen and stored at -40° C until assay, which was performed within 6 weeks. Thromboxane B_2 was extracted from the sample with ethylacetate and measured using a commercially available radioimmunoassay kit (Seragen Inc., Boston, MA). Results are expressed as ng thromboxane B_2 /10⁸ platelets.

Statistics

Data are expressed as mean $\pm$ s.e. mean. Differences between groups were examined by the Wilcoxon rank sum test for paired non-parametric data (Mendenhall, 1973). A *P* value < 0.05 was considered significant.

Results

All subjects completed the study protocol without adverse effects. Baseline platelet aggregation to adrenaline and threshold ADP were $69 \pm 16\%$ and $81 \pm 10\%$ respectively. Platelet aggregation to adrenaline and threshold ADP was significantly ($P < 0.05$) inhibited by all treatments. The platelet aggregation tracings from a representative subject following stimulation with threshold ADP is shown in Figure 1. Diltiazem and propranolol reduced ADP induced platelet aggregation to $45 \pm 9\%$ and $28 \pm 3\%$ respectively (Figure 2). Furthermore, platelet aggregation to ADP following combination treatment was only $15 \pm 6\%$ which was significantly ($P < 0.05$) different from single drug effect alone. Adrenaline induced platelet aggregation was not as sensitive to inhibition by these agents compared with threshold ADP induced aggregation. Adrenaline induced aggregation after diltiazem and propranolol was $57 \pm 13\%$ and $52 \pm 16\%$ respectively (Figure 3). Aggregation was $47 \pm 13\%$ following combination treatment.

Threshold ADP and adrenaline induced ATP release roughly paralleled platelet aggregation, although ATP release was more sensitive to

inhibition by all treatments (Figures 2 and 3). ATP release to threshold ADP was $6.6 \pm 1.6 \mu\text{M}$ for control; after diltiazem $2.8 \pm 1.4 \mu\text{M}$; after propranolol $1.2 \pm 0.3 \mu\text{M}$; and after combination therapy $0.6 \pm 0.2 \mu\text{M}$. Adrenaline induced ATP release for control was $5.5 \pm 1.8 \mu\text{M}$; after diltiazem $3.5 \pm 1.1 \mu\text{M}$; after propranolol $2.4 \pm 0.9 \mu\text{M}$; and after combination treatment $1.5 \pm 0.6 \mu\text{M}$.

Platelet thromboxane A_2 generation induced by threshold ADP, as reflected by measurement of its stable metabolite thromboxane B_2 , was significantly ($P < 0.05$) inhibited by all treatment groups (Figure 4). Thromboxane B_2 production for control was $6.6 \pm 1.4 \text{ ng}/10^8$ platelets compared with $3.9 \pm 1.3 \text{ ng}/10^8$ platelets after diltiazem and $1.2 \pm 0.2 \text{ ng}/10^8$ platelets after propranolol. Combination therapy inhibited thromboxane B_2 production further to $0.7 \pm 0.1 \text{ ng}/10^8$ platelets.

Discussion

The results of this study indicate that a single oral dose of either the calcium entry blocker

Figure 1 Tracings of threshold ADP induced platelet aggregation in a representative subject during control and following treatment with diltiazem, propranolol and their combination.

Figure 2 Platelet aggregation, reflected by maximum light transmission, and ATP release after induction with threshold ADP during control and treatment with diltiazem, propranolol and their combination. Each bar represents the mean of five subjects $\pm$ s.e. mean.

Figure 3 Platelet aggregation, reflected by maximum light transmission, and ATP release after induction with adrenaline during control and treatment with diltiazem, propranolol and their combination.

diltiazem or the β -adrenoceptor blocker propranolol inhibit *ex vivo* platelet aggregation and ATP release to ADP and adrenaline in normal subjects. In addition, ADP induced platelet thromboxane A_2 generation was significantly reduced by all treatments. Adrenaline induced platelet aggregation was less sensitive to inhibition by the treatments than ADP induced aggregation. This may be explained by the use of

threshold concentrations of ADP, which is probably the most sensitive concentration for observing drug effects in platelets.

In general propranolol appeared to have more potent antiplatelet effects than diltiazem, however the differences were not statistically significant. We have observed that chronic treatment with diltiazem results in appreciably greater inhibition of platelet function than is observed

Figure 4 ADP induced platelet thromboxane A₂ generation, as reflected by measurement of its stable metabolite thromboxane B₂, during control and following treatment with diltiazem, propranolol and their combination.

after a single dose (Ring *et al.*, 1986), although conceivably a similar effect may occur with chronic propranolol therapy. Treatment with combination treatment consistently resulted in the greatest inhibitory effect in all platelet function parameters measured.

Propranolol has previously been reported to inhibit platelet aggregation in normal subjects (Keber *et al.*, 1979) and in patients with coronary artery disease (Frishman *et al.*, 1974; Mehta *et al.*, 1978). Winther *et al.* (1985) reported that platelets possess β -adrenoceptor receptors of mainly the β_2 subtype. However it appears that the antiplatelet effects of propranolol are mediated by factors other than β -adrenoceptor blockade (Nathan *et al.*, 1977). Kerry *et al.* (1984) showed that the extent of platelet inhibition by various β -adrenoceptor blockers correlated with the degree of lipid solubility, suggesting that platelet inhibition by these agents is mediated by a membrane effect. This is in agreement with Mehta and coworkers (1983b) who found that an intact platelet membrane is essential for the antiplatelet action of propranolol. Therefore, it is important to recognize that not all β -adrenoceptor blockers have similar antiplatelet properties. In the case of propranolol, it appears that its major metabolite 4-hydroxypropranolol

contributes to the total antiplatelet effect (Mehta *et al.*, 1983b).

Based on the results of this and previous studies (Dale *et al.*, 1983; Cremer *et al.*, 1984; Jones *et al.*, 1985; Ring *et al.*, 1986), it is apparent that oral administration of calcium entry blockers causes inhibition of platelet function measured by *ex vivo* techniques. This is somewhat unexpected since supratherapeutic concentrations of calcium entry blockers are required to inhibit platelet function *in vitro* (Addonizio *et al.*, 1982; Kiyomoto *et al.*, 1983; Han *et al.*, 1983). Oral administration of calcium entry blockers may result in increased concentrations of drug in the platelet membrane or intracellularly. Alternatively, metabolites of these agents may possess more potent antiplatelet properties than the original compound. In the case of diltiazem, almost all of its metabolites have greater antiplatelet potencies than diltiazem itself (Kiyomoto *et al.*, 1983). Experimental evidence supporting an important *in vivo* antithrombotic effect of calcium entry blockers is suggested by a study of Pumphrey *et al.* (1983) which demonstrated that the calcium entry blockers nifedipine and verapamil inhibited platelet deposition on canine vascular grafts and were as effective as dipyridamole in this regard.

In an *in vitro* study examining the effects of propranolol and verapamil on platelet function, Han *et al.* (1982) demonstrated synergistic inhibition of platelet aggregation with the combined use of these agents. In the present study, combined use of a calcium entry blocker and a β -adrenoceptor blocker resulted in a partially additive inhibitory effect on platelet function, rather than a synergistic effect. This may have been a result of the doses employed in our study, however our intent was to study this interaction using clinically relevant doses of each drug. In any case, the additive inhibition of platelet function with combined therapy may have important clinical implications and may account for at least part of the improved clinical efficacy when these agents are combined.

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