THE INFLUENCE OF COMBINED TREATMENT WITH PROPRANOLOL AND ACETYLSALICYLIC ACID ON PLATELET AGGREGATION IN CORONARY HEART DISEASE

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1 Antiaggregating agents are frequently prescribed alone or together with β -adrenergic receptor blocking agents in patients after myocardial infarction. The present study was designed to examine the effects of combined treatment with propranolol and acetylsalicylic acid (ASA) on platelet aggregability.

2 Spontaneous platelet aggregation and aggregation induced by fibrinogen and adrenaline were studied in a group of patients with coronary heart disease before treatment, after treatment with propranolol (0.75–1.0 mg/kg daily), and after combined treatment with propranolol in same dose and ASA (20–25 mg/kg daily). Twenty-one patients with stable angina pectoris and/or after myocardial infarction and twelve healthy men were included in the study.

3 There were no significant differences in both spontaneous and induced platelet aggregation between patient and control group before treatment. Propranolol influenced fibrinogen-induced aggregation: it lowered maximal amplitude of aggregation ($P < 0.05$), but it did not change the reaction time. ASA in combination with propranolol affected both spontaneous and induced aggregation: it diminished the number of pathological spontaneous aggregations ($P < 0.025$), it inhibited the second wave of adrenaline-induced aggregation ($P < 0.01$), prolonged the reaction time of fibrinogen-induced aggregation ($P < 0.01$) and lowered the maximal amplitude of aggregation ($P < 0.05$).

4 The study showed that propranolol besides its well-known hemodynamic effects had an additional antiaggregating property. The action of combined treatment with propranolol and ASA on platelet aggregation is probably additive.

Introduction

In recent years great interest has developed in the role of platelets in the pathogenesis of coronary atherosclerosis and in the origin of the complications associated with coronary heart disease. Platelets initiate thrombosis by aggregating at the site of previous vascular injury and it is speculated that altered platelet aggregability may play a significant role in the development and progression of atherosclerotic lesions (Mustard, 1972; Deykin, 1974).

The formation of platelet aggregates within coronary arteries at points of previous narrowing, or the formation elsewhere and travelling to the points of narrowing in the coronary tree, may be the final event in the origin of a myocardial infarction (Haft, 1976).

Besides thrombosis in the larger coronary vessels more and more attention has been paid to the role of platelet aggregates in the coronary microvasculature, which have been observed in animal models following catecholamine infusion (Haft, Kranz, Albert & Fani, 1972), after acute, severe stress (Haft & Fani, 1973), and also in men after sudden coronary death

(Jørgensen, Haerem, Chandler & Borchgrevink, 1968; Haerem, 1971). By mechanical obstruction of the coronary microcirculation platelet aggregates cause electrical instability of heart and may lead to a fatal ventricular arrhythmia or disturb the collateral blood flow to the ischaemic area, so widening the zone of necrosis.

Therapy directed at lowering platelet aggregability could decrease the incidence of acute thrombotic events in patients with coronary artery disease. Among antiaggregating agents acetylsalicylic acid (ASA) is frequently prescribed after myocardial infarction.

Another promising drug in coronary heart disease is propranolol, a β -adrenoceptor blocking agent with known antianginal effects, but inadequately defined effects on platelet function. β -adrenoceptor blocking agents have been shown to reduce mortality after myocardial infarction (Multicentre International Study, 1975; Vedin, Wilhelmsson & Werkö, 1975; Green, 1977). ASA and propranolol have been

increasingly used together in patients after myocardial infarction, but in spite of this there has been no report of their combined effect on platelet function.

The present study was designed to examine:

1. what are the effects of propranolol treatment on platelet aggregability,
2. what are the effects of combined treatment with propranolol and ASA on platelet aggregability.

Methods

Population

Twenty-one male patients with coronary heart disease aged between 41 and 65 years (mean 52 years) were included in our study. Twelve of them suffered myocardial infarction in the period from 2 to 24 months (mean 11.6 months) before the beginning of the study. The other nine patients had stable angina pectoris with typical history and conclusive ECG changes on exercise stress testing. Twelve male subjects of the same age group with no symptoms of coronary heart disease and negative submaximal exercise stress testing were studied along with the patient group.

Protocol

Platelet aggregation studies in the patient group were done three times: (1) after a 2-week period without medication which might influence platelet aggregation, (2) after 14 days of treatment with propranolol in daily dose of 60 mg orally (0.75–1.0 mg/kg) and (3) after 14 days of combined treatment with propranolol in the same dose and ASA in daily dose of 1.5 g orally (20–25 mg/kg). No other drug treatment, except nitroglycerin if needed, was given. Platelet aggregation of control group receiving no drug therapy was examined on two occasions fourteen days apart.

Platelet aggregation studies

Blood was obtained after a 12-h fast. Blood samples were taken directly from antecubital vein into a plastic tube containing 0.13 M sodium citrate (1 volume of citrate to 9 volumes of blood for induced aggregation and 1.5 volume of citrate to 10 volumes of blood for spontaneous aggregation). The whole blood was centrifuged for 15 min at 750 rev/min at room temperature to obtain platelet rich plasma (PRP) and for 10 min at 2000 rev/min to obtain platelet poor plasma (PPP). Plasma was examined no later than 4 h after its withdrawal. Before testing, the platelet count was measured in each sample by direct counting (Nygaard, 1933). Spontaneous platelet aggregation was measured by method of Breddin & Banke (1965).

An aliquot of 1.5 ml PRP was rotated in a siliconized glass flask at 20 rev/min for 10 min in 37°C water bath. The rotated plasma (0.5 ml) was diluted with 3.5 ml saline-citrate solution (9 volumes of 0.85% NaCl to 1 volume of 3.8% trisodium citrate), poured on a siliconized glass slide and allowed to stand for 30 min. The sample was then fixated with 40% formaldehyde for 5 min, oxidated with 0.1 N potassium permanganate solution and stained with Giemsa staining solution for 60 min. Evaluation under the microscope was done using 100× magnification. Unrotated PRP (0.5 ml) treated identically served as a control. In this test, reversible platelet aggregation means the sticking of platelets to each other without fusion, with their membranes intact. In irreversible aggregation, platelets are fused and membranes are partly destroyed. The platelet aggregation is graded according to the following characteristics: Grade 1: Platelets are seen single with only a few reversible aggregates and no irreversible aggregates. Grade 2: More reversible aggregates are found, but there are no or only very few irreversible aggregates; there is no obvious reduction in single platelets. Grade 3: An increase in reversible aggregates is evident, often with irreversible aggregation at the centre; the number of platelets is clearly diminished. Grade 4: There are more irreversible than reversible aggregates; single platelets are rare. Grade 5: There is complete irreversible aggregation; single platelets are practically missing. The grades 1 and 2 after rotation were taken as normal, and the grades 3, 4, and 5 as pathological aggregability. Induced platelet aggregation studies were performed using turbidometric method of Born (1962) on a Chronolog aggregometer (Havertown). The platelet count of PRP was adjusted to $250 \times 10^9/l$ by suitable dilution with autologous PPP. Samples of 0.4 ml PRP were prewarmed for 1 min in siliconized cuvettes of the aggregometer, and then aggregating agents were added in volume of 0.1 ml. The aggregating agents used were bovine fibrinogen (Behringwerke, Marburg/Lahn) in a final concentration of 0.1 g/l, and adrenaline (Jugoremedija, Zrenjanin) in final concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 4.0 µg/ml. We measured time (s) of maximal change of optical density (the reaction time), and maximal change of optical density (mm) (the maximal amplitude of aggregation) on the platelet aggregation response to fibrinogen. For adrenaline-induced aggregation we determined the lowest concentration producing a full biphasic response of aggregation.

Statistical analysis

For the reaction time and maximal amplitude of fibrinogen-induced aggregation, arithmetic group means with the s.d. of the mean are calculated. For adrenaline concentration data, geometric means are

presented. The data for spontaneous aggregation were analysed by χ^2 test. Because the frequency distribution of induced aggregation values was not normal (Goldenfarb, Cathey & Cooper, 1970), we used the Wilcoxon matched pairs signed rank test for paired values and the Mann Whitney U test for unpaired values (Snedecor & Cochran, 1972).

Table 1 The frequency of normal and pathological spontaneous platelet aggregation in control and patient group

Group	Normal aggregation	Pathological aggregation
Controls:		
occasion 1	8	4
occasion 2	8	2
Patients:		
before therapy	8	13
propranolol	8	12
propranolol + ASA*	14	5

Testing of between group differences by two-tailed χ^2 .

* Significantly lower frequency of pathological aggregations than before therapy and propranolol alone. $P < 0.025$.

Results

Testing of spontaneous and induced platelet aggregation in the control group between two measurements 2 weeks apart did not show a significant difference between the results (Tables 1 and 2). There were no significant differences in spontaneous and induced aggregation between patient and control group before treatment (Tables 1 and 2).

After treatment with propranolol alone the decrease of platelet aggregability was observed in fibrinogen-induced aggregation, in which the maximal amplitude of aggregation was lowered ($P < 0.05$), the reaction time remaining the same (Table 2, Figure 1). There were no changes in other tests.

After combined treatment with propranolol and ASA the lowering of platelet aggregation was demonstrated in all tests used: there was a decrease in number of pathological spontaneous aggregations ($P < 0.025$) (Table 1), in fibrinogen-induced aggregation the reaction time was prolonged ($P < 0.01$), and the maximal amplitude was lowered ($P < 0.05$) (Table 2, Figure 1). In adrenaline-induced aggregation the concentration of adrenaline required to produce the biphasic response was increased ($P < 0.01$) (Table 2), while at equal concentrations the second aggregation wave did not appear (Figure 2).

Comparing the results after treatment with propranolol alone and after treatment with both agents we found that ASA did not further lower the maximal amplitude of fibrinogen-induced aggregation.

Table 2 Values of fibrinogen and adrenaline-induced platelet aggregation in control and patient group

Group	Fibrinogen*		Adrenalin† concentration (µg/ml)
	reaction time (s)	maximal amplitude (mm)	
Controls:			
occasion 1 (n = 12)	320 ± 188	76.8 ± 45.8	0.53
occasion 2 (n = 10)	332 ± 193	72.8 ± 48.5	0.38
Patients:			
before therapy (n = 21)	292 ± 102	82.2 ± 26.2	0.36
propranolol (n = 20)	341 ± 143	69.1 ± 33.7§	0.53
propranolol + ASA (n = 19)	419 ± 138‡	64.1 ± 40.0§	2.77

Testing of between-group differences by the Wilcoxon matched pairs signed rank test for paired values and the Mann Whitney U test for unpaired values.

* Means ± s.d.

† Geometric means.

‡ Significantly longer than before therapy and after propranolol alone, $P < 0.01$.

§ Significantly lower than before therapy, $P < 0.05$.

|| Significantly higher than before therapy and after propranolol alone, $P < 0.01$.

Figure 1 Fibrinogen-induced platelet aggregation response from a representative patient with coronary artery disease before treatment (I), after treatment with propranolol (II), and after combined treatment with propranolol and ASA (III). Arrow indicates the addition of fibrinogen to PRP. Propranolol lowered the maximal amplitude of aggregation while ASA prolonged the reaction time.

Figure 2 Aggregation response of platelet induced by the same concentration of adrenaline (0.5 $\mu\text{g/ml}$) from a representative patient with coronary artery disease before treatment (I), after treatment with propranolol (II), and after combined treatment with propranolol and ASA (III). Arrow indicates the addition of adrenaline to PRP. Propranolol alone has no effect on aggregation, but after combined treatment with ASA the second wave of aggregation was abolished.

Discussion

Increased platelet aggregation in patients with coronary artery disease has been reported several times (O'Brien, Heywood & Heady, 1966; Hampton & Gorlin, 1972; Yamazaki, Takahashi & Sano, 1975), but not in all studies (Steele, Wely, Davies & Genton, 1973; Levites & Haft, 1975). In the present study we have found no difference in spontaneous platelet aggregation and aggregation induced by adrenaline and fibrinogen in patients with coronary artery disease when compared to normal subjects. The reason for these conflicting results may be related to patient selection and to different methods used in the study of platelet aggregability.

In spite of almost complete lack of knowledge of the role of hyperaggregability in the course and prognosis of coronary heart disease, some authors consider it as one of the secondary risk factors, and institute treatment in clinically overt stages of this disease. One of the most potent antiaggregating agents is ASA. It inhibits the release reaction and prevents irreversible aggregation of platelets through inhibition of an enzyme cyclo-oxygenase, which catalyses the conversion of arachidonic acid to labile cyclic endoperoxide forms of prostaglandins (Smith & Willis, 1971; Hamberg, Svensson, Wakabayashi & Samuelson, 1975). Unlike the classical prostaglandins, the cyclic endoperoxides, and especially thromboxane A_2 , derived from the cyclic endoperoxides, are potent inducers of platelet aggregation.

In spite of widespread use of propranolol in many clinical settings ranging from coronary heart disease to hypertension, its effects on platelet function have been studied rarely, and results have been controversial. In *in vitro* studies some authors have demonstrated decreased aggregability (Thomas, 1967), while others have speculated that β -adrenergic receptor blockade can even increase platelet aggregability (Abdulla, 1969; Sacchetti, Bellani, Montanari & Gibelli, 1973). Weksler, Gillick & Pink (1977) have demonstrated that propranolol decreased platelet aggregation by a direct action on platelet membranes and not through β -adrenergic receptor blockade. In an animal model Haft, Fani, Alcorta & Toot (1973) have demonstrated that propranolol prevents isoprenaline induced intravascular platelet aggregation in rats. Frishman, Weksler, Christodoulou, Smithen & Killip (1974) have found that propranolol abolishes platelet hyperresponsiveness to ADP in patients with angina pectoris.

Our results are consistent with the observations that propranolol decreases platelet aggregation, although not to such a degree as ASA. It must be mentioned that doses of propranolol used in our study might be relatively small regarding its influence on platelet aggregation.

The effective action of ASA, reflected in the majority of *in vitro* tests used in our study, was expected. Observation that ASA combined with

propranolol does not decrease the maximal amplitude of aggregation curve more than propranolol, suggests that ASA does not influence this parameter at all. The possibility that it aims at the same receptor site already maximally affected by propranolol, is less probable. It follows that each substance acts at different sites in reducing platelet aggregability and that the combined effect is probably additive. It can be speculated that ASA affects the reaction time of fibrinogen-induced aggregation because of inhibition of platelet release reaction, while propranolol lowers the maximal amplitude of the same aggregation curve

by its membrane stabilizing action that could diminish the extent of aggregation.

We could not find any significant differences in platelet aggregation between two measurements 2 weeks apart in the control group. This shows that temporal variations of platelet aggregability are not large enough to explain the observed differences of platelet reactivity between groups and within patients' group after different treatment.

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