Br. J. clin. Pharmac. (1983), **16**, 663–668

INCREASED PLATELET ADHESION AND AGGREGATION IN HYPERTENSIVE PATIENTS: EFFECT OF ATENOLOL

A. MARKEL, J.G. BROOK, Y. LEVY, M. AVIRAM & M.B.H. YOUDIM[1]

Department of Internal Medicine B and Lipid Research Unit, Rambam Medical Centre, Haifa, and [1]Department of Pharmacology, Faculty of Medicine and Rappaport Family Medical Research Centre, Technion-Israel Institute of Technology, P.O.B. 9649, Haifa 31096, Israel

1 Fourteen patients with established hypertension followed a double-blind crossover-styled trial to study the effects of 100 mg/day atenolol compared to placebo. Atenolol was found to be an effective antihypertensive agent, reducing both systolic and diastolic blood pressure.

2 Hypertensive patients appear to have increased *in vitro* platelet adhesion and aggregation. Atenolol significantly reduced platelet adhesion, but had little effect on aggregation. This may be important in contributing towards the now-recognised cardio-protective effect of the β-adrenoceptor blocking agents.

3 Blood chemistry and haematological parameters were unchanged; but whereas plasma cholesterol and plasma triglyceride levels remained normal, there was a significant fall in plasma high-density lipoprotein cholesterol levels. Side effects were very few.

Keywords atenolol hypertension platelet aggregation

Introduction

Over the last number of years there has been substantial progress in the medical treatment of hypertension. Among the medications, drugs with properties of anti-β-adrenoceptor activity have become the first-line choice in the majority of patients with hypertension (Laragh, 1976; Conway, 1977).

The original β-adrenoceptor blocking drugs (propranolol and others) were not cardio-selective; that is, their blocking effect applied to both the β_1 and β_2 receptors. Much attention has been given to the development of a specific β_1-adrenoceptor blocker. Such a drug is atenolol, which does not have the serious side effects that were attributed to the initial selective β-adrenoceptor blocker practolol (Simpson, 1977; Zacharias, 1977).

Our study was undertaken to confirm the effect of atenolol compared to placebo in patients with essential hypertension. Of particular interest was the effect of the drug on platelet function since any antihypertensive drug possessing also 'anti-platelet' properties would be advantageous. Previous studies with β-adrenoceptor blocking drugs have yielded contradictory results in terms of the effects on platelet function (Frishman *et al.*, 1976; Keber *et al.*, 1979; Leon *et al.*, 1978; Vlachakis & Aledort, 1980). To the best of our knowledge the effect of atenolol on platelet functions has not been reported.

Methods

Fourteen male patients (age range 42–58 years) with essential hypertension were given either atenolol, 100 mg daily, or placebo, in a double-blind fashion. All patients had a systolic blood pressure of 160 mm Hg and above and a diastolic pressure of 95 mm Hg and above on three successive examinations prior to the commencement of the study. All medications were terminated at least 2 weeks before onset of the trial. Half of the group of patients started with the drug and half with the placebo. After 1 month a cross-over took place, whereby those on placebo received the specific drug and *vice versa*. The drug or placebo was administered at 08.00 h each day.

Measurements of blood pressure and pulse rate were undertaken prior to the commencement of the study, after 1 month, and at the end of the study, after 2 months. The blood pressure and pulse were measured at between 07.00 h and 08.00 h, between 12.00 h and 13.00 h and between 16.00 h and 17.00 h. The determination was made with the patient lying, sitting and after exercise which comprised climbing up and down two stairs at a maximum speed over 2 min. The blood pressure was measured on both upper arms by the same examiner, using a standard sphygmomanometer. Of the two measurements, the higher was taken as representative of the blood pressure.

Before commencement of therapy both platelet

adhesion and platelet aggregation were determined in the hypertensive patients, and compared with platelet function in a group of age-matched normotensive controls. No differences existed between the hypertensive patients and controls in terms of smoking, alcohol consumption, degree of physical activity and lipid levels.

On the same morning as the blood pressure measurement, *in vitro* platelet adhesion and platelet aggregation was determined. For purposes of platelet adhesion, whole blood was drawn into 3.8% sodium citrate (v/v = 9/1) and then pumped through an inert silicon tube and applied to a glass slide in the form of a stagnation point flow field (Berkowitz *et al.*, 1981; Viener *et al.*, 1981). The number of platelets adhering to the slide/unit area was determined and represented the degree of platelet adhesion.

Platelet-rich plasma was prepared from citrated blood by low-speed centrifugation (200 *g*, 10 min, room temperature). Platelet aggregation was determined according to the method of Born (1962) using a Chronalog aggregometer. The aggregating agents were adrenaline (10 μM), ADP (10 μM), collagen (1μg/ml) and 5-hydroxytryptamine (10 μM). The extent of aggregation was determined as the area under the aggregation curve. This was weighed and expressed in milligrams. Blood was also taken for determination of cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C), fibrinogen, bilirubin, transminase, alkaline phosphatase, urea, sugar, creatinine, uric acid and electrolytes. A full blood count and electrocardiogram were performed. All biochemical determinations were by conventional methods. HDL-C was determined by the heparin manganous chloride precipitation method (Burstein *et al.*, 1970). Spirometry was performed on all patients before commencement of treatment and subsequent to atenolol or placebo administration. Values for forced vital capacity (FVC) and forced expiratory volume during the first second of expiration (FEV_1) were determined.

On each visit all patients were questioned regarding the appearance of side effects.

Statistics

Student's paired *t*-test and the Wilcoxon rank test were employed in each group.

Results

Blood pressure and pulse rate determinations

There was a significant fall in systolic blood pressure after commencement of atenolol treatment. This was apparent on comparison with values before the treatment commenced ($P < 0.01$) as well as on comparison with values obtained while the patients were on placebo ($P < 0.01$) (Table 1).

Similar results as the above were also obtained on the diastolic blood pressure measurements (Table 1). The average diastolic pressure before atenolol was 105.8 mm Hg, and after treatment 96.3 mm Hg ($P < 0.0.1$). After placebo treatment, no differences whatsoever were apparent.

The pulse rate dropped from an average of 93 beats/min before treatment to 77 beats/min during treatment ($P < 0.001$). No change was noted with the patients on placebo.

The changes in the above three parameters were the same for each position of the patient, lying, sitting or after exercise. They were also completely independent of the time of day.

The long-acting effect of the atenolol was also demonstrated by the fact that the blood pressure and pulse measurements at 08.00 h, i.e. 24 h subsequent to taking the tablets, were significantly lower in the atenolol group compared with the placebo group.

Platelet function

The hypertensive patients revealed both increased platelet adhesion and increased platelet aggregation in comparison with normotensive controls (Table 2). The effect of atenolol is demonstrated in Table 3. There was a reduction in platelet adhesion subse-

Table 1 Systolic and diastolic blood pressure levels before and after atenolol and placebo (means ± s.d.). (Comparisons were made between the values obtained before treatment with those obtained after atenolol and after placebo administration).

		Before treatment		*After atenolol*		*After placebo*	
		Systolic	*Diastolic*	*Systolic*	*Diastolic*	*Systolic*	*Diastolic*
Morning	Lying	159 ± 16	108 ± 6	143 ± 19*	94 ± 10*	152 ± 19	104 ± 9
	Standing	156 ± 19	109 ± 9	141 ± 19*	99 ± 13*	147 ± 17	106 ± 14
	Exercise	204 ± 30	101 ± 10	181 ± 17*	95 ± 14*	184 ± 24	102 ± 13

*$P = (< 0.01$ to $< 0.05)$

N.B. Similar results were obtained at both the afternoon and evening determinations.

Table 2 Platelet function in normal and hypertensive subjects.

	Normals	*Hypertensives*
Platelet adhesion (platelets/2500 M^2)	8.2 ± 3.9	10.6 ± 3.5 ($P < 0.01$)
ADP-induced platelet aggregation (area weight in mg)	2.38 ± 0.88	3.35 ± 0.61 ($P < 0.01$)

quent to treatment, but atenolol evoked little effect on platelet aggregation. The placebo had no effect on either platelet adhesion or platelet aggregation. No further changes in these parameters were noted.

Lipid levels and other laboratory investigations

Plasma cholesterol and triglyceride levels did not change following either atenolol therapy or placebo administration. However, a significant drop in HDL-C levels occurred following the drug which was not apparent after the period on the placebo (Table 4).

The serum levels of glucose, creatinine, uric acid, bilirubin, transaminase, alkaline phosphatase and fibrinogen were unaltered after therapy with atenolol. Similarly, no effect on the blood count was observed.

Pulmonary function tests

Various spirometric parameters were evaluated, and in no case did atenolol result in impairment of respiratory function (Table 5).

Side effects

No serious side effects were demonstrated by any of the patients receiving atenolol. A few complained of weakness, drowsiness and headache. Most of the patients reported feeling better and were more relaxed subsequent to taking the drug.

Discussion

Atenolol has already been established as an effective anti-hypertensive agent. We have confirmed this finding. The effectiveness of the single-dose administration of atenolol has been demonstrated by others (Knapp, 1979; Sleight, 1979). Atenolol also has an effect on exercise-induced tachycardia.

Platelets appear to play an important role in the pathogenesis of atherosclerosis (Mustard & Packham, 1975); and in conditions such as hyperlipidaemia (Aviram & Brook, 1982), diabetes (Kwaan *et al.*, 1972) and chronic renal failure (Viener *et al.*, 1982), in which accelerated atherosclerosis is a feature, enhanced platelet activity has been described. Hypertension is another important risk factor for atherosclerosis. Platelet function in hypertensive individuals has rarely been studied. We report here that our hypertensive patients appear to have increased *in vitro* platelet activation, as evidenced by increased adhesion and increased aggregation in response to ADP. The effect of β-adrenoceptor blockers on platelet aggregation has been determined by others. In most instances propranolol was the β-adrenoceptor blocker tested (Nathan *et al.*, 1977; Frishman *et al.*, 1976, 1978; Vlachakis & Aledort, 1980; Weksler *et al.*, 1977; Leon *et al.*, 1978; Keber *et al.*, 1979), but pindolol (Nathan *et al.*, 1977) and more recently timolol (Thaulow *et al.*, 1981) and carteolol (Small *et al.*, 1982) have been examined. The *in vitro* addition of the β-adrenoceptor blocker invariably resulted in inhibition of platelet aggregation (Nathan *et al.*, 1977; Weksler *et al.*, 1977; Thaulow *et al.*, 1981). However, contradictory results were reported in patients taking β-adrenoceptor blockers. Propranolol induced decreased *in vitro* platelet aggregation in patients with angina pectoris (Frishman *et al.*, 1976, 1978) and hypertension (Vlachakis & Aledort, 1980) in whom a hyperaggregability state had been diagnosed before the onset of β-adrenoceptor blocker therapy. In contrast, propranolol failed to decrease *in vitro* platelet aggregation in either ischaemic heart disease patients (Keber *et al.*, 1970) or healthy volunteers (Leon *et al.*, 1978) who did not demonstrate any underlying hyper-

Table 3 The effect of atenolol treatment on platelet function in hypertensive patients.

	Platelet adhesion (platelet/2500 m^2)	*Platelet aggregation (area in mg)*			
		ADP	*Adrenaline*	*Collagen*	*5-HT*
Before treatment	9.4 ± 3.2	3.85 ± 0.48	0.76 ± 0.70	1.39 ± 1.20	0.31 ± 0.11
After treatment	7.9 ± 3.8 ($P < 0.02$)	3.67 ± 1.31 NS	0.79 ± 0.88 NS	1.71 ± 1.62 NS	0.34 ± 0.26 NS

NS not significant.

Table 4 Plasma cholesterol, triglycerides and high-density lipoprotein cholesterol levels after atenolol and placebo administration (means ± s.d.).

	Before treatment	*After atenolol*	*After placebo*
Total cholesterol (mg/100 ml)	256 ± 36	242 ± 43	244 ± 39
Total triglycerides (mg/100 ml)	187 ± 82	179 ± 65	163 ± 60
HDL-cholesterol (mg/100 ml)	44 ± 13	35 ± 9*	45 ± 13

*$P < 0.01$. HDL is high-density lipoprotein.

aggregability. In patients taking timolol on a long term basis there was no effect on platelet aggregation (Thaulow *et al.*, 1981).

To the best of our knowledge the effect of atenolol on platelet function has not been reported. Certainly the modality of platelet adhesion has not been investigated.

Interestingly, in our patients atenolol significantly reduced platelet adhesion, but there was little effect on platelet aggregation as measured *in vitro*.

Most workers consider the anti-aggregatory properties of the β-adrenoceptor blockers to be related to the membrane stabilizing activity (MSA) of the drug (Nathan *et al.*, 1977; Weksler *et al.*, 1977; Keber *et al.*, 1979) Propanolol which possesses MSA affects platelet aggregation whereas practolol which lacks MSA has no effect on platelet function even when administered at doses ten times greater than that of propranolol (Weksler *et al.*, 1977). Atenolol also devoid of MSA (Barrett, 1977) had no effect on platelet aggregation.

The differential effect of the drug on adhesion but not aggregation is unusual. Its significance is open to speculation, but further investigation with larger groups of patients is indicated before firm conclusions can be reached. Our finding adds a further dimension to the therapeutic application and importance of atenolol. It also indicates a possible mechanism for the cardio-protective effect of the β-adrenoceptor blocking drugs.

The effects of β-adrenoceptor blocking drugs on plasma triglyceride levels appear somewhat confusing. Some workers have described small increases in triglyceride levels in short-term experiments (6 months or less) (Day *et al.*, 1979; Leren *et al.*, 1980). Others have reported no change or, indeed, a small reduction, particularly in studies of a longer duration (Berglund *et al.*, 1976; Tanaka *et al.*, 1976; Berglund & Andersson, 1981). It was suggested that the cardio-selective and non-selective β-adrenoceptor blocking agents may behave differently in their effect on the lipids; but in a recent double-blind, randomized cross-over study on 53 patients by Day *et al.*, (1982), plasma triglycerides increased during 3 months' treatment with propranolol, atenolol, metoprolol and oxprenolol. Both our short-term and long-term results show that atenolol has little effect on plasma triglyceride levels.

It is generally agreed that β-adrenoceptor blocking agents do not affect plasma cholesterol levels; however, HDL-cholesterol concentrations are usually significantly reduced in patients on propranolol (Tanaka *et al.*, 1976; Helgeland, 1978; Streja, 1978). We have confirmed both these observations in this study with atenolol. Day *et al* (1982) described a fall in HDL-cholesterol levels with all four β-adrenoceptor blocking agents tested. The importance of this observation lies in the now well-known inverse correlation between HDL-C levels and prevalence of coronary artery disease (Miller, 1975; Rapoport *et al.*, 1978; Brook *et al.*, 1982). However, there is much evidence in the recent literature favouring a reduction in mortality and morbidity with long-term β-adrenoceptor blockers over an increase as might have been expected from the change in HDL-C (Lambert, 1976; Berglund *et al.*, 1978; Trafford *et al.*, 1981). A small drop in the HDL-C levels may well be an irrelevant price to pay for the effective control of hypertension in any one patient. It would appear, then, that on the basis of morbidity and mortality data during treatment with β-adrenoceptor blockers, the changes observed in HDL-C concentrations are of little consequence.

Table 5 FVC and FEV_1 values before and after atenolol and placebo administration.

	Before treatment	*After atenolol*	*After placebo*
FEV_1 %	76.3 ± 8.4	75.7 ± 6.8	74 ± 11
FVC %	98.1 ± 9.9	94.2 ± 14.3	96.1 ± 17.1

FVC is forced vital capacity; FEV_1 is forced expiratory volume during the first second of expiration.

References

AVIRAM, M. & BROOK, J.G. (1982). The effect of human plasma on platelet function in familial hypercholesterolaemia. *Thromb. Res.*, **26**, 101–109.

BARRETT, A.M. (1977). The pharmacology of atenolol. *Postgrad. med. J.*, **53**, (Suppl. 3), 58–64.

BERGLUND, G. & ANDERSSON, O. (1981). Beta-blockers or diuretics in hypertension? A six-year follow-up of blood pressure and metabolic side effects. *Lancet*, **i**, 744–747.

BERGLUND, G., ANDERSSON, O., LARSSON, O. & WILHELMSEN, L. (1976). Antihypertensive effect and side effect of bendroflumethiazide and propranolol. *Acta med. Scand.*, **199**, 499–506.

BERGLUND, G., WILHELMSEN, L., SANNERSTEDT, R., HANSSON, L., ANDERSSON, O., SIVERTSSON, R., WEDEL, H. & WIKSTRAND, J. (1978). Coronary heart-disease after treatment of hypertension. *Lancet*, **i**, 1–5.

BERKOWITZ, Y., MARMUR, A., AVIRAM, M. & BROOK, J.G. (1981). Determination of platelet adhesion on samples of whole blood. *Isr. J. med. Sci.*, **17**, 1208.

BORN, G.V.R. (1962). Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature*, **194**, 927–929.

BROOK, J.G., AVIRAM, M., VIENER, A., SHILANSKY, E. & MARKEIVITCH, W. (1982). High density lipoprotein subfraction in normolipidaemic patients with coronary atherosclerosis. *Circulation*, **66**, 923–926.

BURSTEIN, M., SCHOLNICK, H.R. & MORFIN, R. (1970). Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J. lipid Res.*, **11**, 538–594.

CONWAY, J. (1977). Problems in the management of hypertension. *Postgrad. med. J.*, **53** (Suppl. 3), 176–178.

DAY, J.L., METCALFE, J. & SIMPSON, C.N. (1982). Adrenergic mechanisms in control of plasma lipid concentrations. *Br. med. J.*, **284**, 1145–1148.

DAY, J.L., SIMPSON, N., METCALFE, J. & PAGE, R.L. (1979). Metabolic consequences of atenolol and propranolol in treatment of essential hypertension. *Br. med. J.*, **278**, 77–80.

FRISCHMAN, W.H., WEKSLER, B., CHRISTODOULOU, J.P., SMITHEN, CH. & KILLIP, T. (1976). Reversal of abnormal platelet aggregability and change in exercise tolerance in patients with angina pectoris following oral propranolol. *Circulation*, **50**, 887–896.

FRISCHMAN, W.H., CHRISTODOULOU, J., WEKSLER, B., SMITHEN, CH., KILLIP, T. & SCHEIDT, S. (1978). Abrupt propranolol withdrawal in angina pectoris: effects on platelet aggregation and exercise tolerance. *Am. Heart J.*, **95**, 169–179.

HELGELAND. A. (1978). High-density lipoprotein cholesterol and anti-hypertensive drugs: The Oslo study. *Br. med. J.*, **277**, 403.

KEBER, I., JERSE, M., KEBER, D. & STEGNAT, M. (1979). The influence of combined treatment with propranolol and acetysalicylic acid on platelet aggregation in coronary heart disease. *Br. J. clin. Pharmac.*, **7**, 287–291.

KNAPP, M.S. (1979). Effect of once-daily atenolol on ambulatory blood pressure. *Br. med. J.*, **278**, 490.

KWAAN, H.C., COLWEL, J.A. & CRUZ, S. (1972). Increased platelet aggregation in diabetes mellitus. *J. lab. clin. Med.*, **80**, 236–246.

LAMBERT, D.M.D. (1976). Effect of propranolol on mortality in patients with angina. *Postgrad. med. J.*, **52** (Suppl. 4), 57–60.

LARAGH, J.H. (1976). Modern system for treating high blood pressure based on renal profiling and vasoconstriction-volume analysis: A primary role for β blocking such as propranolol. *Am. J. Med.*, **61**, 797.

LEON, R., TIARKS, CH. Y. & PECHET, L. (1978). Some observations on the *in vivo* effect of propranolol on platelet aggregation and release. *Am. J. Hem.*, **5**, 117–121.

LEREN, P., FOSS, P.O., HELGELAND, A., HJERMANN, I., HOLME, I. & LUND-LARSEN, P.G. (1980). Effect of propranolol and prazosin on blood lipids: The Oslo study. *Lancet*, **ii**, 4–6.

MILLER, G.J. (1975). Plasma high-density lipoprotein concentration and development of ischaemic heart disease. *Lancet*, **i**, 16–19.

MUSTARD, J.F. & PACKHAM, M.A. (1975). The role of blood and platelets in artherosclerosis and the complications of atherosclerosis. *Thromb. Diath. Haemorrh.*, **33**, 444.

NATHAN, I., DVILANSKY, A., SAGE, J. & KORCZYN, A.D. (1977). Effect of propranolol and pindolol on platelet aggregation and serotonin release. *Life Sci.*, **20**, 407–412.

RAPOPORT, J., AVIRAM, M., CHAIMOVITZ, C. & BROOK, J.G. (1978). Defective high-density lipoprotein composition in patients on chronic haemodialysis. *New Engl. J. Med.*, **299**, 1326–1329.

SIMPSON, E.Y. (1977). Nature and incidence of unwanted effects with atenolol. *Postgrad. med. J.*, **53** (Suppl. 3), 166–167.

SLEIGHT, P. (1979). Effect of once-daily atenolol on ambulatory blood pressure. *Br. med. J.*, **278**, 491.

SMALL, M., DOUGLAS, J.T., AHERNE, G.W., ORR, M., LOWE, G.O., FORTES, C.D. & PRENTICE, C.R. (1982). Effects of the new selective beta-adrenoreceptor blocking agent carteolol on platelet function, blood coagulation and viscosity. *Thromb. Res.*, **25** (Suppl. 4), 351–360.

STREJA, D. (1978). Effect of propranolol on HDL-cholesterol concentration (letter). *Br. med. J.*, **277**, 1495.

TANAKA, H., SAKAGUCHI, S., OSHIGE, K., NIIMURA, T. & KANEHISA, T. (1976). Effect of chronic administration of propranolol on lipoprotein composition. *Metabolism*, **25**, 1071–1075.

THAULOW, E., KJEKSHUS, J. & ERIKSSEN, J. (1981). Effect of timolol on platelet aggregation in coronary heart disease. *Acta med. Scand.*, Suppl. 651, 101–109.

TRAFFORD, J.A.P., HORN, C.R., O'NEAL, H., McGONIGLE, R., HALFORD-HAW, L. & EVANS, R. (1981). Five-year follow-up of effects of treatment of mild and moderate hypertension. *Br. med. J.*, **282**, 1111–1113.

VIENER, A., AVIRAM, M., LEVIN, M., BETTER, O. & BROOK, J.G. (1982). Atherosclerotic risk factors in chronic uraemic patients undergoing maintenance haemodialysis. *Isr. J. med. Sci.*, **18**, 559.

VIENER, A., BERKOWITZ, Y., BRONSTEIN, E., MARMUR, A., AVIRAM, M. & BROOK, J.G. (1981). Platelet adhesion: A description of two new methods. *Isr. J. med. Sci.*, **17**, 396–397.

VLACHAKIS, N.D. & ALEDORT, L. (1980). Hypertension and propranolol therapy: effect on blood pressure,

plasma catecholamines and platelet aggregation. *Am. J. Cardiol.*, **45**, 321–325.

WEKSLER, B.B., GILLICK, M. & PINK, J. (1977). Effect on propranolol on platelet function. *Blood*, **49**, 185–196.

ZACHARIAS, F.J. (1977). Atenolol in hypertension: A study of long-term therapy. *Postgrad. med. J.*, **53** (Suppl. 3), 102–110.

(*Received January 17, 1983, accepted July 18, 1983*)