Platelet sensitivity in vitro to adenosine-5'-diphosphate and prostacyclin and diabetic retinopathy

T. M. E. Davis, E. Bown and R. C. Turner

Diabetes Research Laboratories, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford, UK

Summary. Platelet sensitivity to adenosine diphosphate and prostacyclin in diabetes has been assessed using collision theory and the concept of 'sticking probability' (the probability of particle union). Twenty Type 1 (insulin-dependent) diabetic men (10 with no or minimal retinopathy and a matched group of 10 with proliferative retinopathy) and 10 age-matched non-diabetic men were studied. Platelets from the 20 diabetic patients required, on average, 37% less adenosine diphosphate to achieve a sticking probability of 0.5 (ED_{50}) compared with platelets from the non-diabetic subjects (medians 1.50 and $0.95 \mu\text{mol/l}$, respectively; $p < 0.01$). The platelet prostacyclin

response was assessed by the dissociation constant (K_d) for inhibition of adenosine diphosphate-induced aggregation. Platelets from the diabetic patients had similar prostacyclin sensitivity to those from the non-diabetic subjects (medians 0.42 and 0.42 respectively). Diabetic patients with and without retinopathy had similar platelet sensitivity to both adenosine diphosphate and prostacyclin.

Key words: Type 1 diabetes, retinopathy, platelet aggregation, adenosine diphosphate, prostacyclin.

The results of most of the studies concerned with platelet aggregation in diabetes mellitus suggest that platelets from diabetic subjects are hypersensitive to pro-aggregating agents in vitro [1, 2]. Prostacyclin is thought to counter the pro-aggregatory behaviour of platelets in vivo [3], and, although decreased vascular prostacyclin production might not, as first postulated [4], occur in diabetes [5], platelet prostacyclin sensitivity may be depressed [6, 7].

In the present study, the sensitivity of platelets from normal subjects and from matched diabetic patients both with and without retinopathy to a range of doses of adenosine diphosphate (ADP) and prostacyclin was investigated using a model of platelet aggregation based on particle collision theory [8]. This model allows interpretation of platelet sensitivity to ADP and prostacyclin in terms of 'sticking probability', a measure which reflects the haemostatic function of platelets.

Subjects and methods

Subjects

Twenty Type 1 diabetic men and 10 male non-diabetic subjects were studied (Table 1). Informed consent was obtained from each subject before his participation in the study. The diabetic patients were divided into two subgroups according to severity of retinopathy: 10 had

no or minimal retinopathy, as assessed by ophthalmoscopy and fluorescein angiography, and ten had established proliferative retinopathy. No subject had taken medication known to affect platelet function tests during the fortnight before venepuncture.

Methods

The subjects attended fasting and in random order. Blood was taken for use in aggregation studies [8], for estimation of plasma glucose (glucose oxidase, Boehringer GOD-Perid kit, Lewes, UK), and for determination of glycosylated haemoglobin [9].

Table 1. Clinical details of control and diabetic subjects

	Control subjects (n = 10)	Diabetic patients	
		With no or minimal retinopathy (n = 10)	With proliferative retinopathy (n = 10)
Age (years)	48.5 (37–60)	48.5 (33–65)	45 (35–59)
Duration of diabetes (years)	–	27 (19–36)	26 (17–34)
Body mass index (kg/m ²)	24.5 (22.9–30.5)	23.7 (20.8–26.1)	23.9 (19.7–29.0)
Daily insulin dose (U/kg)	–	0.89 (0.43–1.83)	0.82 (0.65–2.85)
Plasma glucose (mmol/l)	4.7 (4.3–6.2)	13.4 (2.6–20.7)	11.7 (6.2–17.2)
HbA _{1c} (%)	7.3 (5.2–8.1)	8.8 (7.0–10.5)	10.3 (8.4–13.4)

Results given as median with ranges in parentheses

Fig. 1. Values for the final ADP concentration required to produce a 'sticking probability' of 0.5 (ED_{50} – the dose required for a half maximal response) for the 10 non-diabetic and 20 diabetic subjects studied. Group medians are shown as horizontal bars

Fig. 2. Final ADP concentration required to produce a 'sticking probability' of 0.5 (ED_{50}), related to the final prostacyclin concentration in standard platelet-rich plasma (SPRP). Because of the non-normal distribution of the data, medians and upper and lower confidence limits (the data with ranks corresponding to one SD of ranks either side of the median) are shown for the 10 non-diabetic (●—●) and 20 diabetic (○-----○) subjects

Aggregation studies for each subject were performed as described by Davis and Bown [8] using platelet-rich plasma with a standard count of 250,000/ml. A conventional aggregometer (Payton Associates, Buffalo, Illinois, USA) provided the data required for collision theory analysis: a platelet dilution curve (the forms of which were

similar for both diabetic and non-diabetic subjects) and aggregation tracings for ten final ADP concentrations ranging from 0.2 to 4.0 μmol/l. These doses provoked a range of aggregation for minimal to irreversible in all subjects.

The collision theory model was used to calculate a 'sticking probability' (the probability of particle union following collision) for each ADP dose at the point of maximal first-phase aggregation [8]. The model is based on a constant relationship between the collision rate and number of particles present in the aggregometer cuvette. A proportion of such collisions results in particle union following addition of submaximal doses of ADP, with a theoretical 'sticking probability' of unity at an 'infinite' dose. A dose-response curve can be easily constructed.

In addition to a dose-response curve for ADP alone, aggregation tracings for each of four doses of ADP (0.4, 0.8, 1.2, and 3.0 μmol/l) in the presence of each of four doses of prostacyclin (0.2, 0.4, 0.6 and 1.0 ng/ml) were obtained. The prostacyclin was added to standard platelet-rich plasma from a concentrated solution (0.1 mg/μl in 0.05 mol/l Tris/HCl, pH 9.0, at 4°C), with the ADP dose added 30 s later.

The ED_{50} (the dose required for a half-maximal response) of the ADP 'sticking probability' dose-response curve was taken as a measure of platelet sensitivity to ADP for each subject. From the ED_{50} 's of the ADP dose-response curves in the presence of each prostacyclin concentration, an inhibition constant (K_i) for prostacyclin was calculated [10]. This enabled a measure of platelet prostacyclin sensitivity to be obtained for each subject which was independent of platelet sensitivity to ADP alone.

Statistical analysis

All data are expressed as medians and absolute ranges. Non-parametric tests (Mann-Whitney U test) with two-tailed levels of significance were used to analyse the data.

Results

The non-diabetic subjects had significantly higher ED_{50} values for the ADP dose-response curves (where, by definition, the 'sticking probability' is 0.5) than those of the 20 diabetic patients (Mann-Whitney U test, $p < 0.01$; Fig. 1). However, the ED_{50} values of the diabetic patients with no or minimal retinopathy were similar to those with proliferative retinopathy.

The effect of adding prostacyclin to platelets before ADP is seen in Figure 2. The ED_{50} values increased, indicating that more ADP is required to produce a 'sticking probability' of 0.5 in the presence of increasing final prostacyclin concentrations. However, the K_i values for prostacyclin-induced inhibition of ADP-provoked aggregation were similar for the control subjects (median, 0.42, range 0.32–0.69) and diabetic patients (0.42, 0.17–0.65; $p > 0.1$). There was also no significant difference between the K_i values of the diabetic patients with no or minimal retinopathy (0.48, range 0.17–0.65) and those with proliferative retinopathy (0.39, range 0.27–0.57).

There was no significant correlation between age, duration of diabetes, daily insulin dose, fasting plasma glucose concentration or glycosylated haemoglobin levels, and either ED_{50} or K_i for the control subjects or the 20 diabetic patients.

Discussion

Although the control and diabetic groups were well-matched in the present study, platelets from the diabetic subjects were found to be hypersensitive to ADP *in vitro*. This result is consistent with the findings of other authors using alternative methods [1, 2]. The increased 'sticking probability' of platelets from diabetic patients lends weight to the suggestion that increased numbers of platelet aggregates circulate in diabetes [11].

The diabetic subjects with no or minimal retinopathy had similar ED₅₀ values to those of the matched diabetic subjects with proliferative retinopathy. The plasma glucose concentrations of the subjects in these two subgroups were also similar. Poor diabetic control is associated with the development of microvascular disease [12], and the glycosylated haemoglobin levels of the diabetic patients with no or minimal retinopathy tended to be lower than those with proliferative retinopathy. A prospective study might allow better assessment of the relationship between diabetic control, platelet sensitivity to pro-aggregating agents and the progression of diabetic microvascular disease.

The collision theory model allowed a formal comparison of the platelet prostacyclin sensitivity of non-diabetic and diabetic subjects, even though the ADP sensitivity of platelets from the diabetic patients was increased in the absence of this antagonist. The platelet prostacyclin sensitivity of the diabetic subjects in both retinopathy subgroups was similar to that of the non-diabetic subjects. This finding does not support reports of decreased prostacyclin sensitivity in diabetes [6], which has been found to be most marked in the present of vascular complications [7]. These studies were concerned with platelet prostacyclin sensitivity at a single ADP dose, without regard for differences in ADP sensitivity between diabetic and non-diabetic subjects.

The results of the present study support the suggestion that platelets from diabetic patients are hypersensitive to ADP, a situation which might contribute to the development of diabetic angiopathy. However, there seems insufficient evidence to implicate reduced prostacyclin production or anti-aggregatory effect in this process.

Acknowledgements. We are grateful to ICI Ltd, Macclesfield, UK, for the generous provision of synthetic prostacyclin and the aggregometer. We thank Mrs. R. Mullins and Mrs. C. Whittle for technical help, and Dr. J. Aronson for advice on dose-response theory.

References

1. Colwell JA, Halushka PV (1980) Platelet function in diabetes mellitus. *Br J Haematol* 44: 521-526
2. Jones RL, Peterson CM (1981) Hematologic alterations in diabetes mellitus. *Am J Med* 70: 339-352
3. Gryglewski RJ (1980) Prostaglandins, platelets and atherosclerosis. *CRC Crit Rev Biochem* 7: 291-338
4. Silberbauer K, Scherthaner G, Sinzinger H, Piya-Katzer H, Winter M (1979) Decreased vascular prostacyclin in juvenile-onset diabetes. *N Engl J Med* 300: 366-367
5. Davis TME, Bown E, Finch DR, Mitchell MD, Turner RC (1981) In-vitro venous prostacyclin production, plasma 6-keto-prostaglandin F_{1α} concentrations, and diabetic retinopathy. *Br Med J* 282: 1259-1262
6. Davi G, Rini GB, Aversa M, Novo S, Di Fede G, Pinto A, Notarbartolo A, Strano A (1982) Thromboxane B₂ formation and platelet sensitivity to prostacyclin in insulin-dependent and insulin-independent diabetics. *Thromb Res* 26: 359-370
7. Betteridge DJ, El Tahir KEH, Reckless JPD, Williams KI (1982) Platelets from diabetic subjects show diminished sensitivity to prostacyclin. *Eur J Clin Invest* 12: 395-398
8. Davis TME, Bown E (1983) Analysis of platelet aggregation using particle collision theory. *Am J Physiol* 245: R776-R784
9. Jeppson JO, Franzen B, Nilsson KO (1978) Determination of glycosylated haemoglobin fraction HBA1c in diabetes mellitus by thin layer electro-focussing. *Sci Tools* 25: 69-72
10. Goldstein A, Aronow L, Kalman SM (1974) Principles of drug action: the basis of pharmacology. John Wiley, London, pp 82-111
11. Preston FE, Ward JD, Marcola BH, Porter NR, Timperley WR (1978) Elevated B-thromboglobulin levels and circulating platelet aggregates in diabetic microangiopathy. *Lancet* 1: 238-239
12. Jarrett RJ, Keen H (1976) Hyperglycaemia and diabetes mellitus. *Lancet* 1: 1009-1012

Received: 11 June 1984

and in revised form: 23 March 1985

Dr. R. C. Turner
Diabetes Research Laboratories
Radcliffe Infirmary
Oxford OX2 6HE
UK