Platelet Sensitivity to Adenosine Diphosphate and to Prostacyclin in Diabetic Patients

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ONODERA, H., HIRATA, T., SUGAWARA, H., SUGAI, K., YODA, B., TOYOTA, T. and GOTO, Y. Platelet Sensitivity to Adenosine Diphosphate and to Prostacyclin in Diabetic Patients. Tohoku J. exp. Med., 1982, 137 (4), 423-428 — We studied the platelet sensitivity to adenosine diphosphate (ADP) and to prostacyclin (PGI2) in order to clarify the mechanism of the onset or development of diabetic retinopathy. The platelet sensitivity to ADP in diabetics with simple and proliferative retinopathy was significantly increased as compared with that in diabetics without retinopathy and in controls. The platelet sensitivity to PGI2 in diabetics with simple and proliferative retinopathy was decreased significantly as compared with the normal value. There was a significant negative correlation between the platelet sensitivity to ADP and that to PGI2 in all diabetics. Since the effect of PGI2 on platelets is to inhibit its aggregation, the decreased sensitivity to PGI2 implies a tendency to accelerate platelet aggregation. The increased platelet aggregation due to the alteration of the platelet sensitivity is considered as an important factor in diabetic microangiopathy.

Since the discovery of prostacyclin (PGI2) (Moncada et al. 1976) and thromboxane A2 (TXA2) (Hamberg et al. 1975), many authors have reported on the relationship between these two substances and diabetic angiopathy. In diabetic patients, not only PGI2 produced in vessel walls (Harrison et al. 1978; Johnson et al. 1979, 1980; Silberbauer et al. 1979, 1980) but also the serum level of 6-keto-PGF1α, a stable metabolite of PGI2, decreases (Dollery et al. 1979; Subbiah and Deittemeyer 1980). On the other hand, in diabetics thromboxane B2 (TXB2) which is a stable metabolite of TXA2 (Ziboh et al. 1979; Subbiah and Deittemeyer 1980) and enzyme activity of cyclo-oxygenase or thromboxane synthetase (Johnson et al. 1980) increase. Furthermore, platelet sensitivity to adenosine diphosphpate (ADP) increases, and that to PGI2 decreases (Bonne et al. 1978; Betteridge et al. 1980).

We studied platelet sensitivity to ADP and PGI2 in order to clarify the mechanism of the onset or development of diabetic retinopathy.

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Material and Methods

Fifty diabetic patients, 23 male and 27 female, ranging from 15 to 81 years in age (53.6 ±14.3) were included in this study. They were classified into the following three groups: A) 17 diabetic patients without retinopathy, B) 18 diabetics with simple retinopathy and C) 15 diabetics with proliferative retinopathy. All patients were treated with a diet, sulfonylurea or insulin.

Nineteen healthy subjects, 13 male and 6 female, ranging from 25 to 74 years in age (46.2±16.6) were observed as the control group.

Nine ml of blood withdrawn from the antecubital vein was put into a plastic syringe containing 1 ml of 3.8% w/v sodium citrate, and platelet-rich plasma (PRP) was separated after centrifugation at 250 × g for 10 min at 20°C. After decanting the PRP, platelet-poor plasma (PPP) was obtained by centrifugation at 1,800 × g for 10 min at 20°C. Platelet aggregation was measured with a Born type platelet aggregometer (NKK, Tokyo). Synthesized PGI2 sodium salt (molecular weight 374) kindly presented by the Ono Pharmaceutical Co. (Osaka) was solved in glycine buffer (pH 10.5) and then diluted with saline-Tris buffer (pH 8.5) at 4°C.

Platelet sensitivity to ADP was measured as shown in Fig. 1. PRP 225 µl was incubated with a stirrer for 2 min at 37°C and 25 µl of each concentration of ADP (0.5, 1.0, 2.0, 3.0, 4.0, 6.0 µM as a final concentration) was added. After obtaining the maximum platelet aggregation to ADP, the ADP concentration required to provoke a half maximum was determined as the sensitivity to ADP.

![Fig. 1](image1.png)

Fig. 1. Method of calculating ADP concentration inducing half maximum of platelet aggregation (platelet sensitivity to ADP).

![Fig. 2](image2.png)

Fig. 2. Method of calculating PGI2 concentration required for 50% inhibition of maximum platelet aggregation (platelet sensitivity to PGI2). 0.10 ng/assay is equal to 0.77 nM.
Platelet sensitivity to PGI₂ was measured according to the principle that platelet aggregation induced by ADP (5.0 μM as a final concentration) is inhibited by each concentration of PGI₂ (0.1, 0.2, 0.3, 0.4, 0.5 ng/assay, 0.1 ng/assay is equal to 0.77 nM) (Gryglewsky et al. 1978). PRP 100 μl was diluted with 150 μl of a solution containing the following composition (in mM): NaCl 118, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.17, NaHCO₃ 25.0, glucose 5.6. The diluted PRP was incubated with a stirrer for 2 min at 37°C before the addition of PGI₂. After incubation for 3 min, ADP was added, and the final volume of the assay was 350 μl. The sensitivity to PGI₂ was expressed as the PGI₂ concentration required to inhibit 50% of the platelet aggregation (Fig. 2).

Means±S.D. of the platelet sensitivity to ADP and to PGI₂ were calculated, and statistical analysis was carried out according to Student’s t-test.

RESULTS

Platelet sensitivity to ADP in diabetic patients without retinopathy was 1.6±0.8 μM (normal; 1.9±0.7 μM). In diabetics with simple and proliferative retinopathy, the platelet sensitivities to ADP were 0.9±0.4 μM and 0.9±0.5 μM,

| Table 1. Platelet sensitivity to ADP and that to PGI₂ in diabetic patients |
|---------------------------------|-----------------|-----------------|-----------------|
| Number of cases | Platelet sensitivity to ADP (μM) | Platelet sensitivity to PGI₂ (nM) |
| Controls | 19 | 1.9±0.7 | 1.24±0.40 |
| Diabetics without retinopathy | 17 | 1.6±0.8 | 1.46±0.81 |
| Diabetics with simple retinopathy | 18 | 0.9±0.4 | 1.64±0.60 |
| Diabetics with proliferative retinopathy | 15 | 0.9±0.5 | 1.80±0.61 |

* p<0.05, † p<0.01, ‡ p<0.001.

Fig. 3. Correlation between platelet sensitivity to ADP and that to PGI₂ in diabetic patients. Significant negative correlation is demonstrated.
respectively. These differences were significant not only between diabetics with and those without retinopathy \((p<0.01)\), but also in comparison with healthy subjects \((p<0.001)\).

Platelet sensitivity to PGI\(_2\) was 1.46±0.81 nM in diabetic patients without retinopathy, and 1.64±0.60 nM and 1.80±0.61 nM in those with simple and proliferative retinopathy, respectively \((\text{normal}; 1.24±0.40 \text{ nM})\). The platelet sensitivity to PGI\(_2\) in diabetic patients with simple and proliferative retinopathy decreased significantly as compared with the normal value \((p<0.05\) and \(p<0.01\), respectively) \(\text{(Table 1)}\).

In the platelet sensitivity to ADP and to PGI\(_2\), no differences were observed between diabetics with simple and proliferative retinopathy. There was a negative correlation between the platelet sensitivity to ADP and that to PGI\(_2\) in all diabetic patients as shown in Fig. 3 \((r=-0.51, p<0.001)\), but there was no significant correlation between them in the control.

**DISCUSSION**

In diabetic patients, platelet aggregation to aggregating agents (ADP, collagen and epinephrine) was reported to be increased \((\text{Heath et al. 1971; Kwann et al. 1972; Bensoussan et al. 1975; Roux et al. 1977; Bern 1978; Colwell et al. 1978)}\). Colwell et al. (1978) reported that hypersensitivity of platelet to ADP had no effect on vascular diseases. However, Kwann et al. (1972) showed that platelet aggregation was increased in diabetic patients with retinopathy, indicating that the increased sensitivity of platelet to ADP is a factor in causing microangiopathy.

We measured platelet sensitivity to ADP, calculating a half maximum concentration of ADP, which revealed that the sensitivity of platelet to ADP was significantly increased in diabetics with retinopathy.

PGI\(_2\) generation in vessel walls is decreased in diabetic patients \((\text{Johnson et al 1979; Silberbauer et al. 1979)}\). Experiments in animals also showed that the PGI\(_2\) generation was decreased \((\text{Harrison et al. 1978; Johnson et al. 1980; Silberbauer et al. 1980; Sugawara et al. 1982)}\). Subbiah and Deittemeyer (1980) reported the increase of another prostaglandin, TXB\(_2\), which is a stable metabolite of TXA\(_2\). TXA\(_2\) is an antagonist of PGI\(_2\). The increased TXB\(_2\) is considered to reflect over-production of TXA\(_2\). In experimental diabetic animals cyclooxygenase, thromboxane synthetase and malondialdehyde synthesis are increased in platelet \((\text{Johnson et al. 1980)}\), and TXA\(_2\) production is increased in the diabetic state.

Bonne et al. (1978) reported that the platelet sensitivity to PGI\(_2\) was decreased in diabetes mellitus. Betteridge et al. (1980) observed that 20% of diabetic patients showed the decreased sensitivity of platelet to PGI\(_2\). Experimentally Yamada et al. (1981) and Sugawara et al. (1982) discovered that the sensitivity of platelet to PGI\(_2\) was decreased in alloxan-diabetic rabbits.

We clearly demonstrated that the sensitivity of platelet to PGI\(_2\) was decreased in diabetic patients with retinopathy as compared with that of the
control. Since the effect of PGI₂ on platelets is to inhibit its aggregation, the decreased sensitivity to PGI₂ implies a tendency to accelerate platelet aggregation.

As imbalance of prostaglandin metabolism in platelets and vessel walls of diabetes mellitus has been documented, prostaglandins would modulate platelet function. Although the mechanism of the onset and development of diabetic retinopathy remains unsolved, increased platelet aggregation due to the alteration of platelet sensitivity to ADP and to PGI₂ is considered to be an important factor in diabetic microangiopathy.

References

experimentally induced diabetes on swine vascular prostacyclin (PGI₂) synthesis. *Artery, 8*, 30–36.


