Platelet monoamine changes in diabetic patients and streptozotocin-induced diabetic rats

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In the present study we assessed plasma and platelet monoamine content using high performance liquid chromatography (HPLC). The study included 22 subjects consisting of 12 freshly-detected male diabetic patients and 10 age and sex-matched healthy controls. The same parameters were measured in streptozotocin-induced diabetic rat models consisting of controls, diabetic and insulin-treated diabetic rats. The platelet counts were significantly reduced ($P < 0.05$) in rat models as well as human diabetic samples. The plasma norepinephrine (NE) and epinephrine (EPI) concentrations were significantly increased ($P < 0.05$). The platelet showed a significant increase ($P < 0.01$) in NE, EPI and serotonin content. Increase in the plasma and platelet content of neurotransmitters may be due to increased sympathetic function, which is an adaptation for the decreased platelet count observed in our study. The results indicate that changes in the neurotransmitter content of the platelet may be a good index to assess the neurotransmitter status in pathological condition such as diabetes mellitus.

THE role of neurotransmitters in diseased condition has been studied using post-mortem brain samples which are often not reliable since conditions of collection and preservation of such samples are not uniform and there is hardly any choice of age, sex or status of disease. It has therefore become necessary to develop a peripheral model for studying neurotransmitter changes. The blood platelets can be used as a peripheral model since there are structural, morphological and physiological similarities between platelets and synaptosomes[1,2]. Platelets, like synaptosomes are limited by a membrane and contain mitochondria, storage granules, lysosomes, glycogen particles, microtubules and microfilaments[1]. They accumulate monoamines by an active transport mechanism located in the cell membrane and store high concentration of serotonin (5-hydroxytryptamine, 5-HT) in storage granules. The platelets and synaptosomes also possess monamine oxidase which metabolizes serotonin and other amines. Thus we have used platelet as a peripheral model and quantitated NE, EPI and 5-HT by HPLC method to assess monoamine alterations in diabetic patients and streptozotocin-induced diabetes in an animal model.

Three months old male Sprague-Dawley rats of ~180 g body weight were used for all experiments. They were housed in separate cages under 12 h light period and 12 h dark period. They were maintained on standard food pellets and tap water *ad libitum*. Diabetes was induced by a single intrafemoral dose (45 mg/kg body wt.) of STZ prepared in citrate buffer (pH 4.5) (refs 3, 4). The animals were randomly divided into three groups, i.e. controls, diabetic and insulin-treated diabetic groups with 4–6 animals in each group. The insulin-treated diabetic group received a daily dose (1 unit/kg body weight) of Lente insulin. The dose was increased daily according to the blood glucose level. Glucose was measured by GOD-PAP glucose estimation kits (MERCK) (Table 1).

Blood samples from diabetic patients were obtained from freshly-diagnosed cases with a fasting blood sugar level ranging from 150 to 200 mg/dl and with 1.5–2.0 mg urine sugar. None of the patients had any infection or secondary complications and were non-ketotic at the time of sample collection. Only male volunteers between age groups 40 and 60 were selected for the experiment. Patients with any other complications or on drugs were excluded from the study. The controls were age and sex matched.

About 10 ml of blood was collected from human volunteers by ante cubital venipuncture into 1 ml of Alsever's solution (acid/citrate/dextrose pH 5.0). Platelets were prepared according to the method of Steven *et al.*[5] The platelets were counted using a Neubaeur haemocytometer under a phase contrast microscope.

20 µl supernatant from sonicated platelet pellets in 0.1 ml of 0.4 M perchloric acid was injected into an HPLC system integrated with an electrochemical detector to determine the neurotransmitter contents. HPLC separation conditions were followed as previously reported[6]. The monoamine values were expressed per million platelets. 1.0 ml plasma was diluted in equal volume of distilled water. 50 µl of 5 mM sodium

Table 1. Blood glucose levels and body weight of experimental animals

	Blood glucose level (mg/dl)	Body weight (g)
Control $n = 4$	103.28 ± 3.76	174.58 ± 1.69
Diabetic $n = 6$	223.58 ± 20.79*	126.25 ± 4.37*
Diabetic + insulin $n = 5$	140.38 ± 11.24	148.88 ± 3.24

*$P < 0.05$ when compared with control

Values are mean ± S.D. of 4–6 separate animals.

Table 2. Plasma and platelet monoamine content of humans

		Plasma monoamines nmoles/ml		Platelet monoamine content nmoles/million cells		
	Platelet count $\times 10^9$/l	NE $\times 10^{-2}$	EPI $\times 10^{-2}$	NE $\times 10^{-4}$	EPI $\times 10^{-4}$	5-HT $\times 10^{-4}$
Control $n = 10$	327.718 ± 92.15	0.214 ± 0.01	0.039 ± 0.014	0.066 ± 0.02	0.0103 ± 0.003	0.86 ± 0.23
Diabetic $n = 12$	156.745 ± 32.31*	18.96 ± 2.37†	1.78 ± 0.497†	0.63 ± 0.94†	9.2867 ± 1.611†	2.75 ± 0.31†

*$P < 0.05$ compared with controls.
†$P < 0.01$ compared with controls.
Values are mean ± S.D of 10–12 separate determinations.

Table 3. Plasma and platelet monoamine content of experimental animals

		Plasma monoamines nmoles/ml manoamines		Platelet content monoamine nmoles/million cells	
	Platelet count $\times 10^9$/l	NE $\times 10^{-2}$	EPI $\times 10^{-2}$	EPI $\times 10^{-4}$	5-HT $\times 10^{-4}$
Control $n = 4$	245.733 ± 34.0	3.72 ± 0.80	2.39 ± 0.21	17.45 ± 4.53	0.57 ± 1.32
Diabetic $n = 6$	169.800 ± 8.18*	5.08 ± 1.04*	7.29 ± 1.04*	162.08 ± 3.45†	1.579 ± 2.60†
Diabetic+insulin $n = 5$	173.350 ± 9.05*	1.28 ± 0.02*	0.434 ± 0.06†	126.07 ± 15.86†	ND

*$P < 0.05$ compared with controls.
†$P < 0.01$ compared with controls.
ND – not detected.
Values are mean ± S.D of 4–6 separate determinations.

bisulphite was added to it followed by 250 µl of 1 M Tris buffer pH 8.6. Acid alumina (20 mg) was then added, mixed for 20 min, the supernatant aspirated off and alumina was washed with 2.0 ml of sodium bisulphite. To the final pellet of alumina, 0.2 ml of 0.1N perchloric acid was added. The supernatant was filtered and used for HPLC determination of monoamines. 20 µl of extracted sample was injected into a Shimadzu HPLC apparatus connected to a C-18 reverse-phase column. The mobile phase consisted of 75 mM sodium phosphate monobasic, 1 mM sodium octyl sulphonate, 50 µM EDTA and 8% acetonitrile. The pH was adjusted to 3.25 with phosphoric acid and filtered through a Millipore filter. A flow rate of 1 ml/min was used. The monoamines were identified using electrochemical detector. The peaks were identified by relative retention time with standards and the contents were calculated using a Shimadzu integrator (Chromatopac CR6A) interfaced with the detector. Data were statistically analysed by Student's *t* test.

Blood glucose levels of all patients screened were significantly high ($P < 0.05$) compared with controls (diabetic 191.9 ± 7.6 mg/dl, controls 102.2 ± 5.3 mg/dl). Circulating plasma NE and EPI were increased ($P < 0.05$) in human samples. There was a significant increase ($P < 0.01$) in platelet NE, EPI and 5-HT content when compared to controls. The platelet count was significantly reduced ($P < 0.05$) in diabetic patients (Table 2).

Plasma NE and EPI content in experimental animals showed a significant increase ($P < 0.05$), but the increase in plasma NE of diabetic rats was less compared to humans. The EPI and 5-HT content of diabetic rat platelets showed a significant increase ($P < 0.01$). The insulin-treated group showed a decrease in platelet EPI content ($P < 0.01$) compared to diabetic untreated group. There was a significant reduction in platelet counts of diabetic rats ($P < 0.05$) compared to control. The insulin treatment to diabetic rats showed an increasing trend in platelet count but was not significant (Table 3).

The results of the present study showed a diabetic-induced change in the levels of platelet monoamines. In both humans and rats, the plasma NE and EPI levels were significantly high. A similar trend was seen in platelet NE and EPI content of human samples. The content in rat platelets was significantly high, while the NE content could not be detected. The increase in NE and EPI content of human platelets corresponded with the increase in plasma NE and EPI concentration. The same pattern was observed in rat model. But in diabetic rats the increase in plasma NE level was very low com-

pared to human samples. This may be the reason for undetectable level of platelet NE in rats. The increase in plasma NE and EPI observed in experimental diabetes as well as human subjects may be due to the increased output of sympathetic stimulation in both these cases. Previous studies have shown the involvement of sympathetic nervous system in elevating plasma NE and EPI in diabetic condition. The adrenal glands may also be involved in bringing about an increase in EPI[7]. Martin *et al.*[8,9] reported increased serotonin content in human and rat platelet in diabetic condition. Acute myocardial infarction showed a similar platelet 5-HT content[10]. There are also reports of altered NE, EPI and 5-HT in brain regions of diabetic rat models as well as humans[11].

The present study showed a decrease in platelet number in diabetic patients as well as in rat models which supports our earlier report on decreased number of platelets in diabetic rats[12]. The increased content of neurotransmitters observed in plasma and platelets may be similar to the increased sympathetic stimulation observed in our previous studies in pyridoxine-deficient animal models[13]. The increase in monoamine uptake by the platelets can be due to alteration of platelet membrane potential[14]. Kjeldsen *et al.*[15] have reported that a depletion of sodium in the diet can increase the monoamine uptake by the platelets. It appears that the changes in rat and human samples of plasma monoamines are correspondingly reflected in the platelet monoamines. Thus it is suggested that the platelets can be used for measuring the levels of NE, EPI and 5-HT in diabetic condition and the platelet content of these neurotransmitters is altered in untreated diabetes.

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A Mössbauer spectroscopic study of the Pipliya-1 meteorite

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A recently fallen stony type meteorite in Rajasthan namely Pipliya-1 meteorite has been investigated using Mössbauer spectroscopy at 300 K. The obtained Mössbauer parameters resemble pigeonite to a greater extent, among pyroxene. The ratio of Fe^{2+} inner doublet and Fe^{2+} outer doublet confirm the textural interpretations of the geothermal history of the pyroxene. Presence of a weak Fe^{3+} doublet is probably indicating the change in the oxidation state of Fe^{2+} in the meteroite itself because of shock phenomenon.

THE Pipliya-1 meteorite is one of the two Pipliya meteor showers fallen on 20 June 1996 at 20.30 h at village Pipliya Kalan (Lat. 26°2'5": Long 73°56'30"), Pali, Rajasthan, India. A preliminary study of the same has been reported recently[1]. In this paper the Mössbauer spectroscopic study of the Pipliya-1 meteorite is reported.

The Pipliya-1 meteorite has a glossy pitch black crust of nearly 500 μm thickness. Under the fused crust it has typical characters of a stony meteorite. The rock as a whole is a complex breccia consisting chiefly of two types of fragments (type A and type B, welded together), and a few xenoliths[1]. Type A is a fine-grained, gray rock while type B is a coarse-grained, grayish-white rock as reported[1]. The thin sections under petrological microscope reveal[1] that type A is composed of grains of pyroxene (65%), plagioclase (25%), opaque minerals (6%), and some xenoliths (4%). Type B is composed of pyroxenes (55%), plagioclase (40%) and opaque minerals (5%). Pyroxene is clinopyroxene of pale pink colour, nonpleochroic with moderate extinction angles.