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Effects of Gliclazide and Insulin Therapy on Thromboxane B_2 and 6-Keta-$PGF_{1\alpha}$ Levels in Type II Diabetic Patients

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Abstract: Diabetic patients show hemobiological abnormalities such as increased platelet adhesiveness, platelet hyperaggregability, decreased platelet half life, hemorheological abnormalities and altered fibrinolysis, perhaps contributing to a procoagulative state. Gliclazide, a novel sulfonylurea in routine clinical use, was thought to have effects on prostanoid release and platelet function. We studied thromboxane A_2 metabolite; serum thomboxane B_2 (TXB_2) and the prostacyclin metabolite, 6-keto-$PGF_{1\alpha}$ to assess the efficacy of gliclazide on these parameters. Two groups of age and sex matched type II diabetics were examined in the study. There were 16 subjects in each group (F:M= 10/6). The study period was 12 weeks. Gliclazide was given to the first group and insulin to the second. Following parameters were evaluated to see the effect of good control. HbA1c, cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, serum TXB_2, 6-keto-$PGF_{1\alpha}$ levels were measured before and after 3 months of therapy. There was no significant change in TXB_2 (2.24±0.2 to 2.08±0.4 nmol/L) and 6-keto-$PGF_{1\alpha}$ (2.53±0.2 to 2.15±0.1 nmol/L,) levels in patients treated with insulin despite the amelioration in the HbA1c levels. Therapy with gliclazide was followed by a significant decrease in both serum TXB_2 levels (4.18±0.7 to 2.72±0.4, p=0.039) and 6-keto-$PGF_{1\alpha}$ (2.97±0.3 to 2.03±0.1, p=0.0047). TXB_2/6-keto-$PGF_{1\alpha}$ ratio did not change both after insulin (1.09±0.5 to 1.06±0.8) and gliclazide (1.31±0.9 to 1.32±0.4) treatment.

According to the datas in our study, gliclazide therapy decreased TXB_2 levels as well as 6-keto-$PGF_{1\alpha}$ levels so that ratio of TXB_2/6-keto-$PGF_{1\alpha}$ did not change, which would mean that gliclazide has neutral effect on diabetic microvascular complications.

Key Words: Non insulin dependent diabetes, gliclazide, thromboxane B2, 6-keto-$PGF_{1\alpha}$.

Introduction

Non-insulin dependent diabetes mellitus (NIDDM) is associated with an increased risk of macro and microvascular degenerative complications. Although blood glucose levels can now be controlled with some confidence using diet and/or hypoglycaemic agents, many patients with diabetes mellitus still develop long term micro and macro-angiopathies resulting in atherosclerosis, cardiac disease, retinopathy and renal failure. Studies have demonstrated dysfunction of coagulation and fibrinolysis in patients with diabetes mellitus, evidenced by increased platelet adhesiveness and aggregation, raised levels of thromboxane A_2, and reduced prostacyclin (PGI_2) (1, 2). Prostacyclin is unstable and it undergoes a spontaneous hydrolysis to 6-keto-$PGF_{1\alpha}$. Due to this spontaneous hydrolysis of prostacyclin, the quantitation of 6-keto-$PGF_{1\alpha}$ is accepted by many researchers as a measure of prostacyclin formation. Thromboxane A_2 is a labile bicyclic compound formed from prostaglandin endoperoxides. It has half life of about 30 second and is rapidly hydrolyzed to its stable biologically inactive metabolite, thromboxane B_2 (TXB_2). The short life of TXA_2 impedes its measurement at physiological concentrations.

The measurement of TXB_2 is accepted as an indicator of TXA_2 production (3). Prostacyclin is a vasodilatator and a potent inhibitor of platelet aggregation (4) while thromboxane A_2 (TXA_2) is a vasoconstrictor and a promoter of platelet aggregation (5). A physiological balance between the activities of these two effectors is probably important to maintaining a healthy vascular bed. As PGI_2 inhibits platelet aggregation and relaxes vascular

Table 1. Mean age, duration of diabetes, sex distribution and complications of the group I and II.

	n	F/M	Mean age	Duration of diabetes	BSPP (n)	R (n)	CHD (n)
Group I	16	10/6	49.8±7.4	11.8±3.1	13/16	10/16	8/16
Group II	16	11/5	51.5±12.9	12.2±2.9	14/16	11/16	9/16

BSPP: bilateral sensorineural peripheric neuropathy

R: retinopathy

CHD: coronary heart disease

Table 2. Pre- and post-treatment datas in two groups

	GLICLAZIDE		INSULIN	
	Pre-treat.	Post-treat	Pre-treat.	Post-treat
HbA1c(%)	7.15±1.5	7.05±0.8	8.9±2.2	6.9±1.2*
FPG (mmol/L)	9.15±0.61	9.21±0.58	10.48±0.86	6.71±0.81*
Chol.(mmol/L)	5.69±1.15	5.82±0.82	5.45±0.88	5.25±0.96
TG (mmol/L)	1.80±0.75	1.95±0.79	1.58±0.50	1.54±0.75
HDL (mmol/L)	1.13±0.49	1.21±0.36	1.14±0.46	1.19±0.29
LDL (mmol/L)	3.93±1.06	3.90±1.41	3.30±1.19	3.21±0.62

*p<0.001

Chol: Cholesterol TG: Triglyceride FPG: Fasting plasma glucose

smooth muscles, the decrease in PGI_2 levels and the increase in TXA_2 levels in diabetes could facilitate the development of microthrombi, leading to diabetic microvascular complications.

Gliclazide is a second generation sulfonylurea that is widely used in the treatment of NIDDM. In addition to its metabolic effects, there are some reports implicate that gliclazide has beneficial effects on the haemobiological abnormalities of NIDDM, although one report showed no effect (6). In our previous study to investigate the effect of good glycemic control on platelet function, we measured changes in plasma beta thromboglobulin and platelet factor 4 (PF4) levels and platelet aggregation after 3 months of treatment with insulin and gliclazide. We could not demonstrate significant change in platelet functions although there was a significant reduction in HbA1c levels (7). With regard to platelet functions, several groups have demonstrated a significant reduction in serum and intraplatelet beta thromboglobulin and TX B_2. Animal studies have shown a correction of the TXA_2/PGI_2 imbalance, by this drug (8, 9).

Platelet dysfunction, platelet-endothelium interactions in the early stages of diabetes cause microvascular damage and tendency to atherosclerosis. Risk of microangiopathy can be reduced by optimising PGI_2 and TXA_2 levels which are the markers of platelet-endothelium inter reaction. The aim of this study is: - to emphasise the known effects of gliclazide on TXB_2 and 6-keto-$PGF_{1\alpha}$ - to see whether the direct effect of gliclazide on these parameters is different from the metabolic control of diabetes. We switched to insulin treatment in a group of patients who had poor diabetic control, to detect if the normalisation of TXB_2 and 6-keto-$PGF_{1\alpha}$ is due to good diabetic control.

Materials and Methods

Patients

Sixteen patients whom were not achieved good metabolic control with gliclazide therapy in 3 months, were selected for gliclazide treatment group (group I) and 16 NIDDM patients whom were achieved good metabolic control with insulin were selected for insulin treatment group (group II). Details about patients were given in table 1.

In group I, all patients were switched to gliclazide for 3 months. Patients were administered maximum 160 mg/day gliclazide.

In group II, all patients switched to insulin from oral

Table 3. Pre and post-treatment data

	GLICLAZIDE		INSULIN	
	Pre-treat.	Post-treat	Pre-treat.	Post-treat
TXB_2(ng/L)	1550±256	1010±141	832.3±99.1	773.0±155
p value	0.039		NS	
6-keto-$PGF_{1\alpha}$(ng/L)	1102±98.3	752.2±52.1	937.3±94	795.0±54.4
p value	0.0047		NS	
TXB_2/6-keto-$PGF_{1\alpha}$	1.31±0.9	1.32±0.4	1.09±0.5	1.06±0.8
p value	NS		NS	

antidiabetics other than gliclazide for 3 months.

Diabetic patients were identified using the following exclusion criteria: Type I diabetics, patients treated with progtaglandin synthesis inhibitors, adrenocorticoids, salicylates, dipyridamole, theophyline, Vitamin E and antilipemic drugs and patients had good metabolic control after gliclazide treatment.

Diabetic neuropathy was examined by electrophysical examination, retinopathy was assessed by ophthalmoscopic examination and coronary heart disease was examined by anamnesis and ECG.

Method

Assessment of the patients were performed at the baseline and after completion of three months of therapy. These included detailed medical history, clinical examination, and determination of fasting plasma glucose (glucose oxidase method), HbA1c (colorimetric method), serum triglycerides (enzymatic colorimetric method GPO-PAP), total cholesterol (enzymatic colorimetric method CHOD-PAP), HDL cholesterol (Tungstophosphoric acid hydrate-magnesium cloride precipitation method), LDL cholesterol (by Friedewald formula), TXB_2 and 6 keto $PGF_{1\alpha}$ levels (by RIA). We collected the blood for TXB_2 and 6-keto $PGF_{1\alpha}$, in a tube with EDTA, centrifuged immediately and froze the plasma rapidly. If blood samples could not be processed rapidly, indomethacin or asprin was added to the anticoagulant as recommended.

Results are given as medians and ranges are presented as mean±SD. Statistical analyses were performed by using standard t test.

Results

HbA1c values decreased significantly in group I, but there was no change in gliclazide group because these patients were selected from the subjects that metabolic control could not be achieved. Fasting plasma glucose levels were decreased significantly in group I, but not in group II. Cholesterol, triglycerides, HDL and LDL cholesterol levels did not change in both groups (table 2).

Three months of gliclazide therapy resulted in a significant decline in serum TXB_2 and 6-keto-$PGF_{1\alpha}$ levels. In spite of significant decrease in HbA1c levels, neither TXB_2 nor 6-keto-$PGF_{1\alpha}$ levels changed after insulin treatment. TXB_2/6-keto-$PGF_{1\alpha}$ ratio did not change both after gliclazide and insulin treatment (table 3).

Discussion

Abnormalities of arachidonic acid metabolism and of the prostaglandine pathway have been studied following the development of techniques for the assay of thromboxane A_2 and thromboxane B_2. An increase in TXA_2 levels during diabetes has been clearly shown (10,11). This abnormality is confirmed by assay of it's stable metabolite TXB_2 (12, 13). Furthermore PGI_2-platelet interaction is impaired during diabetes because of reduced sensitivity of platelets to prostacyclin (14, 15, 16). TXA_2 and PGI_2 acts on platelets by influencing the activity of adenylate cyclase. The opposing effects of TXA_2 and prostacyclin on adenylate cyclase have fostered the theory that platelet hemeostasis is dependent on a reciprocal regulation of cyclin AMP levels by PGI_2 and TXA_2 (17).

As PGI_2 inhibits platelet aggregation and relaxes vascular smooth muscles the decrease in PGI_2 levels and the increase in TXA_2 levels in diabetes could facilitate the development of microthrombi, leading to diabetic microvascular complications. One study was demonstrated that TXB_2/6 keto-$PGF_{1\alpha}$ ratio was decreased from 4.6 to 1.6 by gliclazide treatment (18).

In diabetics ▲5, ▲6 desaturase activity is reduced, causing a subsidence in arachidonic acid production and its metabolite PGI_2 synthesis (1, 19). Furthermore lipid

peroxidation increases due to oxidative stress, causing inhibition of prostacyclin synthetase which result in the reduction of PGI_2 synthesis and increment in TXA_2 (20). Opposing this concept, there are some studied showing increased PGI_2 synthesis to compensate the increased platelet aggregation in diabetics (21). Increased endothelial PGI_2 synthesis has been shown in the rabbit coronary arteries (22, 23). Likewise, it has been pointed out that TXA_2 synthesis increases in rabbits' aortic endothelium (23) and human vascular endothelium (24). Studies with gliclazide revealed that by suppressing platelet functions, the drug inhibits the formation of vascular complications independent from its effect on the glycaemic control (8, 25, 26). Effects of gliclazide on the TXA_2 synthesis is controversial. As some authors advocate that gliclazide decreases TXA_2 synthesis by reducing arachidonic acid secretion from palatelet membrane phospholipids (8, 18) some others believe that it does not effect TXA_2 synthesis (6).

In vivo and in vitro radical scavenger effect of gliclazide inhibits lipid peroxidation, causing an increase in PGI_2 synthesis (20). According to the datas in our study, gliclazide therapy decreased TXA_2 levels as well as PGI_2 levels. Therefore the same TXA_2/PGI_2 ratio obtained at the end of the study would mean that gliclazide has neutral effect on diabetic microvascular complications. Decrease of TXA_2 levels found in our study is compatible to the results of other investigators (8, 18). Decrease in PGI_2 levels can be explained as follows:

- The time period for gliclazide therapy might not be sufficient to overcome the oxidative stress in endothelium and to start PGI_2 synthesis

- Gliclazide might reduce PGI_2 synthesis by inhibiting the secretion of arachidonic acid from endothelium as well as inhibiting its secretion from platelets.

References

1. Jones DB, Carter RD, Haitas B, Mann JI: Low phospholipid arachidonic acid values in diabetic patients. Br Med J 286: 173-75, 1983.
2. Zigler O, Drovin P: Haemobiological activity of gliclazide in diabetes mellitus. Diabetes Res Clin Pract 14-2P: 85-9, 1991.
3. Fitzpatrick FA: A radioimmunoassay for thromboxane B2. Methods-Enzymol 86: 286-97, 1982.
4. Moncada S, Vane JR: Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A2 and prostacyclin. Pharmac Rev 30: 292-331, 1979.
5. Hamberg M, Sevensson J, Samuelsson B: Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. Proc Natl Acad Sci 72: 2294-2998, 1975.
6. Larkins RG, Jerums G, Taft JL, Godfrey H, Smith IL, Martin TJ: Lack of effect of gliclazide on platelet aggregation in insulin treated and non insulin treated diabetics. Diabetes Res Clin Pract 4: 81-7, 1988.
7. Keskin A, Özgen AG, Sermez Y, Yılmaz C, Büyükkeçeci F. Platelet function in type II diabetes mellitus and the effect of glycemic control on platelet reactivity. Turk J Endoc 5: 179-185, 1995.
8. Tsuboi T, Fujitani B, Maeda T, Yashida K, Shimizu M: Effect of gliclazide on prostaglandin and thromboxane synthesis in guinea pig platelets. Thromb Res 21: 103-110, 1981.
9. Fujitani B, Maeda J, Tsuboi T, Kadokawa T, Shimizu M: Effect of gliclazide on prostaglandin I2 formation in normal and streptozotocin induced diabetic animals. Jpn J Pharmacol 33:965-70, 1983.
10. Ziboh VA, Maruta H, Lord J, Cagle WD, Lucky W: Increased biosynthesis of thromboxane A2 in diabetic platelets. Eur J Clin Invest 1979; 9: 223-228.
11. Legarde M, Budin M, Berclaud P, Blanc M, Velardo B, Dechavanne M: Increase of platelet Thromboxane A2 formation and of its plasmatic half life in diabetes mellitus. Thromb Res 1980; 19: 823-30.
12. Brunner D, Klinger J, Weisbort J, Tuval M, Nakash J, Rosenberg CH, Nissim S: Thromboxane, prostacyclin beta thromboglobulin and diabetes mellitus. Clin Ther 6: 636-42, 1984.
13. DiMinno G, Silver MJ, Cerbone AM, Riccardi G, Rivellese A, Macini M, Thiagarajan P: Increased binding of fibrinogen to platelets in diabetes the role of prostaglandin's and thromboxane. Blood 65: 156-162, 1985.
14. Silberbauer K, Schernthaner G, Sinzinger H, Piza Katzer H, Winer M: Decreased vascular prostacyclin in juvenile onset diabetes. N Eng J Med 300: 306-7, 1979.
15. Davi G, Rini GB, Averna M, Novo S, Di Fede G, Pinto A, Notarbartolo A, Strano A: Thromboxane B2 formation and platelet sensitivity to prostacyclin in insulin dependent and noninsulin-independent diabetics Thromb Res 26: 359-70, 1982.
16. Betteridge DJ, El Tehir KEH, Reckless JPD, Williams KI: Platelets from diabetic subjects show diminished sensitivity to prostacyclin. Eur J Clin Invest 12: 385-88, 1982.
17. Gorman R: Mechanism of action of prostacyclin and thromboxane A2 in prostaglandins in clinical medicine-cardiovascular and thrombotic disorders. Wu KK and Rossi EC eds Year Book Medical Publishers Inc

Chicago 21-34, 1982.

18. Fu ZZ, Yan T, Chen YJ, Sang JQ: Thromboxane/prostacyclin balance in type II diabetes: Gliclazide effects. Metabolism 41: suppl 1: 33-5, 1992.
19. Tilvis RS, Taskinen MR, Miettinen T: Effect of insulin treatment of fatty acids of plasma and erythrocyte membrane lipids in type 2 diabetes. Clin Chim Acta 171: 293-304, 1988.
20. Jennings PE, Scott NA, Sanrabadi AR, Belch JJF: Effects of gliclazide on platelet reactivity free radicals in type II diabetic patients. Clinical Assessment. Metabolism 41: suppl 1, 36-9, 1992.
21. Waitzmann MB: Proposed metabolic dysfunction in diabetic microthrombosis and microangiopathy. Metabolism 28: suppl 1: 401-6, 1979.
22. Tesfamariam B, Jakubowski JA, Cohen RA: Contraction of diabetic rabbit aorta caused by endothelium-derived PGH2 TXA2. Am J Physiol 257 (Heart Circ Physiol 26) H 1327-H1333, 1989.
23. Tesfamariam B, Brown ML, Deykin D, Cohen RA: Elevated glucose promotes generation of endothelium derived vasoconstrictor prostanoids rabbit aorta. J Clin Invest 85: 929-32, 1990.
24. Sakurai T, Goto K: Endothelins vascular actions and clinical implications. Drugs 46: 795-804, 1993.
25. Ponario O, Cıvadı E, Megha S, Pını M, Portioli D, Dettoni AG: Antiplatelet effects of long term treatment with gliclazide in diabetic patients. Thrombosis Research 16: 191-203, 1979.
26. Violi F, De Mattia GC, Alessandrie Perrone A, Vezza E: The effects of gliclazide on platelet function in patients with diabetes mellitus. Current