EFFECT OF GLICLAZIDE ON PROSTAGLANDIN I2 FORMATION IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC ANIMALS

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Abstract—The effect of gliclazide, a hypoglycemic sulfonylurea, on the formation of prostaglandin (PG) I2 by the aortic rings of normal and streptozotocin-induced diabetic animals was studied. In in vitro experiments, gliclazide (100-300 µg/ml) enhanced the spontaneous PG12 formation by the guinea-pig and rat aorta. Gliclazide also enhanced the transformation of both arachidonic acid and PGH2 to PG12 in guinea-pig aorta, indicating that one of the main enhancing sites is the step of converting PGH2 to PGI2. In ex vivo experiments, the formation of PGI2 in the aorta of streptozotocin-diabetic rats was markedly reduced as compared with that of normal rats. An oral administration of gliclazide (100-300 mg/kg) significantly restored this reduced formation of PGI2 without any effect on blood glucose level. This enhancing effect of gliclazide may be favorable to the treatment of diabetic microangiopathy.

Abnormalities of platelet functions in diabetic patients, especially in patients with microangiopathy (1, 2), are known, and these abnormalities have been postulated to contribute to the pathogenesis of diabetic microangiopathy through their pivotal role in the thrombus formation and the proliferation of vascular connective tissue cells (3).

In addition, it has been reported that the formation of prostaglandin (PG) I2 is reduced in vessel walls of experimentally induced diabetic animals (4, 5) and diabetic patients (6). As PGI2 is a potent inhibitor of platelet adhesion (7) and aggregation (8), its deficiency in diabetes may also promote the thrombotic tendency and the development of microangiopathy.

An oral hypoglycemic agent, gliclazide, 1-(4-methylbenzenesulfonyl)-3-[3-azabicyclo (3, 3, 0) octyl] urea has been shown to inhibit platelet functions in experimental animals (9) and diabetic patients (10). Gliclazide also inhibits thrombus formation in rats (11) and prevents the formation of retinal platelet aggregates in dogs induced by the infusion of ADP into their carotid artery (12).

As for the inhibitory mechanism of gliclazide, it activates the adenylate cyclase of human platelets (13) and inhibits the release of arachidonic acid from guinea-pig platelets (14). However, little is known about the effect of gliclazide on PGI2 synthesis in vascular tissues.

The present paper describes the in vitro and ex vivo effects of gliclazide on PGI2 formation in the aorta of normal and streptozotocin-induced diabetic animals.

Materials and Methods

Animals: Male Hartley strain guinea-pigs weighing 500–800 g and male Wistar strain
rats weighing 190–200 g were used. Commercial diets and water were given ad libitum. Diabetic rats were produced by an intravenous injection of streptozotocin at 40 mg/kg. Diabetic rats, whose blood glucose levels ranged 250–450 mg/dl, were used 1 month after the induction of diabetes.

Formation of PGI₂: The formation of PGI₂ in the aorta of guinea-pigs and rats was estimated by the method of Gryglewski et al. (15). Platelet-rich and platelet-poor plasma of guinea-pigs used in platelet aggregation studies were prepared as previously described (14).

Thoracic aorta was isolated under anesthesia, immediately frozen with dry ice and stored at −80°C. Frozen tissues were used 2–3 days after the isolation. The tissue, which was thawed just before use, was cut into rings. The rings (5–20 mg) were incubated with 400–1000 µl of saline buffered with 50 mM Tris (TBS, pH 7.6) for 5 min at 22°C. For the in vitro experiments, test compounds were dissolved in TBS. In the ex vivo experiments, gliclazide was orally given 3 hr before isolating aorta. As for the measurement of released PGI₂, 10–100 µl of the medium was added to platelet-rich plasma preincubated at 37°C for 1 min, and platelet aggregation started by adding ADP (1–2×10⁻⁶ M). Total volume of the aggregation mixture was 250 µl. The extent of aggregation was assessed by the maximum change of light transmittance within 5 min after the addition of ADP. The amount of released PGI₂ was determined by comparison of its anti-aggregatory potency with that of a known amount of synthetic PGI₂ sodium salt. Identification of antiaggregatory potency as PGI₂ was confirmed by comparing its instability with that of synthetic PGI₂ and by reduced formation following acetylsalicylic acid and tranylcypromine treatments.

The transformation of exogenous arachidonic acid and PGH₂ to PGI₂ was examined with the aorta of guinea-pigs given hydrocortisone sodium succinate (200 mg/kg, i.p.) 4 hr before or acetylsalicylic acid (100 mg/kg, p.o.) 3 hr before isolating aorta. Both acetylsalicylic acid and hydrocortisone treatments abolished spontaneous PGI₂ formation in guinea-pig aorta. Aortic rings were incubated with gliclazide for 10 min at 22°C, followed by washing twice with TBS, and further incubated with arachidonic acid (0.1 mM) or PGH₂ (0.47 µM) for 5 min.

Blood glucose: Blood was obtained from the retro-orbital vein of rats. Blood glucose was determined by a method of Lowry et al. (16).

Chemicals: PGI₂ sodium salt was a kind gift of the Ono Pharm. Co. Ltd., Japan. PGH₂ was obtained from Ran Biochem. Ltd., Israel. Arachidonic acid, hydrocortisone sodium succinate, tranylcypromine and ADP were from Sigma Chem. Co., U.S.A. Acetylsalicylic acid was from Aldrich Chem. Co., U.S.A., and streptozotocin was from Calbiochem. Co., U.S.A. Gliclazide was obtained from Servier Co., France, and its potassium salt was prepared by us. Glibenclamide and tolbutamide were from Yamanouchi Pharm. Co. Ltd., Japan. Acetohexamide, chloropropamide and tolazamide were from Shionogi & Co. Ltd., Japan, Ono Pharm. Co. Ltd., Japan and Upjohn Japan Ltd., Japan, respectively.

Results

In vitro effect of gliclazide and other sulfonylureas on PGI₂ formation: Gliclazide (100–300 µg/ml) enhanced the spontaneous PGI₂ formation in guinea-pig aorta in vitro (Table 1). Among sulfonylureas tested, tolazamide (200–1000 µg/ml) and acetohexamide (500–1000 µg/ml) enhanced the spontaneous PGI₂ formation (Table 2). However, chloropropamide (500–1000 µg/ml) did not show this effect, and tolbutamide (500–1000 µg/ml) and glibenclamide (50–500 µg/ml) inhibited the PGI₂ formation.
Gliclazide (30–1000 µg/ml) also enhanced the conversion of exogenous arachidonic acid to PGI₂ in the aorta of hydrocortisone-treated guinea-pigs and PGH₂ to PGI₂ in the aorta of acetylsalicylic acid-treated animals (Table 1). To examine the possibility that gliclazide causes the generation of some anti-aggregatory substances other than PGs, the

**Table 1.** In vitro effect of gliclazide on PGI₂ formation in guinea-pig aorta

| Treatment | Concentration of gliclazide (µg/ml) | PGI₂ released (% of control) | Mean±S.E. |
|-----------|----------------------------------|-----------------------------|-----------|
|           |                                  | 1  | 2  | 3  | 4          |
| Spontaneous PGI₂ formation | 30 | 105 | 126 | 105 | — | 112±7 |
|             | 100 | 153 | 148 | 130 | — | 143±7 |
|             | 300 | 151 | 188 | 197 | — | 179±14 |
| PGI₂ formation from AA in hydrocortisone-treated aorta | 100 | 114 | 75 | 105 | 133 | 107±12 |
|             | 300 | 111 | 196 | 133 | — | 147±25 |
|             | 1000 | 148 | 138 | 140 | — | 142±3 |
| PGI₂ formation from PGH₂ in acetylsalicylic acid-treated aorta | 30 | 103 | 163 | 80 | 86 | 108±19 |
|             | 100 | 133 | 224 | 125 | 127 | 152±24 |
|             | 300 | 225 | 206 | 131 | 230 | 198±23 |

In the experiment on spontaneous PGI₂ formation, aortic rings were incubated in Tris-buffered saline for 5 min at 22°C with or without gliclazide. In the control, aortic tissues released 0.76±0.08 ng/mg tissue/5 min of PGI₂. As for the PGI₂ formation from arachidonic acid (AA) and PGH₂, aortic rings were incubated with gliclazide for 10 min at 22°C, followed by washing twice and then further incubated with AA (0.1 mM) or PGH₂ (0.47 µM). PGI₂ formation from AA or PGH₂ was examined with the aorta of hydrocortisone or acetylsalicylic acid-treated animals, respectively; and these treatments abolished spontaneous PGI₂ formation. In the control, tissues released 1.89±0.20 and 13.9±1.2 ng/mg/5 min of PGI₂ from AA or PGH₂, respectively.

**Table 2.** In vitro effect of sulfonylureas on spontaneous PGI₂ formation in guinea-pig aorta

| Compound   | Concentration (µg/ml) | PGI₂ released (% of control) |
|------------|-----------------------|-------------------------------|
| Glibenclamide | 50 | 88±10 |
|             | 100 | 72±6  |
|             | 200 | 37±6  |
|             | 500 | Not detected |
| Tolbutamide | 500 | 102±15 |
|             | 1000 | 78±7  |
| Acetohexamide | 200 | 121±22 |
|             | 500 | 168±26 |
|             | 1000 | 138±22 |
| Chlorpropamide | 500 | 102±20 |
|             | 1000 | 93±18 |
| Tolazamide | 100 | 92±11 |
|             | 200 | 128±25 |
|             | 500 | 138±12 |
|             | 1000 | 188±10 |

Figures in the table are the means±S.E. of 3 experiments. Spontaneous PGI₂ formation was examined as described in Table 1.
Table 3. In vitro effect of gliclazide on spontaneous PG12 formation in rat aorta

| Concentration of gliclazide (μg/ml) | PG12 released (% of control) |
|-------------------------------------|-----------------------------|
|                                     | 1  | 2  | 3  | Mean±S.E. |
| 30                                  | 73 | 169| 85 | 107±10    |
| 100                                 | 167| 147| 144| 152±24    |
| 300                                 | 182| 172| 130| 161±16    |

In the control, the tissue released 16.8±1.7 ng/mg tissue/5 min of PG12. Spontaneous PG12 formation was examined as described in Table 1.

Table 4. Effect of oral administration of gliclazide on aortic PG12 formation and blood glucose levels in streptozotocin-induced diabetic rats

| Treatment          | Dose (mg/kg) | PG12 released (ng/mg aorta/5 min) | Blood glucose (mg/dl) |
|--------------------|--------------|-----------------------------------|-----------------------|
| Normal             |              | 21.1±1.6**                        | 94±3                  |
| Diabetic control   |              | 5.7±0.9                           | 320±12                |
| Diabetic with gliclazide | 30 | 9.6±1.5                           | 295±6                 |
|                     | 100          | 14.8±2.3**                        | 308±12                |
|                     | 300          | 15.7±2.5**                        | 317±18                |

Figures in the table are the means±S.E. of 5–6 animals. **: Differences from the diabetic control are statistically significant with P<0.01. Gliclazide was orally given 3 hr before isolating aorta.

effect of gliclazide on the generation of antiaggregatory potency was tested in the aorta of acetylsalicylic acid-treated animals under the presence or absence of arachidonic acid. However, gliclazide did not induce the generation of any antiaggregatory potency under these conditions (Data not shown).

Gliclazide also enhanced the spontaneous formation of PG12 in rat aorta (Table 3). The enhancing effect in rat aorta was comparable with that in guinea-pig aorta, though the basal formation of PG12 in rat aorta was about 25 times higher than that in guinea-pig aorta.

Ex vivo effect of gliclazide in streptozotocin-induced diabetic rats: The spontaneous formation of PG12 in the aorta of diabetic rats was reduced to approx. 25% of that in normal rats. Gliclazide (30–300 mg/kg) restored this reduced formation in a dose-dependent manner (Table 4). On the other hand, blood glucose levels of diabetic rats were about 3 times higher than those of normal rats, and gliclazide did not alter the blood glucose levels of diabetic rats (Table 4).

Discussion

Gliclazide enhanced the spontaneous PG12 formation in the aorta of guinea-pigs and rats in vitro. A similar effect was observed by some sulfonylureas such as tolazamide and acetohexamide, but not by chlorpropamide, tolbutamide and glibenclamide, indicating that the enhancing effect on PG12 formation is not common to all hypoglycemic sulfonylureas.

PG12 is synthetized from membrane phospholipids through sequential steps of 1) liberation of arachidonic acid from phospholipids, 2) conversion of arachidonic acid to PG endoperoxides and 3) transformation of endoperoxides to PG12. As gliclazide enhanced the transformation of both arachidonic acid and PGH2 to PG12, one of the main enhancing sites by gliclazide may be a step of converting endoperoxides to PG12, though possibilities that gliclazide influences the preceding steps can not be excluded.

Recent studies demonstrated the reduced
formation of PGI₂ in vessel walls of experimentally induced diabetic animals (4, 5) and diabetic patients (6). Harrison et al. (4, 5) observed an inverse relationship between blood glucose level and the amount of PGI₂ formed in vascular tissues of diabetic rats and observed that insulin increased PGI₂ formation in diabetic animals, suggesting that abnormal carbohydrate metabolism contributes to the reduced PGI₂ formation in diabetics.

We confirmed the marked reduction of PGI₂ formation in streptozotocin-diabetic rats. An oral administration of gliclazide at 100–300 mg/kg significantly restored this reduced formation of PGI₂ without any effect on blood glucose level. Terauchi et al. (17) reported that serum concentration of gliclazide reached to about 100 and 300 µg/ml at 3 hr after the oral administration of gliclazide at 50 and 200 mg/kg, respectively; thus, serum concentration of gliclazide after its oral administration at 100–300 mg/kg may be comparable to its concentration used in invitro studies and may be sufficient to enhance PGI₂ formation.

As for the blood glucose lowering and insulin-releasing effects of gliclazide, gliclazide at doses or concentrations used in this study sufficiently lowers blood glucose or stimulates insulin secretion in normal rats (18). However, it is well known that sulfonylureas do not show the hypoglycemic and insulin-releasing effects in alloxan- and streptozotocin-diabetic animals. Gliclazide also neither stimulates insulin secretion nor lowers blood glucose levels in alloxan-diabetic rabbits (19). Thus, the restoring effect of gliclazide on PGI₂ formation in diabetic rats may be independent of its insulin-releasing and hypoglycemic effects. The in vitro and ex vivo enhancements of PGI₂ formation by gliclazide suggest its direct action on vascular PGI₂ formation, though the precise mechanism remained to be elucidated.

Extensive studies demonstrated the enhancement of platelet adhesion and aggregation in diabetics, especially in those with microangiopathy (1, 2), and the enhancement of platelet functions seems closely linked to vascular abnormality in diabetic microangiopathy. As PGI₂ is a potent inhibitor of platelet functions produced by vessel walls (7, 8), the restoration of reduced PGI₂ formation may prevent the thrombotic tendency and microangiopathy in diabetic patients.

Gliclazide restored the reduced formation of PGI₂ in the aorta of streptozotocin-diabetic rats. Gliclazide also inhibits platelet functions in experimental animals and diabetic patients (9, 10, 14, 17). Although these effects in experimental animals are observed at relatively high doses of gliclazide as used in this study, clinical doses of gliclazide also inhibit platelet functions in diabetic patients. Thus, these concomitant effects of gliclazide on PGI₂ formation and platelet functions may be favorable for the treatment of diabetic microangiopathy.

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