A BRIEF COMMUNICATION

Antiglycation Effect of Gliclazide on *In Vitro* AGE Formation from Glucose and Methylglyoxal

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Gliclazide, a sulfonylurea widely used for treatment of diabetes mellitus, is known to scavenge reactive oxygen species. To clarify whether its antioxidative ability interferes with the glycation processes, we incubated bovine serum albumin (BSA) with 1 *M* glucose or 1 m*M* methylglyoxal, in the presence or absence of gliclazide, and observed the formation of advanced glycation end products (AGEs). AGE production was assessed by AGE-specific fluorescence, an enzyme-linked immunosorbent assay (ELISA), and Western blotting. The fluorescence at excitation/emission wavelengths of 320/383 nm and 335/385 nm was definitely increased by incubating BSA with 1 *M* glucose or 1 m*M* methylglyoxal, and 1 m*M* gliclazide significantly blunted the fluorescent augmentation, in both wavelengths, in a dose-dependent fashion. Gliclazide almost equaled to aminoguanidine, a putative antiglycation agent, in the inhibitory effect on the glucose-induced fluorescence, while the methylglyoxal-derived fluorescent formation was less suppressed by gliclazide than by aminoguanidine. The AGE concentrations determined by ELISA showed similar results. Incubation of BSA with 1 *M* glucose or 1 m*M* methylglyoxal yielded an apparent increase in carboxymethyllysine or argpyrimidine. Both AGEs were significantly lowered by 1 m*M* gliclazide and a reduction of glucose-derived carboxymethyllysine was comparable to that caused by aminoguanidine. The results of Western blotting supported the findings in ELISA. To our knowledge, the present study provides the first evidence of the antiglycation effect of gliclazide on *in vitro* AGE formation from glucose and methylglyoxal. Exp Biol Med 233:176–179, 2008

Key words: gliclazide; oxidative stress; glycation; advanced glycation end products

Introduction

Advanced glycation end products (AGEs) and oxidative stress have been implicated in the pathogenesis of diabetic complications. Both are known to interact with each other (i.e., reactive oxygen species [ROS] accelerate AGE formation and, inversely, AGEs increase the ROS) (1).

Gliclazide, a sulfonylurea widely used as a hypoglycemic agent for the treatment of diabetes mellitus, is known to possess scavenging ability against ROS (2), and this antioxidative characteristic has been thought to account for, at least in part, its favorable effects on diabetic complications (3). In theory, as described above, the antioxidative action may influence glycation processes as well, yet the effects of gliclazide on AGE formation have remained uninvestigated. Here, we report the first evidence of the antiglycation action of gliclazide on *in vitro* AGE formation from glucose and methylglyoxal.

Materials and Methods

Materials. Gliclazide, bovine serum albumin (BSA) and dimethyl sulfoxide (DMSO) were purchased from

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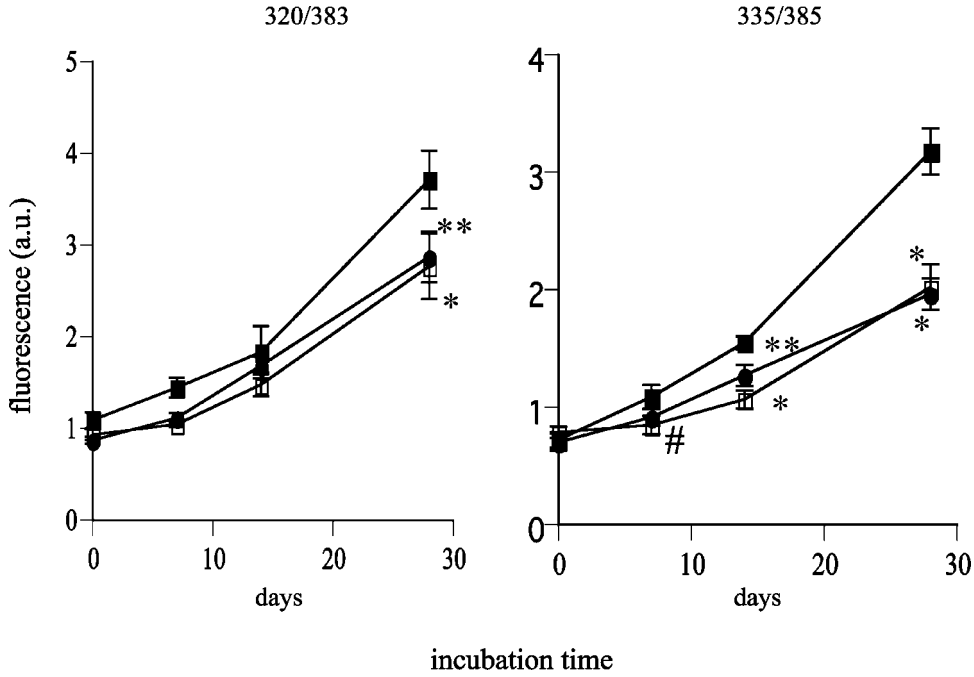

Figure 1. Fluorescence augmentation by incubating BSA with 1 *M* glucose in the absence (■) or presence of 1 m*M* gliclazide (●) or 1 m*M* aminoguanidine (□). Error bars represent SD. * $P < 0.001$, ** $P < 0.01$, and # $P < 0.05$ versus fluorescence with BSA and glucose at the same incubation time, respectively.

Wako (Osaka, Japan). Anti-AGE monoclonal antibody (6D12) and anti-argpyrimidine antibody were obtained from Trans Genic (Kumamoto, Japan) and NOF (Tokyo, Japan), respectively. Aminoguanidine and other chemicals were purchased from Sigma (St. Louis, MO).

Preparation of BSA Solutions. Gliclazide and aminoguanidine, a putative antiglycation agent, were dissolved in DMSO and then added to 10 m*M* phosphate-buffered saline containing 10 mg/ml BSA and either 1 *M* glucose or 1 m*M* methylglyoxal to acquire final concentrations of 10, 100, and 1000 μ*M*. After sterilization, using a Millex GV filter (Millipore, Cork, Ireland) to prevent bacterial growth, the solutions were incubated at 37°C for up to 28 days (glucose) or 7 days (methylglyoxal). Each experimental condition was performed in triplicate.

Determination of AGEs. After incubation for the required time, AGE production was assessed by fluorescence, an enzyme-linked immunosorbent assay (ELISA), and Western blotting. The fluorescence at excitation/emission wavelengths of 320/383 nm and 335/385 nm was detected using the RF-1500 Spectro fluorophotometer (Shimadzu, Kyoto, Japan). The amounts of carboxymethyllysine and argpyrimidine were measured by competitive ELISA using anti-AGE antibody (6D12) and anti-argpyrimidine antibody, as described previously (4). Western blotting was performed, as described previously (5), with the same antibodies as used for ELISA.

Statistical Analysis. Differences among conditions were assessed by analysis of variance.

Results

Figure 1 depicts the time course of AGE production from BSA and 1 *M* glucose. The fluorescence at excitation/emission wavelengths of 320/383 nm and 335/385 nm is thought to mainly reflect the amounts of argpyrimidine and pentosidine, respectively (6). The fluorescence at both wavelengths was successively increased through Day 28. Gliclazide (1 m*M*) significantly blunted the fluorescent augmentation in both wavelengths, and the inhibitory effect was nearly equivalent to that of 1 m*M* aminoguanidine.

There was a clear dose dependency in both gliclazide and aminoguanidine inhibition of the fluorescent augmentation, not only from 1 *M* glucose, but also from 1 m*M* methylglyoxal (Fig. 2). The inhibitory effects on the glucose-induced fluorescence were similar between glicalzide and aminoguanidine, while gliclazide was less suppressive on the methylglyoxal-derived fluorescent formation than was aminoguanidine. None of the agents interfered *per se* with the fluorescent measurement at either wavelength.

Quantification of AGEs by ELISA showed comparable results to those seen in the fluorescent changes. The AGE formation from glucose (Fig. 3A) was detected by 6D12, which has been reported to mainly react with carboxymethyllysine (7). The incubation of BSA with 1 *M* glucose demonstrated an obvious increase in carboxymethyllysine levels. In addition, 1 m*M* gliclazide reduced carboxymethyllysine formation by approximately 47%, which was greater than the 33% reduction observed following 1 m*M* aminoguanidine treatment. Gliclazide also significantly inhibited argpyrimidine production from methylglyoxal

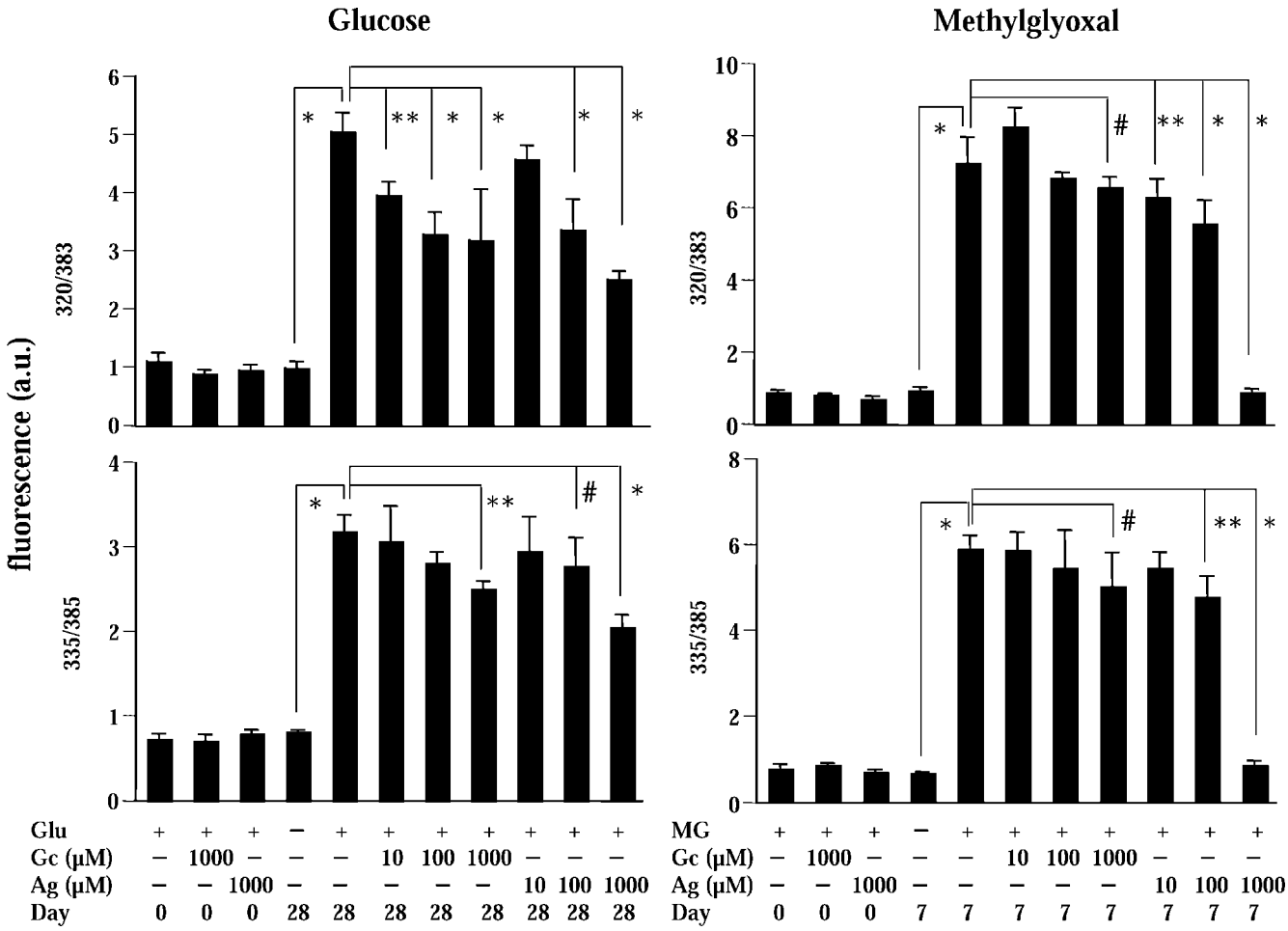

Figure 2. Dose dependency of gliclazide and aminoguanidine on fluorescence from glucose and methylglyoxal. Error bars represent SD. Ag, Aminoguanidine; Gc, gliclazide; Glu, D-glucose; MG, methylglyoxal. * $P < 0.001$, ** $P < 0.01$, and # $P < 0.05$.

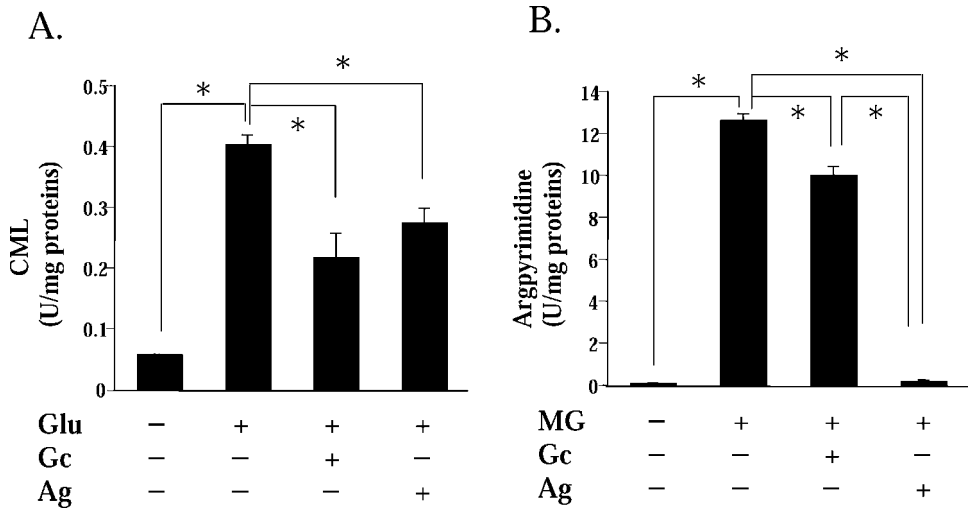

Figure 3. The concentrations of CML from glucose after 28-day incubation (A) and argpyrimidine from methylglyoxal after 7-day incubation (B). The AGE amounts were determined by competitive ELISA. Error bars represent SD. Ag, Aminoguanidine; Gc, gliclazide; Glu, D-glucose; MG, methylglyoxal. * $P < 0.001$.

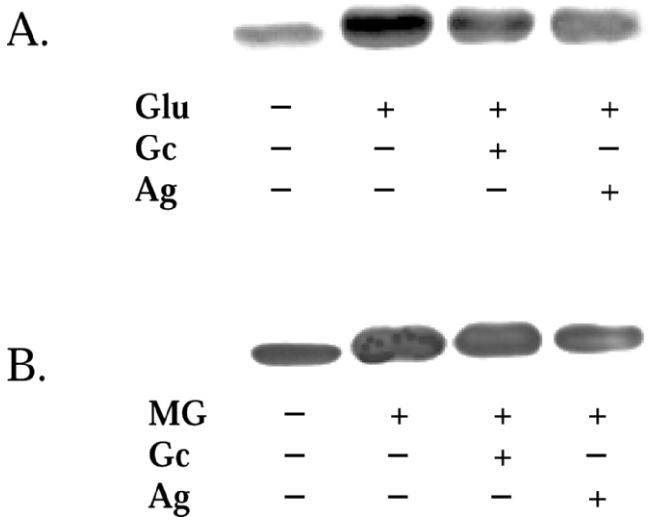

Figure 4. Western blotting of CML from glucose after 28-day incubation (A) and argpyrimidine from methylglyoxal after 7-day incubation (B). Ag, Aminoguanidine; Gc, gliclazide; Glu, D-glucose; MG, methylglyoxal.

(Fig. 3B), although the inhibition was apparently weaker than that for aminoguanidine when compared with the glucose-derived AGE formation. The results of Western blotting supported the findings in ELISA (Fig. 4).

Discussion

Independent of hypoglycemic effects, the well-documented antioxidative action of gliclazide has attracted attention in terms of its therapeutic benefit for the treatment of diabetic complications. In fact, there are some interesting data showing that gliclazide prevents atherosclerosis (8), diabetic neuropathy (9), or diabetic retinopathy (10, 11). These favorable effects have been attributable to its antioxidative feature (12). Although the amelioration of AGE-induced cellular responses by gliclazide has been reported (13), the drug effect on AGE formation itself has been neglected. Our present data clearly reveal that gliclazide possesses an antiglycation effect comparable to that of aminoguanidine in glucose-induced AGE formation *in vitro*. The suppression of AGEs possibly results from ROS scavenging, while the reduced AGE formation may inversely diminish the ROS production. The intervention of gliclazide in the connection between glycation and oxidation will likely contribute to the blunted progression of diabetic complications.

The reported concentration of gliclazide in the serum of diabetic patients is around 24 μ*M* after 7 days of successive administration of 80 mg/day (14), which is much lower than the doses used in the present experiments. Thus, the application of the present results to clinical efficacy may be limited. However, we also used glucose and methylglyoxal at more than 100-times higher concentrations than physiological levels (15) to yield detectable amounts of AGEs. This made the concentration ratios of gliclazide to glucose and methylglyoxal less than 1:1000 and 1:1, respectively, mimicking physiological conditions in terms of the drug-to-metabolite ratio. *In vivo* study remains to be performed, although the hypoglycemic action of gliclazide may interfere in the antiglycation action due to lowered glucose itself.

1. Taniguchi N, Takahashi M, Sakiyama H, Park YS, Asahi M, Misonou Y, Miyamoto Y. A common pathway for intracellular reactive oxygen species production by glycoxidative and nitroxidative stress in vascular endothelial cells and smooth muscle cells. Ann N Y Acad Sci 1043: 521–528, 2005.
2. Noda Y, Mori A, Packer L. Gliclazide scavenges hydroxyl, superoxide and nitric oxide radicals: an ESR study. Res Commun Mol Pathol Pharmacol 96:115–124, 1997.
3. Ceriello A. Effects of gliclazide beyond metabolic control. Metabolism 55:S10–S15, 2006.
4. Hamada Y, Nakamura J, Naruse K, Komori T, Kato K, Kasuya Y, Nagai R, Horiuchi S, Hotta N. Epalrestat, an aldose reductase inhibitor, reduces the levels of Nε-(carboxymethyl)lysine protein adducts and their precursors in erythrocytes from diabetic patients. Diabetes Care 23:1539–1544, 2000.
5. Ota K, Nakamura J, Li W, Kozakae M, Watarai A, Nakamura N, Yasuda Y, Nakashima E, Naruse K, Watabe K, Kato K, Oiso Y, Hamada Y. Metformin prevents methylglyoxal-induced apoptosis of mouse Schwann cells. Biochem Biophys Res Commun 357:270–275, 2007.
6. Shipanova IN, Glomb MA. Nagaraj RH. Protein modification by methylglyoxal: chemical nature and synthetic mechanism of a major fluorescent adduct. Arch Biochem Biophys 344:29–36, 1997.
7. Ikeda K, Higashi T, Sano H, Jinnouchi Y, Yoshida M, Araki T, Ueda S, Horiuchi S. N (epsilon)-(carboxymethyl)lysine protein adduct is a major immunological epitope in proteins modified with advanced glycation end products of the Maillard reaction. Biochemistry 35:8075–8083, 1996.
8. Jennings PE. The potential of gliclazide, a sulphonylurea to influence the oxidative processes within the pathogenesis of diabetic vascular disease. Adv Exp Med Biol 366:313–324, 1994.
9. Qiang X, Satoh J, Sagara M, Fukuzawa M, Masuda T, Miyaguchi S, Takahashi K, Toyota T. Gliclazide inhibits diabetic neuropathy irrespective of blood glucose levels in streptozotocin-induced diabetic rats. Metabolism 47:977–981, 1998.
10. Akanuma Y, Kosaka K, Kanazawa Y, Kasuga M, Fukuda M, Aoki S. Long-term comparison of oral hypoglycemic agents in diabetic retinopathy: gliclazide vs. other sulfonylureas. Diabetes Res Clin Pract 5:81–90, 1988.
11. Kinoshita N, Kakehashi A, Inoda S, Itou Y, Kuroki M, Yasu T, Kawakami M, Kanazawa Y. Effective and selective prevention of retinal leukostasis in streptozotocin-induced diabetic rats using gliclazide. Diabetologia 45:735–739, 2002.
12. Fava D, Cassone-Faldetta M, Laurenti O, De Luca O, Ghiselli A, De Mattia G. Gliclazide improves anti-oxidant status and nitric oxide–mediated vasodilation in type 2 diabetes. Diabet Med 19:752–757, 2002.
13. Mamputu JC, Renier G. Signalling pathways involved in retinal endothelial cell proliferation induced by advanced glycation end products: inhibitory effect of gliclazide. Diabetes Obes Metab 6:95–103, 2004.
14. Kobayashi K, Kimura M, Sakoguchi T, Hase A, Matsuoka A, Kaneko S. Pharmacokinetics of gliclazide in healthy and diabetic subjects. J Pharm Sci 73:1684–1687, 1984.
15. Beisswenger PJ, Howell SK, Touchette AD, Lal S, Szwergold BS. Metformin reduces systemic methylglyoxal levels in type 2 diabetes. Diabetes 48:198–202, 1999.