Vascular Benefits of Gliclazide Beyond Glycemic Control

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Advanced glycolysation end products (AGEs) and the free radicals generated in this process can both be implicated in the accelerated atherosclerosis and vascular and prothrombotic microangiopathic changes typified by diabetes. The rate of formation of free radicals is dependent on the rate of protein glycosylation and therefore the level and duration of hyperglycemia. Glycation and oxidation are inextricably linked. Increased oxidative stress due to excess free radical activity may be central to diabetic vascular disease, since endothelial cell damage, lipoprotein oxidation, and modification of platelet reactivity and the arachidonic acid cascade are all properties of free radicals and their reaction products, lipid peroxides. The importance of the demonstration of the mechanism whereby hyperglycemia contributes to vascular damage opens the possibility of scavenging free radicals, which will have effects independently of improving diabetic control. Over the past 15 years, studies have shown that gliclazide not only lowers blood glucose but also confers beneficial effects on the hemorrheologic abnormalities seen in diabetic vascular disease. Clinically, gliclazide reduces platelet reactivity and stimulates endothelial prostacyclin synthesis; it also increases fibrinolysis by its effects on tissue plasminogen activator. These effects, seen both in vitro and in vivo, are independent of glycemic control and are not seen with other sulfonylureas. In clinical studies, the beneficial effects of gliclazide on platelets have been related to a reduction in oxidative stress. This property is due to gliclazide's free radical scavenging ability that relates to the unique aminoazabicyclo-octane ring grafted onto the sulfonylurea. It is fully maintained by the gliclazide modified-release preparation. In diabetes, therefore, where increased glycation and oxidation are fundamental to the pathogenesis of diabetic vascular disease, agents such as gliclazide with its antioxidant activities may have an enhanced therapeutic role.

THE ETIOLOGY of diabetic vascular complications remains in doubt, although the development of vascular abnormalities is positively associated with prolonged hyperglycemia; improving diabetic control delays the progression of complications.[1,2] Many factors have been implicated in the development of complications, including functional abnormalities within the microcirculation, the physiologic consequences of enhanced glucose metabolism via different pathways of nonglycolytic metabolism, and genetic susceptibility. Both microangiopathy and macroangiopathy share factors in common, such as changes in endothelial cell structure and function, enhanced platelet reactivity, and an increase in oxidative stress. However, damage to endothelial cells, modification of platelet reactivity, and modulation of the arachidonic acid cascade are all properties of free radicals and their reaction products, lipid peroxides. These products are also directly cytotoxic to vascular endothelial cells.[3] Free radicals also modulate the arachidonic acid cascade, reducing the synthesis of prostacyclin while stimulating cyclooxygenase to promote platelet production of thromboxane A_2 (TXA_2).[4] This demonstrates that there is a relationship between oxidative stress and the thrombotic tendency.

OXIDATIVE STRESS

Oxidative stress occurs when there is an imbalance between oxygen-derived free radical production and scavenging. Free radicals are naturally produced by normal metabolism, but are scavenged effectively. In diabetes, however, several factors lead to an increase in the production of free radicals and a reduction in the ability to scavenge them, resulting in oxidative stress. Evidence for the involvement of reactive oxygen species in diabetes has recently been reviewed.[5] There is considerable evidence that diabetic patients are under oxidative stress.[6] Most of the early evidence came from measurement of lipid peroxidation,[7] protein modification,[8] evidence of DNA damage, and lower antioxidant defences. Superoxide dismutase (SOD), for example, is a specific antioxidant which is widespread, but its activity is impaired by glycosylation.[6] Similarly, reduced glutathione detoxifies organic peroxides producing oxidized glutathione, which is rapidly reduced back to its active form by reactions utilizing NADPH generated from "redox cycling." NADPH may be lacking in hyperglycemia, as it is a cofactor for aldose reductase in the polyol pathway.[9] Consumption of NADPH by increased flux through this pathway leaves insufficient NADPH to generate antioxidants, such as glutathione or vitamin C, and renders the tissues susceptible to free reactive oxygen species attack. There is thus considerable evidence for an increase in oxidative stress in diabetic patients, and these abnormalities are more marked in association with vascular damage, both microvascular and macrovascular.

ENDOTHELIAL DYSFUNCTION AND VASCULAR CHANGES

In addition to inducing oxidative stress, hyperglycemia leads to the accumulation of advanced glycosylation end products (AGEs). The process leading to protein glycosylation not only generates free radicals, but produces a product that reacts with nitric oxide (NO) to prevent its vasodilator and anticoagulant actions. AGE modification of proteins in the vascular wall changes its structure and function, leading, for example, to the trapping of proteins. The AGE modification of low-density lipoprotein (LDL) promotes its uptake by macrophages, leading to the formation of foam cells, which are important in the development of atherosclerotic plaques.[10]

Both oxidative stress and AGE have been linked to endothelial cell dysfunction, particularly an imbalance which has led to defective endothelium-dependent vasodilatation and increased

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endothelium-dependent vasoconstriction. The evidence supporting the abnormalities in vascular function in type 2 diabetes has recently been reviewed.[11] One of the most important factors leading to impaired vasodilatation is quenching of NO by free radicals, thereby preventing NO from diffusing effectively into target smooth muscle cells. This abnormality can be improved by adding free radical scavengers in studies on isolated blood vessels from diabetic animals.[12]

Other factors responsible for abnormalities of vascular endothelium in type 2 diabetes include abnormalities of tumor necrosis factor-α (TNF-α), transforming growth factor-β, and vascular endothelial growth factor. On top of the abnormalities of the vascular endothelium is the demonstration that diabetic patients are in a hypercoagulable state. There is a shift in the balance between coagulation and fibrinolysis, and several different mechanisms contribute to these abnormalities.[13] Type 2 diabetic patients have elevated levels of fibrinogen, coagulation factors, and inhibitors of plasminogen activation. Some patients have low levels of plasminogen activator, which is important in fibrinolysis.[14] In addition, the fibrin network within clots may be altered in diabetic patients, making this network more resistant to fibrinolysis.[15] Therefore, evidence indicates that diabetic patients exhibit a shift in the balance between coagulation and fibrinolysis towards increased blood clot formation. Furthermore, the platelets in diabetic patients are hyperreactive, showing a greater than normal tendency to adhere and aggregate.[16] This factor may well be due to increased oxidative stress as a result of the known effects of lipid peroxidation on the thromboxane to prostacyclin ratio.[4,17] Moreover, NO, which normally inhibits platelet activation, is not as available in diabetes because of the reduced output of NO by the endothelial cells.

Thus, in type 2 diabetes a substantial body of evidence shows increased free radical production and reduced antioxidant defences, with the promotion of oxidation of LDL cholesterol. This associated with AGE deposition around the blood vessels, which is linked to structural and functional dysfunction of the endothelial cells with reduced production of NO and impaired vasodilatation and increased platelet aggregation, leads to a highly prothrombotic situation. This pathologic process is clearly manifested in the diabetic patient by the widespread microvascular changes readily appreciated in the retina and kidney. In addition, these changes promote accelerated atherosclerosis, which is the main reason for the reduced life expectancy in patients with type 2 diabetes. These vascular abnormalities can be improved or even eliminated by maintaining normal blood glucose levels, as has been demonstrated in the landmark clinical studies, the Diabetes Control and Complications Trial (DCCT)[2] and the United Kingdom Prospective Diabetes Study (UKPDS).[1] In addition, agents that have antioxidant activity or prevent platelet aggregation have an enhanced therapeutic role in the management of type 2 diabetes.

NONHYPOGLYCEMIC EFFECTS OF GLICLAZIDE

Gliclazide is a second-generation sulfonylurea that not only lowers blood glucose, but has also been shown in vitro to be a general free radical scavenger. This has initially been demonstrated by the inhibition of the photo-oxidation of dianisidine with concentrations well below the expected therapeutic level.[18] However, glibenclamide and other sulfonylureas have no free radical scavenging effect, even at very high concentrations in vitro. This effect against specific reactive oxygen species has been assayed using electron spin resonance spectroscopy,[19] showing that not only did gliclazide scavenge the superoxide radical but also was effective against the more reactive hydroxyl radical. Again, this was in a dose-dependent manner, whereas glibenclamide was not effective. The free radical scavenging area of the molecule is the aminoazabicyclo-octane ring, which is not found on other sulfonylureas.

In addition to direct free radical scavenging in vitro, gliclazide is also effective against the formation in vitro of oxidized LDL cholesterol, similar to that seen with vitamin C; this is in contrast to other sulfonylureas, which had no effect.[20] Further dose-dependent antioxidant effects of gliclazide were in cell-mediated oxidation, which also inhibited the oxidized LDL-induced adhesion of monocytes to endothelial cells.[21] Monocytes produce TNF-α, which has been implicated in the progression of type 2 diabetes and its complications. Poorly controlled glibenclamide-treated diabetic patients show marked elevation in the production of TNF-α, which returned to normal following 3 months of treatment with gliclazide.[22,23]

Experimentally induced diabetes in animals produces abnormalities of endothelial function, including impairment of NO-mediated endothelium-dependent vasodilatation. This can be improved if the diabetic animals are treated with gliclazide,[24] even in streptozotocin-induced diabetes, suggesting that the action must be independent of any modification of insulin secretion.[25]

Restoration of endothelial function in diabetes by gliclazide has also been documented in human microvessels. Omental microvessels were obtained from normotensive nonsmokers undergoing surgical intervention, and endothelial dysfunction induced by the addition of glycated oxyhemoglobin. This dysfunction is thought to be NO-mediated and is mirrored by the effects of L-NAME (N^G-nitro-L-arginine methyl ester), but not by indomethacin. Gliclazide, in therapeutic concentrations, dose-dependently restored endothelial function, an effect mediated via reduced oxidative stress, as equimolar vitamin C and 100 U/L SOD have similar effects. Neither glibenclamide nor indomethacin has any effect on endothelial dysfunction in this model.[26]

CLINICAL STUDIES WITH GLICLAZIDE

Hemovascular effects of gliclazide are independent of its hypoglycemic action. For example, in a study of the effect of gliclazide on the balance between thromboxane and prostacyclin, in which patients crossed over from glibenclamide to gliclazide, diabetic control at 3 months remained constant. Both fasting plasma glucose and insulin levels had not changed, but the TXA_2:prostacyclin ratio had decreased almost to the normal range in the gliclazide-treated patients.[27] Similarly, an open study examining the effect on TXA_2 and parameters of free radical activity such as lipid peroxidation, again documented a decline in the vasoconstrictor TXA_2 together with a decline in lipid peroxides, which was independent of glycemic control.[28] The hypothesis that the beneficial hemovascular effects were

secondary to its free radical scavenging and independent of glycemic control was further investigated in a blinded glibenclamide-controlled study over a 6-month period.[29] Thirty patients with type 2 diabetes, 20 of whom were male, with a mean age of 58 years, were recruited to the study. All patients had been treated for diabetes for more than 2 years (mean, 8 years) and had been established on glibenclamide for at least 2 years, with or without adjunctive metformin. On entry, half were randomly allocated to change to gliclazide at a dose equipotent to the dose of those remaining on glibenclamide. The baseline characteristics of the patients are listed in Table 1.

At 3 months, diabetic control was unaltered, but there were significant improvements in the oxidative status of the gliclazide-treated patients. Lipid peroxides decreased (8.3 ± 1.1 to 7.0 ± 0.6 μmol/L, $P < .01$) and red blood cell SOD increased (135 ± 21 to 152 ± 36 μg/mL, $P < .05$). Plasma thiol (PSH) levels were unaltered at 458 ± 38 μmol/L, while they had decreased significantly in the glibenclamide-treated patients (414 ± 34 μmol/L, $P < .05$), resulting in a significant difference between the 2 treatment groups ($P < .004$). Platelet reactivity to collagen also improved in the gliclazide-treated patients, decreasing from 65.1% ± 14% to 50.8% ± 24% ($P < .01$). Reactivity of the platelets was unaltered in the glibenclamide-treated patients. At 6 months, the significant differences between the 2 treatment groups remained, although there were no further improvements in the gliclazide-treated patients (Table 2).

In a recent double-blind study conducted in Australia, significant improvements in a number of oxidative parameters (8-isoprostanes, total plasma antioxidant capacity, SOD, PSH) were seen over 10 months of treatment of type 2 diabetic patients with gliclazide. Support for the glycemia-independent nature of this effect comes from the observation that improvements continued between the fourth and tenth month, whereas there was a plateau in glycemic control. The new gliclazide-modified release, given once daily in the morning, was at least as effective as the existing gliclazide preparation on these parameters.[30]

This study also showed that gliclazide reduces the hyperreactivity of the platelets. This was first shown in a short-term study in 1982 on 18 diabetic patients treated for 30 days.[31] In addition to these effects seen in the clinical studies on platelet activity and free radicals, benefits have also been shown in correcting the imbalance between blood coagulation and fibrinolysis. For example, patients switched from chlorpropamide to gliclazide normalized their previously low vascular plasminogen activator activity, and the inhibitors of plasminogen activator were significantly reduced after 48 months of therapy.[32] A similar study showed significant improvements in tissue plasminogen activator activity.[14]

Table 1. Baseline Characteristics of the Patient Groups

	Gliclazide-Treated (n = 15)	P*	Glibenclamide-Treated (n = 14)
Blood glucose (mmol/L)	8.6 ± 3.1	.16	10.6 ± 4.7
HbA_1 (%)	8.2 ± 2.3	.23	9.1 ± 2.0
Lipid peroxides (μmol/L)	8.3 ± 1.1	.11	9.0 ± 1.2
PSH (μmol/L)	458 ± 42	.70	451 ± 63
SOD (μg/mL)	135 ± 21	.75	132 ± 19
Platelet aggregation (%)	65.1 ± 14	.97	70.2 ± 14

NOTE. Results are presented as the mean ± SD.

*Significance tested by Mann-Whitney *U* test comparing gliclazide-treated with glibenclamide-treated patients.

Abbreviations: HbA_1, glycosylated hemoglobin; PSH, plasma thiols; SOD, superoxide dismutase.

Table 2. Six-Month Characteristics of the Patient Groups

	Gliclazide-Treated (n = 15)	P*	Glibenclamide-Treated (n = 14)
Blood glucose (mmol/L)	8.8 ± 2.9	.63	9.6 ± 4.5
HbA_1 (%)	8.8 ± 2.3	.51	9.3 ± 2.3
Lipid peroxides (μmol/L)	7.2 ± 0.7	.009	8.8 ± 1.9
PSH (μmol/L)	449 ± 42	.007	418 ± 30
SOD (μg/mL)	158 ± 33	.003	117 ± 27
Platelet aggregation (%)	49.3 ± 22	.004	72.4 ± 19

NOTE. Results are presented as the mean ± SD.

*Significance tested by Mann-Whitney *U* test comparing gliclazide-treated with glibenclamide-treated patients.

SUMMARY

Much of the morbidity and mortality of type 2 diabetes is due to the microvascular and macrovascular abnormalities. The changes induced by prolonged hyperglycemia are wide-ranging. Fundamental to these are effects of excess reactive oxygen species. This increase in free radical activity coupled to an impairment of antioxidant defences produces changes in the function of the endothelium and the endothelial cells which may be central to the process, leading to end-stage vascular disease. These vascular complications can be prevented or their progression slowed by achieving good diabetic control. However, in type 2 diabetes, clinical benefit has been difficult to demonstrate because of the multifactorial nature of large vessel complications, ischemic heart disease in particular. Nevertheless, improving the oxidative status of patients by using gliclazide for its hemovascular properties independent of its hypoglycemic action has led to improvements in oxidative stress, hypercoagulability, endothelial function, and platelet reactivity, which have not been demonstrated by other sulfonylureas. Gliclazide modified release has been demonstrated in double-blind trial conditions to retain the antioxidant properties of the twice-daily gliclazide form.[30] This may have clinical relevance, as suggested by the Japanese Retinopathy Programme, which studied the progression of retinopathy over a 5-year period and found a lower rate of deterioration of retinopathy with significantly lower incidence of preproliferative retinopathy occurring in the group of patients receiving gliclazide, compared with other patients, despite equivalent metabolic control.[33]

In conclusion, in type 2 diabetes, atherosclerosis coexists in the majority of patients and often predates the clinical diagnosis of diabetes. The presence of atherosclerosis, which often determines the ultimate fate of the patient, further increases the level of lipid peroxidation, thus amplifying the effects of hyperglycemia and potentiating vascular damage. Therefore, in type 2 diabetes, where hyperglycemia and oxidation are fundamental to the ultimate thrombotic complications of diabetes, agents such as gliclazide, and gliclazide modified release, with antioxidant activities may have an enhanced therapeutic role.

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