Effect of α-lipoic acid on gliclazide-induced hypoglycemia/antihyperglycemia in normal/alloxan-induced diabetic rats

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Background: Gliclazide is a widely used drug for the treatment of Type 2 diabetes. α-lipoic acid is an ingredient of antioxidant formulations used as health supplements along with antidiabetic drugs. The role of α-lipoic acid on the hypoglycemic activity of gliclazide is not currently known. **Objective**: The objective of this study was to examine the effect of oral administration of α-lipoic acid on blood glucose and investigate its potential influence on gliclazide-induced hypoglycemia in normal and diabetic rats. **Materials & methods:** Albino rats of either sex were divided into three groups of six each and were fasted for 18 h prior to the experiment with water *ad libitum* – the rats continued to fast throughout. Groups I/II/III were treated with α-lipoic acid at half the therapeutic dose (½TD)/TD/twice TD of humans extended to 200 g rat, respectively. Later in the study, group II rats were treated with gliclazide TD (1.44 mg/200 g body weight of rat)/α-lipoic acid TD + gliclazide TD with a washout period of 1 week between treatments. Diabetes was induced by alloxan monohydrate 100–150 mg/kg body weight intraperitoneally. A group of six rats showing fasting blood glucose levels above 200 mg/dl were selected for the study. Rats were treated with α-lipoic acid TD, gliclazide TD and α-lipoic acid TD + gliclazide TD with a washout period of 1 week between treatments. Blood samples were collected from the retro-orbital plexus at 0, 1, 2, 3, 4, 6, 8, 10 and 12 h and were analyzed for blood glucose by the Glucose Oxidase/Peroxidase method. **Results:** α-lipoic acid TD produced hypoglycemia with peak effect at 6 h in normal and diabetic rats. The percentage blood sugar reduction was greater in diabetic than normal rats. Gliclazide TD produced hypoglycemia with peak effects at 2 and 8 h in normal and diabetic rats. In combination, α-lipoic acid TD enhanced and significantly prolonged the glucose-lowering effect of gliclazide TD at 1 and 6 h in normal rats and 2, 6 and 8 h in diabetic rats without hypoglycemic convulsions. **Conclusion:** Thus, it can be concluded that the use of lipoic acid in supplemental formulations in conjunction with gliclazide may be safe in diabetic patients. However, further studies are warranted.

Keywords: antihyperglycemia, blood glucose, diabetes, hypoglycemia

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose levels and disturbances in carbohydrate, fat and protein metabolism. It may be due to a decrease in the synthesis of insulin (Type 1) or decrease in the secretion of insulin (Type 2) from the β-cells of the islets of Langerhan's in the pancreas. Type 2 DM constitutes more than 95% of diabetic patients in India. Its prevalence is constantly increasing and has already reached epidemic proportions, particularly in urban India. It is thought that by the year 2025, India will have approximately 57.2 million diabetic patients, the most in any one country [1].

The initial treatment of Type 2 DM has always been optimization of diet and physical activity. However, the benefits of nonpharmacological therapy are not dependable due to the noncompliance of patients to the prescribed diet and exercise. Currently available sulfonylureas and biguanides initially work well as therapeutic agents, but failure rates are increasing on long-term therapy [2]. The limitations of currently available pharmacological agents for the control of blood glucose has promoted doctors to prescribe supplemental agents with antidiabetic drugs. Since DM is associated with stress, antioxidants are used as one of these supplemental agents. However, their influence on antidiabetic therapy is not known. Recent investigations in our laboratory were aimed at the effect of antioxidants, such as vitamin C and selenium, on antidiabetic therapy in normal and diabetic rats [3,4]. In the present study, the effect of α-lipoic acid (LA) on gliclazide activity in normal and diabetic rats was studied.

Materials & methods

Albino rats of either sex procured from Mahaveer Enterprises (Hyderabad, India) were used in the study. They were maintained under standard

Future Drugs Ltd

Table 1. Mean percent blood glucose reduction in normal rats (n = 6).

Time (h)	α-lipoicacid (TD)	Gliclazide (TD)	α-lipoicacid (TD) + gliclazide (TD)
0			
1	6.66 ± 1.06	23.83 ± 0.98	33.52 ± 2.16*
2	9.90 ± 0.87	31.95 ± 1.28	41.06 ± 4.32
3	14.33 ± 1.10	27.64 ± 1.84	30.73 ± 2.37
4	17.75 ± 1.38	21.10 ± 1.94	27.20 ± 2.66
6	18.90 ± 1.21	25.87 ± 1.20	34.24 ± 2.64*
8	12.64 ± 1.65	34.06 ± 1.08	42.22 ± 3.47
10	9.5 ± 1.68	24.66 ± 1.60	32.46 ± 3.60
12	8.43 ± 1.66	16.26 ± 2.10	23.62 ± 2.84

Significant at $p < 0.05$, gliclazide response compared with combination. TD: Therapeutic dose.

laboratory conditions at ambient temperature of 25 ± 2°C and 50 ± 15% relative humidity with a 12-h light/12-h dark cycle. Rats were fed with a commercial pellet diet (Rayan's Biotechnologies Pvt. Ltd) and water *ad libitum*. The experimental protocol was approved by the Institutional Ethics Committee and the regulatory body of the government (Reg. NO.516/01/A/CPCSEA). Rats were fasted for 18 h prior to the experiment (allowing access to water) and, during the experiment, food and water were withdrawn.

Gliclazide was supplied by Aristo Pharma, Mumbai, India. Glucose kits (Span diagnostics) were purchased from the local pharmacy. Alloxan monohydrate was purchased from LOBA Chemie, Mumbai, India.

Study design

In clinical practice, LA is used as a supplement in several disorders and gliclazide is used as an oral hypoglycemic agent in DM. Hence, human therapeutic doses (TDs) of gliclazide extrapolated to rat based on body surface area [5] were used in the study and the influence of LA on the response of gliclazide at the TD)was determined in normal and diabetic rats.

Study in normal rats

Group I/II/III were treated with LA at half-TD (0.9 mg/200 g rat)/TD (1.8 mg/200 g rat)/2TD (3.6 mg/200 g rat), respectively. Later, group II rats were treated with gliclazide TD (1.44 mg/200 g rat)/LA TD + gliclazide TD with a washout period of 1 week between treatments.

Study in diabetic rats

Albino rats of either sex were treated with alloxan monohydrate 100–150 mg/kg body weight intraperitoneally to induce DM. A group of six rats showing fasting blood glucose levels above 200 mg/dl were selected for the study. Rats were treated with LA TD, gliclazide TD and LA TD + gliclazide TD with a washout period of 1 week between treatments.

Collection of blood samples

Blood samples were collected from the retro-orbital plexus at 0, 1, 2, 3, 4, 6, 8, 10 and 12 h. Blood glucose was estimated by the Glucose Oxidase/Peroxidase (GOD–POD) method [6].

Data & statistical analysis

Data were expressed as mean ± standard error of mean (SEM). The significance of blood glucose reduction produced by LA TD + gliclazide TD compared with gliclazide TD was determined by applying student's paired t test. p values of less than 0.05 were considered to be statistically significant.

Results

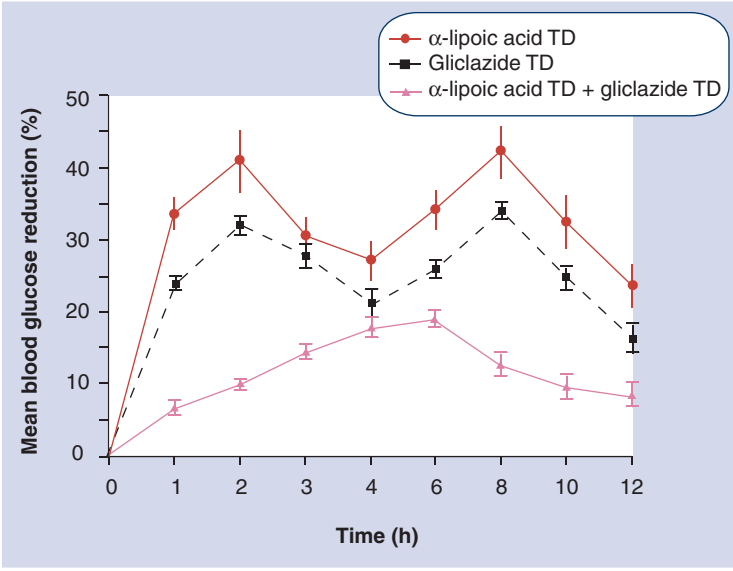
Effect in normal rats

LA half-TD, TD and 2TD produced dose-dependent hypoglycemia with peak effect at 6 h in group I/II/III, respectively. Gliclazide TD produced hypoglycemia with a peak effects at 2 and 8 h in group II. In the same group, prior administration of LA TD enhanced gliclazide TD response with peak effects at 2 and 8 h and prolonged the hypoglycemic effect. The percentage values of blood glucose reduction with LA TD, gliclazide TD and LA TD + gliclazide TD are given in **Table 1** and represented in **Figure 1**. Significant variation in blood glucose reduction was observed at 1 and 6 h.

Effect in diabetic rats

LA TD produced peak antihyperglycemic effect at 6 h with a 10–29% reduction in blood glucose at the rest of the intervals. Gliclazide TD produced a

Figure 1. Effect of α-lipoic acid/gliclazide and their combination on blood glucose reduction in normal rats (n = 6).



TD: Therapeutic dose.

22–34% reduction in blood glucose at other intervals with peak antihyperglycemic effects at 2 and 8 h. The combination produced a 24–39% reduction in blood glucose at other intervals with peak antihyperglycemic effects at 2 and 8 h with significant variation at 2, 6 and 8 h. The percentage blood glucose reduction with LA TD, gliclazide TD, LA TD + gliclazide TD are given in **Table 2** and represented in **Figure 2**.

Discussion

Oxidative stress has been implicated in the pathogenesis of various diseases [7]. Consequently, the potential therapeutic or preventive effects of antioxidants has been raised [8]. Both poorly controlled Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) DM are characterized by reduced capacity of peripheral tissues (i.e., skeletal muscle and adipose tissue) to respond to the metabolic effects of insulin. The causes and cellular mechanisms responsible for this abnormality are still not fully understood despite intense investigative effort. Several lines of evidence suggest that increased oxidative stress occurs in DM and could have a role in the development or deterioration of peripheral insulin resistance. Markers of increased oxidative stress [8–13] and reduced levels of antioxidants [14–19] are found in blood and tissue in both human and experimental DM. Evidence has been accumulated in the past few years supporting the theory that DM is precipitated by stress [20,21]. Additionally, it has also been reported that hyperglycemia itself increases stress [22]. Thus, it is logical to think that antioxidants can prevent precipitation of DM and also control hyperglycemia. Recently, LA has attracted interest due to its diverse biological actions, attributed to its chemical properties [15,16]. It is both lipid and water soluble [14], has a potent antioxidative capacity in a wide variety of experimental systems and is a cofactor of key regulatory enzymes, including the pyruvate dehydrogenase complex [16]. LA has been shown to improve glucose metabolism in diabetic subjects [23,24] and has long been used in Germany for the relief of symptoms of diabetic neuropathy [25–28].

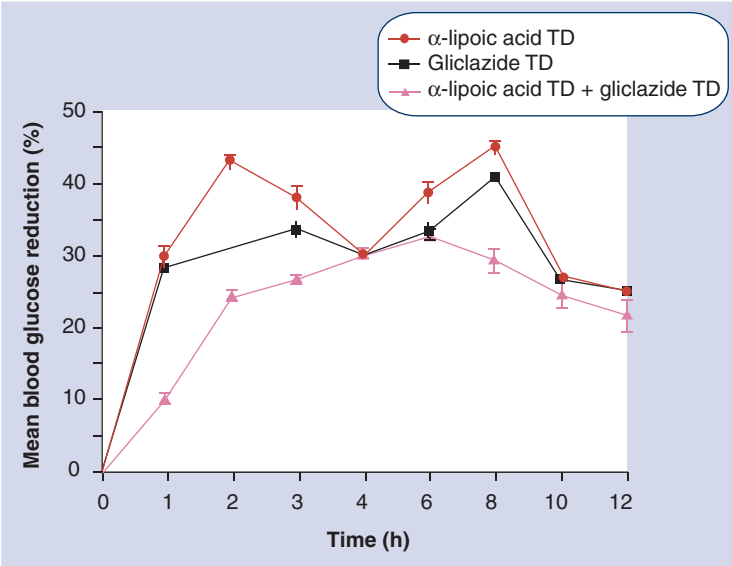
The present study was conducted in rats with LA as an antioxidant. LA was selected because it is used as an ingredient in antioxidant formulations that are used in therapy. Rat was used since it is an animal model used routinely for quick screening of drugs for their hypoglycemic/antihyperglycemic action. Since small amounts of blood were required for glucose analysis, the blood samples were collected by

Table 2. Mean percent blood glucose reduction in diabetic rats (n = 6).

Time (h)	α-lipoicacid (TD)	Gliclazide (TD)	α-lipoicacid (TD) + gliclazide (TD)
0			
1	10.43 ± 0.68	28.41 ± 1.32	30.16 ± 1.27
2	24.63 ± 0.68	39.74 ± 0.47	43.17 ± 0.92*
3	26.97 ± 0.40	33.72 ± 0.90	38.13 ± 1.62
4	29.79 ± 0.82	29.26 ± 0.55	29.95 ± 0.98
6	33.07 ± 1.04	33.76 ± 0.95	38.83 ± 1.36*
8	29.38 ± 1.62	40.88 ± 0.54	45.17 ± 0.89*
10	24.92 ± 2.10	26.63 ± 1.30	27.55 ± 0.70
12	21.80 ± 2.28	22.01 ± 0.81	24.88 ± 0.91

**Significant at p < 0.05 , gliclazide response compared with combination.*
TD: Therapeutic dose.

Figure 2. Effect of α-lipoic acid/gliclazide and their combination on blood glucose reduction in diabetic rats (n = 6).



TD: Therapeutic dose.

retro-orbital puncture as it has been reported to be a good method when small samples of blood are required [29]. DM was induced with alloxan monohydrate since it was more economical, easily available and has been reported to cause DM by damaging the pancreas due to free radical-related mechanisms [30].

Highlights

- The effect of α-lipoic acid (LA) on blood glucose and gliclazide-induced hypoglycemia in normal and diabetic rats was studied.
- LA produced dose-dependent hypoglycemia in normal rats.
- Gliclazide produced biphasic hypoglycemia in normal and diabetic rats.
- LA enhanced and prolonged the hypoglycemic activity of gliclazide in normal and diabetic rats without convulsions.
- From the study, it is concluded that LA may be a safe and effective antioxidant supplement when used with gliclazide in diabetic patients.

Conclusions

The results indicate that oral administration of LA alone dose-dependently reduced blood glucose levels. Gliclazide is known to produce hypoglycemic/antihyperglycemic activity by pancreatic [31–33] and extrapancreatic [34–36] mechanisms. The biphasic peak action on blood glucose reduction may be due to the enterohepatic cycling of gliclazide in rats, partly due to its biliary excretion [37,38]. Supplementation of LA TD to gliclazide TD enhanced and prolonged the gliclazide activity in both normal and diabetic rats without affecting the biphasic effect of gliclazide, indicating that it does not interfere with its enterohepatic circulation. LA was reported to act as a hypoglycemic agent through stimulation of basal and insulin-activated glucose uptake and resulted in improvement of glucose metabolism in diabetic subjects [25,39]. Evidence from existing studies also indicates that it can improve glucose transport into the cells [40,41]. However, the fate of glucose after transport in the presence of LA is understood poorly [42]. LA TD enhanced and prolonged the effect of gliclazide TD without producing hypoglycemic convulsions. Thus, it can be concluded that it may be safe for use as a supplement with gliclazide.

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