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SHORT COMMUNICATION

METABOLIC EFFECTS OF THIOCTIC ACID IN RODENT MODELS OF INSULIN RESISTANCE AND DIABETES

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SUMMARY

1. The antioxidant thioctic acid (TA) has been used in the treatment of diabetic neuropathy and recent studies have suggested that TA also has pancreatic and peripheral effects that improve glucose transport and metabolism. In the present study, the metabolic effects of TA were evaluated in rodent models of insulin resistance (fructose-fed Sprague-Dawley rat) and insulin deficiency (streptozotocin (STZ)-induced diabetic rat). Oral and intravenous glucose tolerance tests (OGTT and IVGTT, respectively) were performed in conscious rats after treatment with 50 mg/kg per day TA or vehicle for 5 days.

2. Fructose feeding for 7 days induced insulin resistance and impaired glucose tolerance and hypertriglycerideaemia. Treatment of fructose-fed rats with TA had no significant effect on fasting or stimulated glucose levels or on fasting triglyceride concentrations (e.g. the area under the curve for glucose (AUC_{glu}) following OGTT was 1233±67 and 1284±59 in fructose-fed rats treated with either TA ($n = 12$) or vehicle ($n = 12$), respectively). Similarly, TA had no significant effect on IVGTT profiles in fructose-induced insulin resistance.

3. Low-dose STZ (80 mg/kg, i.p, over 2 days) induced hyperglycaemia, but TA had no significant glucose-lowering effects in STZ-diabetic rats (AUC_{glu} (OGTT) following oral administration was 5507±27 and 5450±27 in TA ($n = 12$) and vehicle-treated ($n = 12$) rats, respectively). Nor did pretreatment with TA affect the diabetogenic response to STZ.

4. In contrast with previous *in vitro* studies reporting favourable metabolic effects of TA, the present study shows that after short-term oral therapy there are no significant improvements in glucose tolerance in rodent models of insulin resistance and insulin deficiency. Thioctic acid is unlikely to be of therapeutic benefit as an anti-diabetic drug in clinical practice.

Key words: diabetes, insulin resistance, thioctic acid.

INTRODUCTION

The naturally occurring antioxidant thioctic acid (TA; 1,2-dithiolane-3-pentanoic acid), also known as α-lipoic acid, is a cofactor in multi-enzyme complexes that catalyse the oxidative decarboxylation of α-keto acids, such as pyruvate, α-ketoglutarate and branched chain α-keto acids.[1] Thioctic acid is transported across the cell membrane and is converted by NADPH-dependent enzymes to dihydrolipoic acid (DHLA), which has a number of cellular actions, including free radical scavenging,[2] decreased lipid peroxidation, increased glutathione levels and reduced tumour necrosis factor (TNF)-α-induced nuclear factor (NF)-κB activation in T lymphocytes.[3] Because exogenous TA is readily absorbed from the diet,[1] pharmacological studies of TA supplementation have been undertaken in a variety of conditions where free radicals are thought to play an important role in symptoms and disease progression.[2]

Thioctic acid is licensed in parts of Europe for the treatment of patients with painful diabetic neuropathy,[4] but recent animal and human studies have suggested that TA may also improve glucose metabolism and insulin secretion via effects on peripheral tissues and the pancreas.[5–7] In particular, it has been shown that TA enhances insulin-mediated glucose transport in a genetic rodent model of obesity and type II diabetes[5] as well as in isolated muscle cells[6] and that the antioxidant effects of TA preserve pancreatic function in the cyclophosphamide-induced model of type I diabetes in NOD mice.[8]

The purpose of these present studies was to evaluate the short-term effects of TA on glucose and lipid metabolism in rodent models of insulin deficiency and insulin resistance. The fructose-fed rat[9,10] is an established dietary induced model of insulin resistance and hyperinsulinaemia independent of obesity and low-dose streptozotocin (STZ), via destruction of pancreatic beta-cells, and provides an experimental model of type 1 (insulin-deficient) diabetes.

METHODS

A total of 120 male Sprague-Dawley (SD) rats (Gore Hill Research Laboratories, Sydney, NSW, Australia), aged 5–6 weeks, were used in a series of protocols that were approved by the Animal Care Ethics Committee of the University of Sydney. Animals were housed in cages and were maintained in a temperature-controlled environment (22°C) with a 12 h light–dark cycle and free access to water and diet. Rats fed a fructose-enriched diet had their usual laboratory chow substituted with a peletted high-fructose diet (TD78463; Teklad Laboratories, Madison, WI, USA), as described previously.[10] Thioctic acid was suspended in 5% polyethylene glycol (PEG) for administration by oral gavage. In each study, TA (50 mg/kg

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per day) or vehicle 5% PEG was given for 5 days with experimental procedures undertaken on the fifth day.

Effects of TA on glucose tolerance

Oral and intravenous glucose tolerance tests (OGTT and IVGTT, respectively) were performed in groups of normal and fructose-fed SD rats after 5 days treatment with TA (50 mg/kg per day) or PEG. A fructose-enriched diet was commenced 7 days before starting treatment with TA and continued throughout the period of drug administration. The OGTT (glucose 3 g/kg bodyweight) was performed at baseline (prior to fructose feeding) and at the end of the TA treatment period in overnight-fasted rats; tail vein blood samples were collected before and at 30 min intervals for 3 h after glucose administration.

The IVGTT (glucose 0.5 mg/kg bodyweight) was performed in conscious animals at the end of the TA treatment period; each animal was anaesthetized (ketamine and xylazine) 3 days earlier for implantation of a jugular venous cannula and allowed to recover. Blood samples were withdrawn from the cannula before and at 2, 5, 10, 15, 30 and 60 min after bolus administration of glucose.

Effects of TA on STZ-induced diabetes

In one study, the effects of TA in STZ-induced diabetic rats were assessed while a second protocol was undertaken to explore whether pretreatment with TA would ameliorate the hyperglycaemic effect of STZ (80 mg/kg, i.p. over 2 days). Oral glucose tolerance was measured at baseline, prior to STZ administration (40 mg/kg bodyweight, i.p., on 2 consecutive days), then fasting serum glucose levels were measured 1 week later to confirm the development of diabetes. Animals with STZ-induced diabetes were then treated for 5 days with TA 50 mg/kg per day or 5% PEG and the OGTT was repeated at the end of the treatment period.

In the second study, the effects of pretreatment with TA on the hyperglycaemic effect of STZ were investigated. Groups of normal SD rats were treated with TA or 5% PEG for 5 days prior to administration of STZ (40 mg/kg bodyweight, i.p. on 2 consecutive days) with OGTT performed before and 7 days after STZ administration.

Laboratory methods

Blood samples were collected for measurement of serum glucose and triglyceride concentrations using standard enzymatic assays, as described previously.[9]

Statistical analysis

Area under curve (AUC_{glu}) values were obtained for each glucose tolerance test and data were compared by two-way analysis of variance. Fasting glucose and triglyceride levels were compared between TA- and vehicle-treated animals using paired *t*-tests and all data are presented as the mean±SEM. Statistical significance was set at the 5% level.

RESULTS

Thioctic acid was generally well tolerated and there were no differences in weight gain or behaviour between TA- and vehicle-treated rats. As reported previously,[9] fructose feeding was associated with marked increases in fasting triglyceride levels and impaired glucose tolerance. Treatment with TA for 5 days had no significant effect on fasting and peak stimulated glucose levels or AUC_{glu} after OGTT and IGTT in fructose-fed rats (Table 1). Similarly, in normal SD rats, there was no detectable effect of TA on intravenous glucose tolerance: for example, AUC_{glu} was 652±41 and 622±27 in TA- and vehicle-treated animals, respectively.

Administration of low-dose STZ (80 mg/kg bodyweight) produced hyperglycaemia without inducing a severe catabolic state and weight loss, but in STZ-induced diabetic rats TA had no significant glucose-lowering effect; for example, AUC_{glu} after OGTT was 5507±27 and 5450±27 in TA and vehicle-treated rats, respectively. In addition, there was no evidence to support the hypothesis that pretreatment with TA would ameliorate the diabetogenic effect of STZ. Glucose tolerance tests were similar in rats pretreated with TA compared with vehicle (Table 1).

DISCUSSION

These studies have shown that short-term treatment with TA at 50 mg/kg per day for 5 days has no significant glucose-lowering effect in well-established rodent models of insulin resistance and insulin deficiency. The IVGTT is usually the most sensitive method for detecting modest effects of drug treatment on glucose tolerance, but IVGTT profiles in both normal and diabetic rats were similar in TA- compared with vehicle-treated groups. These results contrast with recent *in vitro* studies that have shown improvements in both glucose transport and metabolism in muscle and liver tissues in a variety of experimental models,[5,6] as well as a preliminary clinical study showing that TA has favourable metabolic effects in patients with type II diabetes.[7] For example, glucose-lowering effects with TA were reported in diabetic rats and rabbits with secondary improvements in glycogen and fat synthesis.[11] More recently, increased rates of glycogen synthesis and glucose oxidation were reported in

Table 1 Oral and intravenous glucose tolerance tests following thioctic acid treatment

	Normal SD		Fructose-fed SD		STZ-diabetic rats		Pretreated STZ-diabetic rats*	
	TA (n = 12)	Vehicle (n = 12)	TA (n = 12)	Vehicle (n = 12)	TA (n = 12)	Vehicle (n = 12)	TA (n = 12)	Vehicle (n = 12)
OGTT								
Fasting Glu (mmol/L)	–	–	6.6±0.7	6.6±0.6	22.9±0.3	24.9±0.3	25.1±1.4	25.8±1.1
Peak Glu (mmol/L)	–	–	9.4±0.4	8.9±0.5	40.4±0.3	40.1±0.3	40.3±1.1	38.6±1.1
AUC_{Glu}	–	–	1233±67	1284±59	5507±27	5450±27	5741±103	5783±156
Fasting TG (mmol/L)			4.0±0.5	6.1±1.3				
IVGTT								
Fasting Glu (mmol/L)	7.4±0.7	7.2±0.6	16.7±1.5	17.7±1.2				
Peak Glu (mmol/L)	20.7±1.1	23.5±2.6	34.2±1.4	41.5±5.6				
AUC_{Glu}	652±41	622±27	1701±119	1830±84				

*Rats were pretreated with thioctic acid (50 mg/kg per day) for 5 days prior to the tests.

SD, Sprague-Dawley rats; STZ; streptozotocin; OGTT, oral glucose tolerance test; IVGTT, intravenous glucose tolerance test; Glu, glucose; TG, triglycerides.

muscle tissues from obese Zucker rats (a genetic model of obesity and type II diabetes), with associated reductions in free fatty acid levels.[5] Similarly, in isolated liver[12] and muscle[6] cells, TA appears to enhance insulin-mediated effects. In models of type I diabetes, Khamaisi *et al.*[13] showed reductions in glucose levels associated with increased GLUT 4 expression in STZ-induced diabetic rats. In addition, the antioxidant effects of TA have been linked to preservation of pancreatic beta-cells in cyclophosphamide-induced diabetes,[8] but in the present study there was no evidence that pretreatment with TA for 5 days protected the rat pancreas from beta-cell destruction by low-dose STZ.

The negative results of the present study may reflect differences in the dose and route of administration of TA compared with previous studies. Our studies investigated short-term (5 days) treatment with TA at 50 mg/kg per day, but previous studies in which favourable metabolic effects of TA have been reported used similar or higher doses of TA for longer treatment periods[14] and often intraperitoneal rather than oral administration.[7,8] In addition, the metabolic effects of R(+)-TA are greater than S(−)-TA or the racemic mixture[6] and it is possible that isoform-specific effects were obscured in the present study.

Nevertheless, the results of the present study suggest that oral treatment with TA is unlikely to have clinically useful anti-diabetic effects in patients with hyperglycaemia.

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