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Long-term taurine supplementation reduces mortality rate in streptozotocin-induced diabetic rats

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Summary. Oxidative stress is implicated in the pathogenesis of diabetes mellitus. Taurine and vitamin E + selenium supplementation has some benefits in experimental models of diabetes mellitus. This study evaluates whether taurine and vitamin E + selenium supplementations reduce a hard end-point such as mortality due to diabetes. Streptozotocin-induced diabetic rats were fed with standard diet or taurine (5%, w/w) or vitamin E (500 UI/Kg) + selenium (8 mg/Kg) enriched diets. Taurine significantly decreased mortality rate ($p < 0.04$), while vitamin E failed to increase survival. In the late phase of the disease, taurine significantly decreased glycaemia, being vitamin E ineffective. No correlation between glycaemia and survival was found. None of supplementations modified body weight. Thus, only taurine decreases the mortality rate and glycaemia. These results encourage new research in the field, since classical hypoglycaemic agents are unable to decrease mortality in diabetic patients.

Keywords: Taurine – Streptozotocin – Diabetes mellitus – Selenium – Vitamin E – Survival – Glycaemia

Introduction

In the last years, diabetes mellitus has reached epidemic proportion and is now becoming cause of premature mortality and morbidity (Zimmet, 2002). Indeed, the use of intensive hypoglycaemic therapies such as insulin, sulfonylureas appears unsuccessful UK Prospective Diabetes Study (UKPDS) Group (1998) in reducing mortality.

Antioxidants has been proposed in the therapy of diabetic patients (Devaraj and Jialal, 2000) because this metabolic disease is characterised by an increased production of oxidative species and by a decrease in antioxidant defences (Baynes and Thorpe, 1999; Lipinski, 2001; Santini et al., 1997; Seghieri et al., 1998; Marra et al., 2002). Consequently, the correction of oxidative stress may have important implications in preventing diabetes-induced complications and in reducing diabetic mortality.

Taurine is a scavenger of hypochlorite and of carbonyl radicals with ancillary properties (Huxtable, 1992; Eppler and Dawson, 2001; Franconi et al., 2004). This amino acid seems also to have indirect antioxidant effects (Nonaka et al., 2001; Keys and Zimmerman, 1999; Schaffer et al., 2000). In addition, *in vivo* and *in vitro*, taurine is antihypoxic (Franconi et al., 1985; Malcangio et al., 1989). Taurine treatment shows beneficial effects in experimental diabetes mellitus models (Hansen, 2001; Franconi et al., 2004; Mozafari et al., 2003) and in clinical short study (Hansen, 2001; Franconi et al., 2004). On the other hand, in a double-blind trial of one-year duration, taurine failed to prevent kidney complications associated with type 2 diabetes mellitus (Odetti et al., 2002), and 4 months of taurine supplementation, in 2 type diabetic patients with bad metabolic control, failed to decrease glycosilated haemoglobin and glucose (Chauncey et al., 2003).

Vitamin E is a fat soluble vitamin known to be one of the most potent antioxidant that breaks the propagation of the free radical chain reaction in the lipid molecules (Sies et al., 1992). Selenium is essential for glutathione peroxidase activity. Previously, it has been shown that the vitamin E + selenium supplementation in relatively short-term studies delays retinal and renal alterations in streptozotocin

Table 1. Body weight (g) in diabetic rats fed with standard (D-rats) or taurine (D-Taurine rats) or vitamin E + selenium (D-vitamin E + selenium rats) diets

	Time (days) from the onset of diabetes					
	0	60	120	360	450	540
D-rats	239 ± 12 (23)	261 ± 27 (19)	299 ± 14 (19)	303 ± 51 (9)	317 ± 95 (7)	313 ± 29 (3)
D-Taurine rats	239 ± 12 (23)	285 ± 91 (23)	262 ± 31 (22)	302 ± 50 (18)	316 ± 95 (12)	337 ± 42 (8)
D-vitamin E + selenium rats	239 ± 12 (24)	254 ± 23 (22)	225 ± 27 (20)	311 ± 42 (7)	321 ± 27 (5)	336 ± 26 (4)

Values are means ± SD, in brackets the number of experiments

(STZ)-diabetic rats (Di Leo et al., 2003; Douillet et al., 1996).

Therefore, we investigated whether taurine or vitamin E + selenium supplementations, started after the onset of diabetes mellitus, reduce the mortality in STZ diabetic rats.

Materials and methods

Male Wistar rats, eight week-old, were purchased from Harlan-Nossan (Milan, Italy), they were housed under controlled temperature conditions (22 ± 1°C) and humidity (60%) and in a controlled environment with alternating 12-hour reversed light–dark cycle. The rats had free access to food and water. Riefer (Bolzano, Italy) supplied all diets. All experiments were performed according to the Guidelines of the American Physiology Society. After 1 week of acclimatization, rats were injected with a single intraperitoneal injection of 60 mg/Kg STZ (Sigma, St. Louis, MO, USA). Induction of diabetes was confirmed by determining of glucose (Lifescan, Milpits, CA, USA) in blood obtained from the tail vein. Rats with glucose above >16 mmol/L were considered diabetic.

Soon after the STZ injection, animals were randomly assigned to different three experimental groups. Three independent experimental groups were studied: diabetic rats fed with standard diet (which includes 80 UI/Kg of alpha-tocopherol but not selenium), diabetic rats fed with a 5% w/w enriched taurine diet, and diabetic rats fed with a 500 IU vitamin E + 8 mg selenium/Kg enriched diet. The doses of antioxidants were selected according to Di Leo (2003). Mortality rate, glycaemia and body weight were measured. Rats were followed for over two years until the death of each one. A group of 10 non-diabetic rats was selected for evaluating the intercurrent period between the birth and the death. Only two normal rats died before two years for little-known causes. The rats naturally died within 800 days.

Statistical analysis

Survival data were examined using Kaplan-Meier survival analysis. Comparisons between groups were made using one-way ANOVA values corrected for multiple comparisons by Fisher method. Significance was defined at $p < 0.05$.

Results

Taurine and vitamin E + selenium supplementation did not influence the body weight in diabetic animals (Table 1). As shown in Fig. 1, glucose concentrations in rats, one month after STZ treatment, increased significantly. In rats fed with standard diet and in vitamin E + selenium supplemented rats, glucose remained constant for 12 months, then it tended to decrease without reaching the significance. In taurine supplemented rats, glycaemia was reduced in a time-dependent manner and the decrease reached the statistical significance ($p < 0.05$) after 15 months supplementation. No significant difference between the 0 time and 15 months after the beginning of diabetes in taurine-treated rats was found, although higher blood levels of glycaemia at the end of the study have been shown in this group.

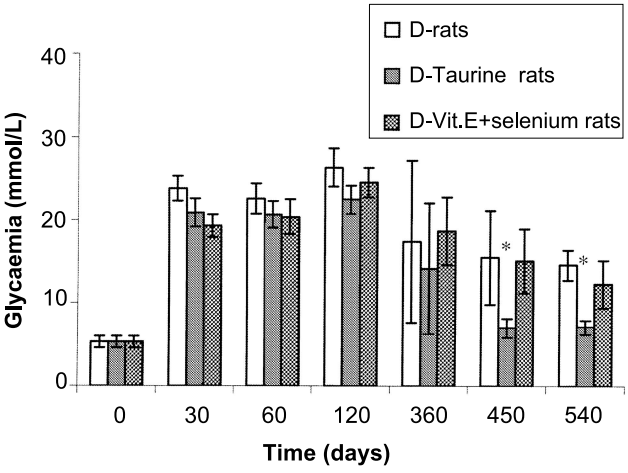

Fig. 1. Variation of glycaemia during the illness. Values are mean ± SD. *$p < 0.05$ versus non supplemented rats. For the number of animals see Table 1

As shown in Fig. 2, survival rate were significantly ($p < 0.04$) higher in the 5% taurine-supplemented rats than in untreated diabetic rats. No correlation between glycaemic reduction and survival was found. While, vitamin E + selenium supplementation did not modify survival of diabetic animals.

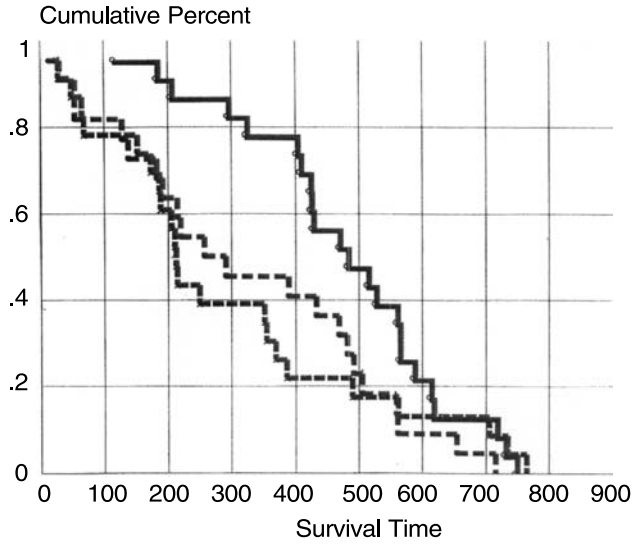

Fig. 2. Kaplan-Maier survival rate in streptozotocin-induced diabetic rats supplemented by 5% taurine enriched-diet (normal line), vitamin E + selenium enriched-diet (narrow dashed line) and normal diet (wide dashed line). Survival time was expressed as days

Discussion

Taurine supplementation reduces mortality rate in diabetic rats without influencing body weight. The increase in survival is a very "hard" end point that many antidiabetic drugs fail to achieve (UK Prospective Diabetes Study (UKPDS) Group, 1998). Consequently, new therapeutic approaches should affect long-term diabetic mortality, also considering that certain agents have detrimental effects, despite their ability to reduce glycaemia.

In the late phase of disease, taurine supplementation reduces blood glucose. Actually, a hypoglycemic effects of taurine in the alloxan-treated rabbit has been already seen (Tenner et al., 2003). Our results are also in line with previous studies, which show that taurine supplementation is able to reduce the haematic glucose and non enzymatic glycosilation of haemoglobin only after long-term supplementation (Odetti et al., 2002; Alvarado-Vasquez et al., 2003). However, in some short studies taurine does not modify glycaemia (Hansen, 2001; Di Leo et al., 2003; Franconi et al., 2004). It has been suggested that, at least *in vitro*, this amino acid could interact with the insulin receptor and could stimulate glucose and amino acid uptake in skeletal muscle and in adipocytes (Maturo and Kulakowski, 1988). Recently, it has been suggested that taurine improves the sensitivity to insulin (Nakaya et al., 2000).

In non-treated STZ rats, a non significant decrease in glycaemia is also observed. No clear explanations are available for this reduction, but it has been also seen by others (Alvarado-Vasquez et al., 2003). At this regard, it has been suggested that after STZ there is a spontaneous regeneration of pancreas (Movassat and Portha, 1999). However, it is not possible to exclude that after one year only animals that are less hyperglycaemic may survive.

Vitamin E plus selenium does not affect mortality rate and glycaemia in diabetic animals. The lack of activity of vitamin E is in line with the results of human interventional trials (Heart Protection Study Collaborative Group, 2002). Thus, our data suggest that the choice of the right antioxidant is a critical point for antioxidant therapy in diabetes, and taurine seems to be a good choice. The amino acid is mainly localized in the cytoplasm (Huxtable, 1992), reacts poorly with superoxide, peroxide and hydroxyl radicals (Tadolini et al., 1995), but it is a good scavenger of HOCl generating taurochloramines (Huxtable, 1992; Cunningham et al., 1998; Cantin, 1994; Kearns and Dawson, 2000). The availability of a HOCl scavenger is useful because hyperglycaemia-mediated vascular inflammation is associated with an increased production of HOCl (Omi et al., 2002).

In vitro, taurine reacts with aldehydes (Ogasawara et al., 1994), nevertheless the *in vivo* studies give contradictory results (Trachtman et al., 1994; Li et al., 1996). Indeed, *in vivo* taurine reduces oxidative stress induced by hyperglycaemia (Haber et al., 2003; Franconi et al., 2004), inhibits the nonenzymatic glycosylation of aortic collagen in diabetic rats (Li et al., 1996) and attenuates the age related increase of oxidative damage, decreasing carbonyl group production (Eppler and Dawson, 2001). All these data suggest that taurine could be a glycation scavenger. The enhanced formation of glycosylated proteins observed in diabetes mellitus may be the result of an overload of detoxification mechanisms (Baynes and Thorpe, 1999). The taurine depletion could promote the accumulation of reactive carbonyl and advanced glycosilation end products (Hansen, 2001; Franconi et al., 2004).

Classically, vitamin E is considered a chain-breaking antioxidant and selenium is essential for glutathione peroxidase activity. However, recent findings suggest that vitamin E may act as a promoter of oxidation (Neuzil et al., 2001). This could involve other metabolic pathways and could be dependent on its localisation within biological systems (Neuzil et al., 2001). Therefore, the protective effect of vitamin E could be observed only if other prooxidant factors are relatively weak and this is not the case of diabetes mellitus where oxidative stress is increased (Baynes and Thorpe, 1999; Lipinski, 2001; Santini et al., 1997). The lack of activity of vitamin E is in line with findings in the retina of diabetic animals where the effects

of vitamin E + selenium are present mainly at the first phase of illness (Di Leo et al., 2003).

Another point that we observed was that, although hyperglycaemia in taurine treated rats was significantly reduced after 15 months from the beginning of diabetes, the effects of disease were obviously evident in this group. Survival in taurine-treated rats was improved, whereas vitamin E + selenium did not demonstrate an effective tool protecting against diabetes. However, also in taurine-treated animals, it is prerequisite a good metabolic control to achieve a survival in diabetic rats comparable to non-diabetic rats.

In conclusion, the present findings suggest that there are no real reason for considering vitamin E + selenium a recommendable strategy in decreasing diabetic mortality, while taurine supplementation reduces this major end point in diabetic rats. Our findings also indicate that the key for a successful antioxidant therapy could rely on the choice of the right antioxidant or antioxidant mixtures. Whether our results will be extended to humans, taurine, a low cost molecule, will have staggering implications in terms of both human suffering and financial cost.

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