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ORIGINAL ARTICLE

Effect of L-carnitine on diabetogenic action of streptozotocin in rats

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Abstract

OBJECTIVES: L-carnitine is a naturally compound widely distributed in the body. It has an antiradical effect and decreases lipid peroxidation. In acute or chronic streptozotocin (STZ)-induced diabetic rats, the pancreatic content of carnitine was found to be significantly lower than nondiabetic group. We investigated the effects of L-carnitine on the development of STZ-induced diabetes in rats, to determine if L-carnitine can prevent the onset of diabetes or reduce the severity of hyperglicemia and this prevention/reduction is associated with the reduction in oxidative stress.
SETTING AND DESIGN: The rats were divided into 3 groups: Control, STZ-treated (65 mg/kg intraperitoneally) and L-carnitine (500 mg/kg) and STZ-treated.
METHODS: Oxidative stress was assessed by measuring pancreatic thiobarbituric acid reactive substance (TBARS) formation levels using the method of Rehncrona et al, pancreatic superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities using a Randox test combination (RANSOD and RANDOX).
RESULTS: L-carnitine did not prevent the onset of diabetes at this dose. Development of diabetes was associated with an increase in pancreatic TBARS (0.028 ± 0.008 and 0.046 ± 0.017 nmol/mg Protein, respectively), and GPx activity (0.067 ± 0.011 and 0.098 ± 0.016 U/mg Protein, respectively).
MAIN FINDINGS: L-carnitine prevented this increase induced by diabetes; TBARS (0.039 ± 0.006 nmol/mg Protein) and GPx activity (0.053 ± 0.011 U/mg Protein).
CONCLUSION: These results suggest that L-carnitine exerts anti-oxidative effect in experimental diabetes.

Abbereviations

STZ:	Streptozotocin
SOD:	Superoxide dismutase
GPx:	Glutathione peroxidase
TBARS:	Thiobarbituric acid reactive substance
ROS:	Reactive oxygen substances
DNA:	Deoxyribonucleic acid
ADP:	Adenin diphosphate
NAD:	Nicotinamide adenin dinucleotide
NADP:	Nicotinamide adenin dinucleotide phosphate
INT:	2-4-iodophenyl-3-4-nitrophenol-5 phenyl tetrazolium chloride
NADPH:	Reduced nicotinamide adenin dinucleotide phosphate hydrogene
GOD/PAP:	4-aminophenazone, glucose oxidase, peroxidase

Introduction

Animal models of diabetes mellitus can be produced by use of chemicals such as streptozotocin (STZ). It is an agent widely employed to induce experimental diabetes due to its ability to selectively targets and destroys insulin producing pancreatic islet β-cells [1, 2]. Its diabetogenic action is considered as follows; STZ causes DNA strand breaks in pancreatic islets and stimulates nuclear poly (ADP ribose) synthetase and thus depletes the intracellular NAD and NADP levels. NAD

depletion by STZ inhibits proinsulin synthesis and thus induces diabetes [3]. On the other hand, reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen, have been implicated to play important roles in diabetes. Also in the case of diabetic models induced by STZ, ROS were proposed to formed and involved in the death of β-cells [4, 5].

Superoxide radicals are converted to hydrogen peroxide by superoxide dismutase (SOD) and hydrogen peroxide is converted to water by glutathione peroxidase (GPx) and/or catalase [6]. Hydrogen peroxide can react with transition metals in Fenton reactions, thereby being converted into hydroxyl radicals. Hydrogen peroxide can cross all membranes and lead to hydroxyl radical formation at more distant sites. The hydroxyl radical can cause lipid peroxidation, which in turn leads to damage of cellular organels and membranes, thus causing cell death [6].

With respect to oxidative stress, an increased free radical generation was reported in diabetic plasma and tissues. In diabetes, oxidative stress seems mainly due to both increased free radical concentrations and sharp reduction of antioxidative defences [7].

L-carnitine is a naturally occurring compound and an antiradical widely distributed in the body, and decreases lipid peroxidation [8]. L-Carnitine prevents TBARS formation and elevation of antioxidants in aged rats brain [8]. Many physiologic and pathologic conditions affect blood, tissue and urinary concentrations of carnitine in both animal and human subjects. In acute or chronic STZ-induced diabetic rats, the pancreatic content of carnitine was found to be significantly lower than in nondiabetic control rats [9].

Studies on the antioxidant effect of carnitine on diabetes mellitus are not found. We investigated the effect of L-carnitine on the diabetogenic action of STZ in rats.

Materials and methods

Animals and chemicals

Male Wistar Albino rats weighing 290–350 g were used. Drugs used were streptozotocin (Sigma Chemical Co.), L-carnitine (Sigma Tau Pharmaceuticals.)

Rats were divided into three groups, as follows:

Group I: Saline-treated rats,
GroupII: Saline plus streptozotocin-treated,
Group III: L-carnitine plus streptozotocin-treated rats.

Group III rats were injected intraperitoneally with L-carnitine a dose of 500 mg/kg (dissolved physiologic solution) for 30 consecutive days [10]. Group II and Group I rats received an equal volume of serum physiologic. On day 31, Group II and group III rats received a single dose streptozotocin (Sigma, Deisenhofen, Germany), 65-mg/kg bodyweight [9], intraperitoneally. Streptozotocin was dissolved in 1.0 ml of 0.1mol/l citrate buffer pH 4.5. L-carnitine injections were performed 18 consecutive days after STZ injection at Group III rats. Blood glucose from the tail vein was assayed 24 and 48 hours later from streptozotocin injection. Blood glucose assays were performed weekly.

Tissue sampling

Eighteen days after the STZ injection, animals were killed by cervical dislocation. Their pancreatic tissues were quickly removed. Tissues were washed in cold homogenate medium and visible clots removed to minimise blood contamination. Tissue homogenates were prepared as described by Carrillo et al [11]. An aliquot of the homogenate and supernatant was stored –70 °C until the determination of TBARS levels and enzyme activities. Blood samples were drawn from the left ventricle of heart.

Determination of SOD activity

SOD activity was determined using a Randox test combination (RANSOD). Xanthine and xanthine oxidase were used to generate superoxide radicals that react with 2-4-iodophenyl-3-4-nitrophenol-5 phenyl tetrazolium chloride (INT) to form a red formazan dye. Concentration substrates were 0.075 μmol for xanthine and 0.037 μmol for INT. SOD inhibits this reaction by converting the superoxide radical to oxygen. A SOD unit inhibits the rate of reduction of INT by %50 in a complex system with xanthine and xanthine oxidase. Due to small linearity range of the test, the sample must be diluted so that the percentage of inhibition falls between %30 and %60. A standard curve was prepared using the standard provided in the kit, and the value for the supernatant was read from this curve. SOD activity was measured at 505 nm on a Shimadzu UV-1201V spectrometer in the supernatant. Results were expressed as SOD U/mg protein.

Determination of GPx activity

GPx was determined using a Randox test combination (RANSEL). GPx catalyses the oxidation of glutathione (at a concentration of 5 μmol) by cumene hydroperoxyde according to method of Paglia et al [12]. In the presence of glutathione reductase (at a concentration >0.75. 10^{-3} U) and 0.35 μmol of NADPH, the oxidized glutathione is immediately converted to reduced form with a concomitant oxidation of NADPH to NAD+. The decrease in absorbance at 340 nm was measured at 37 °C. The assay was performed on a supernatant. The GPx unit was defined as the enzyme activity necessary to convert 1 μmol of NADPH to NADP in 1 min. Results were expressed as GPx U/mg protein.

Determination of TBARS level

TBARS level was estimated according to the method of Rehncrona et al [13]. 0.5 ml of homogenates were extracted with 0.5 ml of trichloroacetic acid (20% wt/vol). After centrifugation, 0.9 ml of supernatant was added to 1 ml of thiobarbituric acid (0.67% wt/vol). The samples were heated in boiling water for 10 min. After cooling the absorbance was recorded at 532 nm. A standard curve was prepared using 1, 1, 3, 3-tetraethoxypropane and the value for the homogenate was read

from this curve. The results were expressed as nmol/mg protein.

Determination of protein concentration

Protein contents of supernatant and homogenate were determined using the modification proposed by Markwell et al [14] of the method of Lowry using bovine serum albumin as a standard.

Determination of plasma glucose concentration

Plasma glucose was determined using a Randox Lab. GOD/PAP (4-aminophenazone, glucose oxidase, peroxidase) Liquid kit. Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator. The absorbance at 500nm was measured. Normal serum or plasma values are 4.2–6.4 mmol/L or 75–115 mg/dl.

Statistics

The results of the experiments are expressed as mean ± SD. Statistical analysis was performed with ANOVA post hoc Tukey HSD, with $p<0.05$ regarded as significant.

Results

Blood glucose levels were within normal limits in the control group. Animals from Group 2 became diabetic upon STZ treatment. Group 3 animals blood glucose levels were within diabetic limits after STZ treatment (*Table 1*). Animals were considered as being diabetic when glycaemia exceeded 11 mmol/L [19]. It appears from the data that injection with 500 mg/kg L-carnitine prior to STZ treatment did not prevent the incidence of diabetes.

The SOD activity in rats treated with streptozotocin was not different from that in the control group and L-carnitine treated rats (*Table 2*).

The GPx activity in Group II was increased in comparison to the control group. GPx activity in Group III rats was below the activity in Group II rats (*Table 2*).

The TBARS formation levels in Group II rats were increased in comparison to the control group. The administration of L-carnitine to STZ-treated rats caused a decrease in TBARS levels in comparison to the diabetic group (*Table 2*).

Discussion

The present study suggests that streptozotocin causes an increase of GPx enzyme activity and TBARS formation levels. Exogenous L-carnitine causes a decrease in TBARS formation levels and GPx enzyme activity in diabetic rats. Streptozotocin is widely used in studies of experimental diabetes because it selectively destroys the pancreatic β-cell. A considerable body of evidence indicates that the generation of ROS may mediate this cytotoxic effect. An increase of ROS productions has also been pointed out as a main instrument of destruction in streptozotocin-damaged pancreatic islets [4, 5]. These results indicate the important role of L-carnitine in the development of oxidative stress.

Diabetes mellitus-associated increase in lipid peroxidation was observed in our study. Upon L-carnitine administration, a decrease in lipid peroxidation was observed. This may be due to its active role in the transport of fatty acids for energy production, thereby lowering the availability of lipids for peroxidation [15].

The production of highly reactive oxygen species such as $O2^{\bullet -}$, H_2O_2 and $OH^{\bullet}$ are also catalysed by free iron through Haber-Weiss reaction [16]. Carnitine and its esters have been shown to partially inhibit

Table 1 Blood glucose concentrations (mmol/dl) in rats1

Groups	Before experiment (mmol/dl)	48 hours after STZ injection (mmol/dl)	End of the experiment (mmol/dl)
Control	6.544 ± 0.442	6.659 ± 0.325	7.272 ± 1.185
STZ	5.774 ± 0.178	11.542 ± 0.121*	12.367 ± 0.887*
STZ + L-carnitine	6.090 ± 1.067	11.952 ± 0.189 *	12.419 ± 0.153*

1 The values represent mean ± S.E.M of seven animals per group.
* Means, $p<0.001$, in comparison with saline-treated rats (by Tukey HSD test)

Table 2 Pancreas Superoxide Dismutase (SOD) (U/mg Protein), Glutathione Peroxidase (GPx) (U/mg Protein) enzim activities and thiobarbituric acid reactive substance (TBARS) (nmol/mg Protein) levels1

Groups	SOD U/mg Protein	GPx U/mg Protein	TBARS nmol/mg Protein
Control	0.979 ± 0.082	0.067 ± 0.011	0.028 ± 0.008
STZ	1.088 ± 0.103	0.098 ± 0.016*	0.046 ± 0.017*
STZ + L-carnitine	1.155 ± 0.110	0.053 ± 0.011**	0.039 ± 0.006**

1 The values represent mean ± S.E.M of seven animals per group.
* Means, $p<0.001$, in comparison with saline-treated rats (by Tukey HSD test)
** Means, $p< 0.05$, in comparison with STZ-treated rats (by Tukey HSD test)

iron-induced lipid peroxidation in liposomes [17] by forming complexes with free iron. Thus, the reduction in lipid peroxidation in the present study is due to the iron-chelating property of L-carnitine.

Diabetes-related increase in lipid peroxidation might be the reflection of the increase in enzymatic and non-enzymatic antioxidant protection. The antioxidant defense system is composed mainly of three enzymes; glutathione peroxidase, superoxide dismutase and catalase [18]. Kakkar R et al reported that glutathione peroxidase activities increased in pancreas in diabetic rats [18]. In the present study, TBARS levels and GPx activity were increased in diabetic rats. The increase in reactive oxygen species (ROS) may induce GPx activity. However, L-carnitine prevented such increase. The decrease in GPx activity can be the result of the decrease of ROS.

SOD activity is not altered significantly by diabetes; L-carnitin supplementation did not alter this situation.

Many physiologic and pathologic conditions affect blood, tissue and urinary concentrations of carnitine in both animal and human subjects. Reddi et al. reported that both short- and long-term diabetes caused a significant decrease in pancreatic carnitine content, which is probably due to the decrease in uptake or the enhance in degradation [9]. For this reason, we aimed to administrate L-carnitine in diabetic rats. 500 mg/kg L-carnitine administration does not alter blood glucose levels after streptozotocin injection in rats. Thus, 500mg/kg L-carnitine did not prevent streptozotocin-induced diabetes in rats.

In conclusion, this study suggests that STZ-induced diabetes is mediated through oxidative stress and that L-carnitine is not effective at this dose in reducing the STZ-induced diabetes mellitus. However, L-carnitine administration significantly decreases TBARS levels and GPx activity in this model.

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