Acetyl-L-Carnitine Corrects the Altered Peripheral Nerve Function of Experimental Diabetes

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Acetyl-L-carnitine (ALC) has been shown to facilitate the repair of transected sciatic nerves. The effect of ALC (50 mg/kg/d) on the diminished nerve conduction velocity (NCV) of rats with streptozotocin (STZ)-induced hyperglycemia of 3 weeks' duration was evaluated. The aldose reductase inhibitor, sorbinil, which is reported to normalize the impaired NCV associated with experimental diabetes, was used as a positive control. Aldose reductase inhibitors are thought to have an effect by decreasing peripheral nerve sorbitol content and increasing nerve *myo*-inositol. Treatment of STZ-diabetic rats with either ALC or sorbinil resulted in normal NCV. Sorbinil treatment was associated with normalized sciatic nerve sorbitol and *myo*-inositol; ALC treatment did not reduce the elevated sorbitol levels, but sciatic nerve *myo*-inositol content was no different from nondiabetic levels. Both ALC and sorbinil treatment of STZ-diabetic rats were associated with a reduction in the elevated malondialdehyde (MDA) content of diabetic sciatic nerve, indicating reduced lipid peroxidation. The beneficial effects of sorbinil and ALC on the altered peripheral nerve function associated with diabetes were similar, but their effects on the polyol pathway (frequently implicated in the pathogenesis of peripheral neuropathy) were different.

REDUCED CONDUCTION velocity of peripheral nerves has been associated with hyperglycemia and increased activity of the polyol pathway.[1-3] This increased activity is indicated by elevated nerve sorbitol and fructose concentrations. Aldose reductase inhibitors such as sorbinil have been shown to reduce polyol pathway activity and improve nerve conduction velocities (NCVs) in hyperglycemic animals and diabetic subjects.[1-5] The acutely reduced peripheral nerve function associated with diabetes therefore has been linked to biochemical abnormalities. Acetyl-L-carnitine (ALC) has been shown to enhance regeneration of transected peripheral nerves[6,7] without specific biochemical alterations. Since NCV measures signal transmission in peripheral nerves of animals, it seemed appropriate to compare the influence of two different agents (sorbinil and ALC) on the altered peripheral nerve function of rats with streptozotocin (STZ)-induced hyperglycemia.

MATERIALS AND METHODS

Fasted male Wistar rats (Charles River Laboratories, Wilmington, MA) weighing 150 to 210 g were made diabetic by intraperitoneal injection of STZ (Upjohn, Kalamazoo, MI) 50 mg/kg in cold buffered sodium citrate (pH 4.5).[8] Seven and 14 days after STZ injection, a tail blood glucose level greater than 20 mmol/L was used to identify animals with experimental diabetes mellitus. Seven days after STZ injection, hyperglycemic animals were divided into three groups for 14-day intraperitoneal administration of (1) 50 mg/kg/d ALC (Sigma Tau, Rome, Italy) freshly prepared each day in distilled water just before injection (pH adjusted to 7.4 with NaOH); (2) 40 mg/kg/d sorbinil (an aldose reductase inhibitor; Pfizer Central Research, Groton, CT); or (3) buffer alone. Animals were provided abundant food and water and were weighed every third day.

Sciatic Motor NCV Measurements

Rats were anesthetized with sodium pentobarbital (60 mg/kg intraperitoneally). Rear-limb temperature was monitored with a thermistor probe (Sensortek Instruments, Clifton, NJ) placed subcutaneously in the gastrocnemius muscle and maintained at 36°C with a radiant heater. Stimulating electrodes were inserted at the sciatic notch for proximal stimulation and at the ankle for distal stimulation.[9,10] Subcutaneous recording electrodes were placed transversely over the plantar intrinsic foot muscles. Stimuli were supramaximal square-wave pulses of 0.05-millisecond duration and 10- to 20-V amplitude. A Grass stimulus isolation unit (SIU-5, Grass Instrument, Quincy, MA) eliminated stimulus artifacts. A-D 12-bit–resolution hardware and "Scopedriver" waveform acquisition software (RC Electronics, Santa Barbara, CA) were used to capture and analyze the elicited waveform. The hindlimb was extended, and the distance between the two points of stimulation was measured with a micrometer and conduction velocities were calculated: NCV = (distance between two points of stimulation)/(proximal latency − distal latency).

Biochemistry

After all physiologic measurements were made, animals were anesthetized with 100 mg/kg sodium pentobarbital. The sciatic nerve was removed from the live animal, and the epineurium was removed. The nerve was immediately frozen in liquid nitrogen and stored in an atmosphere of nitrogen to prevent auto-oxidation. Five milliliters of blood was removed by heart puncture. The blood was centrifuged, and the packed red blood cells were washed three times in 0.9% sodium chloride solution and frozen at −20°C.

The glucose content of rat plasma was measured in a Beckman Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA). Aliquots of packed red blood cells were analyzed for glycosylated hemoglobin by affinity chromatography using Glyc-Affin GHb columns (Isolab, Akron, OH). Packed red blood cells (1 mL) were mixed with 2 mL distilled water and vortexed, and sciatic nerves were homogenized in cold (4°C) deionized water with a Polytron PT3000 homogenizer (Brinkmann Instruments, Lucerne, Switzerland). One milliliter of these suspensions was deproteinized by precipitation with 2 mL 0.17-mol/L barium hydroxide and 2 mL 0.17-mol/L zinc sulfate. Sorbitol and *myo*-inositol (as acetate derivatives in these samples) were measured by gas-liquid chromatography[11] with two modifications: (1) a 30-m capillary column

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Submitted April 25, 1994; accepted August 16, 1994.

Supported by the Health Program Office, Health and Rehabilitative Services, State of Florida, and Sigma Tau, Rome, Italy.

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0026-0495/95/4405-0019$03.00/0

J & W Scientific, Folsom, CA) with a DB-225 liquid phase was used, and (2) the gas-liquid chromatograph was model 5890 (Hewlett Packard Instruments, Avondale, PA).

The malondialdehyde (MDA) level was measured in segments of tibial nerve using the method reported by Low and Nickander[12] with one modification: the pH of the thiobarbituric acid solution was changed from 6.7 to 2.0 to promote better extraction into the hydrophobic phase. Taurine level was measured by high-performance liquid chromatography using a column containing Aminex A-6 resin (Bio-Rad Laboratories, Melville, NY) and a post-column reaction with *o*-phthaldialdehyde.[13]

Data are expressed as the mean ± SEM. Data analyses were performed using SAS-PC (SAS Institute, Cary, NC). Differences among three or more means were tested by ANOVA (using Proc GLM; SAS Institute), coupled with the least-significant difference multiple range test.

RESULTS

The sciatic NCVs for rats that were hyperglycemic for 3 weeks (41.4 ± 1.0 m/s) were significantly less than for nondiabetic rats (46.6 ± 0.5, $P < .01$; Fig 1). Animals that were hyperglycemic for 3 weeks and treated with either ALC or sorbinil for the last 2 weeks had NCVs (47.5 ± 2.1 and 44.9 ± 1.2 m/s) significantly greater than those for untreated diabetic animals ($P < .01$ and $P < .05$, respectively).

During the 21 days of this experiment, nondiabetic rats gained an average 122 g (from 177 ± 6.0 to 299 ± 5.2 g), whereas diabetic rats gained only 48 g (from 180 ± 6.6 to 228 ± 5.6 g). There was no difference in weight gain among diabetic rats whether they received ALC, sorbinil, or placebo. Plasma glucose concentrations and glycosylated hemoglobin levels were higher in diabetic rats (treated and untreated) as compared with nondiabetic rats ($P < .01$; Table 1). Red blood cell sorbitol, which was elevated in diabetic animals ($P < .01$), was unchanged in rats treated with ALC but was reduced in diabetic rats treated for 2 weeks with sorbinil (Table 1).

Sciatic nerve sorbitol content was increased in untreated diabetic rats (0.81 ± 0.13 nmol/mg dry weight, $P < .025$) and diabetic rats receiving ALC (0.57 ± .14, $P < .05$), but was reduced in diabetic rats receiving sorbinil (0.46 ± 0.11). The *myo*-inositol content of diabetic rat nerves was less than (2.61 ± 0.38 nmol/mg dry weight) that found in normal nondiabetic animals ($P < .01$, Table 1). The sciatic nerve *myo*-inositol content of sorbinil- and ALC-treated diabetic rats was no different from that of nondiabetic rats (Table 1).

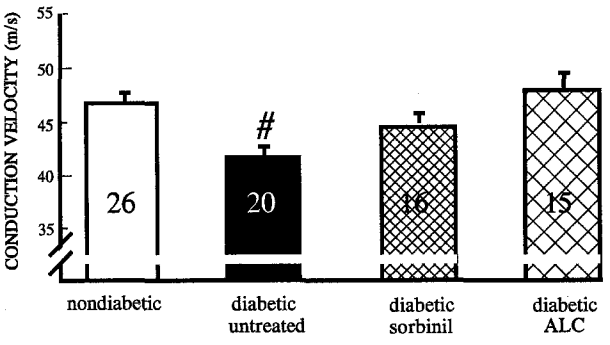

Fig 1. Mean sciatic NCV for 9-week-old male Wistar rats. Diabetic rats were hyperglycemic for 3 weeks. Treated rats were treated for 2 weeks. #Different from nondiabetic and ALC-treated diabetic rats, $P < .01$.

The MDA content of sciatic nerves from untreated diabetic rats was greater than (257.6 ± 49.8 pmol/mg dry weight) that found in nondiabetic sciatic nerve (170.3 ± 18.6, $P < .05$). ALC and sorbinil treatment of diabetic rats was associated with reduced nerve MDA content (168.0 ± 26.3 and 157.3 ± 26.0 pmol/mg dry weight), which did not differ from the nondiabetic animals (Table 1).

The sciatic nerve taurine content of untreated diabetic rats (2.25 ± 0.13 nmol/mg dry weight) was less than that found in nondiabetic rats (3.12 ± 0.25, $P < .05$). The sciatic nerve taurine content of sorbinil-treated diabetic rats did not differ from that of nondiabetic rats. The taurine content of sciatic nerves from ALC-treated rats was less than that from nondiabetic rats ($P < .05$, Table 1).

DISCUSSION

Both ALC and sorbinil prevented the reduced NCV in peripheral nerves of rats with STZ-induced hyperglycemia. Measurements of plasma glucose and glycosylated hemoglobin levels indicated that neither ALC nor sorbinil reduced the experimental hyperglycemia. Sciatic nerve and red blood cell sorbitol contents were normalized in hyperglycemic animals receiving sorbinil, indicating a reduction in polyol pathway activity. ALC did not alter the sorbitol content of either the red blood cells or sciatic nerves of these hyperglycemic animals, indicating that its effect on peripheral nerve function was not mediated by the polyol pathway.

In the present study, sciatic nerve *myo*-inositol content decreased in untreated diabetic animals, but returned to normal levels in diabetic animals treated with sorbinil and ALC. Many investigators have reported that peripheral nerve function in diabetes is linked to nerve polyol pathway activity and its influence on tissue *myo*-inositol content.[1,2] The improved NCV response to ALC in these STZ-diabetic rats was not specifically related to a reduction of nerve sorbitol, but was associated with physiologic nerve *myo*-inositol content. ALC apparently preserved nerve *myo*-inositol content without affecting polyol pathway activity.

It has been postulated that aldose reductase inhibitor–induced increased peripheral nerve *myo*-inositol content in STZ-diabetic animals may cause an observed increase in the activity of Na^+,K^+-adenosine triphosphatase (ATPase), which in turn contributes to improvements in NCV.[2,14] Sima et al[15] and Ido et al.[16] recently reported that ALC treatment of diabetic BB rats was associated with normalization of sciatic nerve Na^+,K^+-ATPase activity and improved peripheral NCV. We did not measure Na^+,K^+-ATPase activity in this study, but if nerve *myo*-inositol content mediates Na^+,K^+-ATPase activity, our observations are consistent with those of Ido et al and suggest a mechanism of tissue *myo*-inositol repletion independent of reduced sorbitol and aldose reductase inhibition.

Table 1. Sciatic Nerve Sorbitol, Inositol, Taurine, and MDA, Plasma Glucose and Hemoglobin A_{1c}, and RBC Sorbitol Contents in Male Wistar Rats Subjected to Different Treatment Regimens

		Diabetic		
Sciatic nerve	Nondiabetic	Untreated	Sorbinil-Treated (40 40/kg/d)	ALC-Treated (50 mg/kg/d)
Sciatic nerve				
Sorbitol (nmol/mg dry weight)	0.37 ± .07	0.81 ± 0.13†	0.46 ± 0.11§	.57 ± 0.14*
No. of rats	26	20	16	14
Inositol (nmol/mg dry weight)	5.49 ± .57	2.61 ± 0.38‡	4.83 ± 0.58§	4.08 ± 0.70§
No. of rats	26	20	16	11
Taurine (nmol/mg dry weight)	3.12 ± .25	2.25 ± .13*	2.55 ± .47	2.07 ± .36*
No. of rats	26	20	16	15
MDA (pmol/mg dry weight)	170.3 ± 18.6	257.6 ± 49.8*	157.3 ± 26.0§	168.0 ± 26.3§
No. of rats	25	19	16	15
Plasma glucose (mmol/L)	5.31 ± 0.35	18.43 ± 0.88‡	18.65 ± 1.02‡	19.80 ± 0.90‡
No. of rats	26	20	16	15
Glycosylated hemoglobin (%)	4.9 ± 0.2	12.5 ± 0.5‡	14.3 ± 0.5‡	15.4 ± 1.0‡
No. of rats	26	20	16	15
RBC sorbitol (nmol/mL)	1.24 ± 0.34	27.9 ± 2.6‡	7.42 ± 1.90§	24.0 ± 2.9‡
No. of rats	26	20	10	15

*Different from nondiabetic, $P < .05$.
†Different from nondiabetic, $P < .025$.
‡Different from nondiabetic, $P < .01$.
§Difference from nondiabetic nonsignificant.

Sciatic nerve MDA content was measured to look for evidence of increased lipid peroxidation in peripheral nerve of STZ-diabetic animals. Lipid peroxidation of peripheral nerve could damage the myelin sheath and interfere with nerve function. Low and Nickander have reported that the MDA content of diabetic nerve was not different from that of nondiabetic sciatic nerve after 4 weeks of experimental diabetes.[12] In the present study, after 3 weeks of hyperglycemia, the MDA content of sciatic nerves was higher than that found in nondiabetic age-matched rats. Two weeks of sorbinil or ALC administration to diabetic animals resulted in a nerve MDA content similar to that in nondiabetic control rats.

Tissue taurine levels have been shown to influence MDA levels in the rabbit lens.[13] Previous reports indicated that nerve taurine was reduced in animals with STZ-diabetes.[17,18] The taurine content of sciatic nerves from STZ-diabetic animals evaluated in this study was less than that of nerves from nondiabetic animals ($P < .05$). Sorbinil-treated diabetic animals had normal nerve taurine levels, but rats treated with ALC continued to have elevated sorbitol and reduced taurine content. Thus, nerve taurine levels seemed to be influenced by the activity of the polyol pathway, possibly as an osmoregulator,[19,20] but taurine content did not explain the beneficial effect of both ALC and sorbinil on the impaired NCVs associated with diabetes mellitus.

Increases in free radical production and accompanying peroxidative damage have been proposed as a mechanism for diabetes-associated tissue damage.[13] The elevated MDA in nerves of STZ-diabetic rats indicates that lipid peroxidation was occurring in sciatic nerves of these animals. This differs from a previous report[12] where MDA was increased, but not significantly, in diabetic animals. This may be due to the improved MDA extraction procedure used in the current study. ALC- and sorbinil-treated hyperglycemic animals did not have elevated sciatic nerve MDA levels. It appears that both ALC and sorbinil protected the nerves of hyperglycemic animals from lipid peroxidation. Fariello and Calabrese[21] have reported that ALC reduces the MDA content of ischemic mouse brain. A possible mechanism for this ALC effect is the generation of increased mouse brain glutathione,[22] which partially protects against increased free radical activity. Aldose reductase inhibitors such as sorbinil may have a similar effect by blocking the hyperglycemia-induced decrease in the concentration of $NADPH^+$.[23] Another recent report indicates that ALC protected cultured fibroblasts against the effects of high levels of free radicals.[24] The role of free radical damage of peripheral nerve in STZ-induced diabetes has not been well evaluated to date. This observed effect of ALC on peripheral nerve MDA levels and nerve function indicates the value of further consideration of an oxidative mechanism for peripheral nerve dysfunction associated with hyperglycemia.

The apparent link of peripheral nerve function (NCV) to nerve *myo*-inositol and MDA levels, plus the lack of a consistent association with sorbitol and taurine levels, indicates the importance of evaluating the simultaneous influence of a variety of parameters on peripheral nerve function in hyperglycemic animals. Diabetic peripheral nerve dysfunction is likely to have a number of mechanisms initiated by hyperglycemia.

ACKNOWLEDGMENT

We thank Charles Edwards for his help with sciatic NCV measurements and Candy Miranda and Sarfaraz Dhanji for animal care. Sorbinil was provided by Dr N.J. Hutson, Pfizer Central Research, Groton, CT. STZ (Zanosar) was provided by Thomas R. Klobucar, Upjohn Laboratories, Kalamazoo, MI.

REFERENCES

1. Gillon KRW, Hawthorne JN, Tomlinson DR: *Myo*-inositol and sorbitol metabolism in relation to peripheral nerve function in experimental diabetes in the rat: The effect of aldose reductase inhibition. Diabetologia 25:365-371, 1983
2. Greene DA, Lattimer SA: Action of sorbinil in diabetic peripheral nerve: Relationship of polyol (sorbitol) pathway inhibition to a *myo*-inositol–mediated defect in sodium-potassium ATPase activity. Diabetes 33:712-716, 1984
3. Sima AAF, Prashar A, Zhang W-X, et al: Preventive effect of long-term aldose reductase inhibition (ponalrestat) on nerve conduction and sural nerve structure in the spontaneously diabetic bio-breeding rat. J Clin Invest 85:1410-1420, 1990
4. Gieron MA, Malone JI, Lowitt S, et al: Improvement in peripheral nerve function after one year of sorbinil. NeuroReport 2:348-350, 1991
5. Sima AAF, Bril V, Nathaniel V, et al: Regeneration and repair of myelinated fibers in sural-nerve biopsy specimens from patients with diabetic neuropathy treated with sorbinil. N Engl J Med 319:548-555, 1988
6. Fernandez E, Pallini R, Gangitano C, et al: Effects of L-carnitine, L-acetylcarnitine and gangliosides on the regeneration of the transected sciatic nerve in rats. Neurol Res 11:57-62, 1989
7. Fernandez E, Pallini R, Gangitano C, et al: Studies on the degenerative and regenerative phenomena occurring after transection and repair of the sciatic nerve in rats: Effects of acetyl-L-carnitine. Int J Clin Pharmacol Res 10:85-99, 1990
8. Rerup CC: Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol Rev 22:485-518, 1970
9. Mayer JH, Tomlinson DR: Prevention of defects of axonal transport and nerve conduction velocity by oral administration of *myo*-inositol or an aldose reductase inhibitor in streptozotocin-diabetic rats. Diabetologia 25:433-438, 1983
10. Julu POO: The correlation between sensory nerve conduction velocities and three metabolic indices in rats treated with streptozotocin. Diabetologia 31:247-253, 1988
11. Malone JI, Knox G, Benford S, et al: Red cell sorbitol: An indicator of diabetic control. Diabetes 29:861-864, 1980
12. Low PA, Nickander KK: Oxygen free radical effects in sciatic nerve in experimental diabetes. Diabetes 40:873-877, 1991
13. Malone JI, Benford SA, Malone J Jr: Taurine prevents galactose-induced cataracts. J Diabetic Complications 7:44-48, 1993
14. Winegrad AI: Does a common mechanism induce the diverse complications of diabetes? Diabetes 36:396-406, 1987
15. Sima AAF, Kamijo M, Cherian PV, et al: Diabetic neuropathy in the BB/W-rat is prevented by acetyl-L-carnitine treatment. Muscle Nerve 1:S244, 1994 (suppl, abstr)
16. Ido Y, Rattarasarn M, Allison W, et al: Effects of acetyl-L-carnitine on functional and metabolic abnormalities in diabetic peripheral nerve. Diabetes 42:101A, 1993 (suppl 1, abstr)
17. Malone JI, Harvey R, Benford S, et al: Taurine deficiency in the sciatic nerve and lens of streptozotocin-diabetic rats. Diabetes 34:102A, 1985 (abstr)
18. Stevens MJ, Lattimer SA, Kamijo M, et al: Osmotically-induced nerve taurine depletion and the compatible osmolyte hypothesis in experimental diabetic neuropathy in the rat. Diabetologia 36:608-614, 1993
19. Thurston JH, Hauhart RE, Dirgo JA: Taurine: A role in osmotic regulation of mammalian brain and possible clinical significance. Life Sci 26:1561-1568, 1980
20. Trachtman H, Barbour R, Sturman JA, et al: Taurine and osmoregulation: Taurine is a cerebral osmoprotective molecule in chronic hypernatremic dehydration. Pediatr Res 23:35-39, 1988
21. Fariello RG, Calabrese F: Prevention of ischemia-induced increase of malonaldehyde by acetyl carnitine. Ann Neurol 24: P114, 1988
22. Fariello RG, Ferraro TN, Golden GT, et al: Systemic acetyl-L-carnitine elevates nigral levels of glutathione and GABA. Life Sci 43:289-292, 1988
23. Kador PF: The role of aldose reductase in the development of diabetic complications. Med Res Rev 8:325-352, 1988
24. Tesco G, Latorraca S, Piersanti P, et al: Protection from oxygen radical damage in human diploid fibroblasts by acetyl-L-carnitine. Dementia 3:58-60, 1992