Cardioprotective Effects of Troglitazone in Streptozotocin-Induced Diabetic Rats

Michio Shimabukuro, Satoshi Higa, Tatsushi Shinzato, Fumio Nagamine, Ichiro Komiya, and Nobuyuki Takasu

Troglitazone, a new oral antidiabetic agent, shows hypoglycemic effects in insulin-resistant animal models and humans. This study was conducted to evaluate the effects of troglitazone on the heart of diabetic animals. Streptozotocin (STZ)-induced diabetic rats and age-matched controls were treated with troglitazone as a 0.2% food admixture for 6 weeks. Basal and postischemic cardiac functions at 14 weeks of age were then examined in isolated working heart. Troglitazone treatment did not attenuate the insulinopenia and hyperglycemia of diabetic rats, but it partially improved the hypertriglyceridemia. Troglitazone treatment partially restored the basal heart rate and cardiac work of diabetic rats to nearly control values. Troglitazone also improved the postischemic functional deficits of diabetic rats: heart rate (untreated 61% of baseline at 30-minute reperfusion *v* treated 92%, $P < .001$), left ventricular (LV) developed pressure (54% *v* 94%, $P < .001$), peak positive ([LV +dP/dt] 54% *v* 93%, $P < .001$) and negative ([LV −dP/dt] 53% *v* 94%, $P < .001$) first derivative of LV, and cardiac work (44% *v* 98%, $P < .001$). Diabetic animals showed ultrastructural damage including disarray of sarcomere, disorganization of mitochondrial matrix, cytoplasmic vacuolization, and invagination of nuclear membrane; these were partially normalized by troglitazone treatment. Our results suggest that troglitazone treatment has a cardioprotective effect on the basal and postischemic cardiac function of STZ-induced diabetic rats.

A VARIETY OF experimental models of diabetes have been shown to induce characteristic functional and structural changes in the heart.[1-4] Extensive studies have been performed to clarify the mechanisms underlying such changes. Metabolic disturbances,[5] impaired Ca^{2+} metabolism in subcellular organelles,[6-9] and elevations of plasma and intramembrane lipid and increased lipid peroxidation[10] are possible mechanisms of the functional changes in the heart of diabetic animals. Ultrastructural studies of diabetic cardiomyopathy showed significant alterations in the myofibrils, mitochondria, nuclear membrane, and cytoplasm.[3,4] Such diabetes-induced functional and structural alterations are reversed by insulin[2,4] and also by several other agents such as probucol,[10] ω-3 fatty acid,[11] vanadyl sulfate,[12] sulfonylureas,[13,14] and Ca^{2+}-channel antagonists,[15-17] but the underlying mechanisms are not clearly understood.

Troglitazone is a member of a new class of oral antidiabetic agents, thiazolidinediones, that show hypoglycemic effects in obese or insulin-resistant diabetic animals[18,19] and in non–insulin-dependent diabetic[20] or obese[21] humans. The hypoglycemic effects of the agents are currently thought to be achieved through augmentation of insulin actions in the liver, skeletal muscle, and adipose tissues.[18,19,22] Recently, troglitazone was shown to possess in vitro direct effects on cardiac myocytes,[23] and this agent is therefore hypothesized to modulate in vivo cardiac performance.

The present study was undertaken to examine long-term effects of troglitazone on the basal and postischemic cardiac function and myocardial structure of streptozotocin (STZ)-induced diabetic and nondiabetic rats.

From the Second Department of Internal Medicine, Faculty of Medicine, University of The Ryukyus, Okinawa, Japan.

Submitted January 5, 1996; accepted March 22, 1996.

Supported in part by Grant No. 7770519 from the Ministry of Education, Science, and Culture of Japan.

Presented in part at the 55th Annual Meeting of the American Diabetes Association, June 10-13, 1995, Atlanta, GA.

Address reprint requests to Michio Shimabukuro, MD, Second Department of Internal Medicine, Faculty of Medicine, University of The Ryukyus, 207 Uehara, Nishihara, Okinawa 903-01, Japan.

0026-0495/96/4509-0019$03.00/0

MATERIALS AND METHODS

Animals and Treatment

Male Sprague-Dawley rats aged 6 weeks and initially weighing 200 to 210 g were randomly divided into four groups: (1) an untreated control group (n = 10), (2) an untreated diabetic group (n = 10), (3) a troglitazone-treated control group (n = 10), and (4) a troglitazone-treated diabetic group (n = 10). At 7 weeks of age, diabetes was induced by a single intravenous injection of STZ 60 mg/kg.[14] Rat chow with or without troglitazone (0.2% vol/vol) was given ad libitum from ages 8 to 14 weeks. At 14 weeks of age, the four groups of animals were used for blood sampling or for the isolated working-heart experiment. The investigation conformed with the guidelines of the University of The Ryukyus committee on animal care and handling.

Heart Perfusion Protocol

At age 14 weeks, the four groups of animals were fed chow without troglitazone for 24 hours. They were then killed, and cardiac function was assessed using the isolated working-heart model as described by Neely et al[24] with some modifications.[14] Briefly, rats were anesthetized with sodium pentobarbital (30 mg/kg intraperitoneally), and the abdomen and sternum were incised. Hearts were rapidly removed, placed on a noncirculating Langendorff apparatus, and perfused for a 5-minute washout period. Hearts were then transferred to a working-heart system for a 20-minute control period, with a preload pressure of 3 to 5 mm Hg and an afterload pressure of 60 mm Hg. At the end of the basal period, ischemia was induced by decreasing the afterload pressure to 0 mm Hg. After a 10-minute ischemic period, hearts were reperfused for another 30 minutes with the afterload reversed to the initial level. Modified Krebs-Hensleit bicarbonate buffer (contents in millimolars: NaCl 118.0, KCl 4.70, $CaCl_2$ 2.50, $MgSO_4$ 1.20, KH_2PO_4 1.20, $NaHCO_3$ 25.0, and glucose 11.0) was equilibrated with 95% O_2 and 5% CO_2 (pH 7.40 at 37°C).

Measurement of Hemodynamic Parameters

Left ventricular (LV) pressure was monitored with an 18-gauge cannula attached to a pressure transducer (AP601G; Nihon Kohden, Tokyo, Japan) inserted into the ventricle via the left atrium, and the peak positive and negative first derivatives of LV pressure

(LV +dP/dt and LV −dP/dt) were calculated with a differentiator (ED601G; Nihon Kohden). LV developed pressure was calculated as follows: LV developed pressure = LV systolic pressure − LV end-diastolic pressure (all as millimeters mercury). The electrocardiogram was recorded from the cardiac surface, and heart rate was monitored with a cardiotachometer (AT601G; Nihon Kohden). Aortic flow was measured with a flow meter (MFV1200; Nihon Kohden), and coronary flow was determined by collecting the perfusate from the pulmonary artery. Cardiac output (milliliters per minute) was calculated with the sum of both. Cardiac work was calculated as follows: cardiac work (milliliters per millimeter mercury per gram wet weight) = cardiac output (milliliters per minute) × LV developed pressure (millimeters mercury)/wet heart weight (grams).

Biochemical Analysis

Under pentobarbital sodium anesthesia, nonfasting blood samples were obtained via the inferior vena cava. Plasma glucose level was measured using the glucose oxidase method (Glu Neo Shinotest; Shinotest, Tokyo, Japan), total cholesterol by an enzymatic method (T-CHO Neo Shinotest; Shinotest), triglyceride by the glycerol-3-phosphate oxidase method (TG-II Shinotest; Shinotest), and serum insulin by enzyme immunoassay (Immunoball-IRI; Ono, Osaka, Japan). Plasma troglitazone concentration was measured by high-performance liquid chromatography using the LC-10A system (Shimadzu, Kyoto, Japan), with a column of 4.6 mm × 150 mm (Cosmoseal 5C18-P; Nakaraitesque, Kyoto, Japan) with the spectrophotometer set at 230 nm. The mobile phase consisted of CH_3CN:H_2O:KH_2PO_4 (51:49:0.05); the flow rate was maintained at 1.0 mL/min (40°C).

Histological Examination

A part of the LV free wall was resected from each heart and subjected to electron microscopic examination. The tissue was immersion-fixed in 2% glutaraldehyde, 2% OsO_2, dehydrated in graded alcohol, and embedded in Epon for electron microscopy. Ultrathin sections of the tissues were stained with uranyl acetate and lead citrate and examined under an electron microscope (H600; Hitachi, Tokyo, Japan).

Drugs

STZ was purchased from Sigma (St Louis, MO), and all other chemicals were from Wako (Osaka, Japan). Troglitazone was supplied by Sankyo (Tokyo, Japan).

Statistical Analysis

All data are presented as the mean ± SEM. Statistical analyses were performed using one-way or two-way ANOVA followed by Bonferroni's multiple comparison test.

RESULTS

Body and Heart Weight Changes

Daily chow intake of untreated diabetic rats (119 ± 9 g/kg/d) was higher than that of untreated controls (67 ± 4 g/kg/d), and addition of troglitazone did not change chow intake in control (57 ± 5 g/kg/d) or diabetic (99 ± 9 g/kg/d) rats. Troglitazone was administered at a daily dose of approximately 114 and 198 mg/kg/d in control and diabetic groups, respectively. Plasma troglitazone concentrations in control and diabetic rats were 1.65 ± 0.59 and 1.39 ± 1.18 μg/mL. Fourteen-week-old diabetic rats with diabetes of 6 weeks' duration showed decreased body weight but an increased ratio of heart weight to body weight compared with controls. Treatment with troglitazone did not alter body weight or the ratio of heart weight to body weight in control or diabetic rats (Table 1).

Plasma Glucose, Total Cholesterol, and Triglyceride and Serum Insulin

Compared with controls, diabetic rats showed higher plasma levels of glucose, triglyceride, and total cholesterol together with a lower serum insulin concentration (Table 1). Troglitazone treatment decreased plasma triglycerides in diabetic rats, but the other parameters were not altered in either control or diabetic rats.

Basal Cardiac Function

Isolated perfused hearts of diabetic rats showed significant decreases in heart rate and cardiac work compared with control rats (Table 2). Troglitazone treatment of diabetic rats partially restored the heart rate and cardiac work toward control levels, but did not improve the other parameters.

Postischemic Cardiac Function

Coronary flow in all groups of animals was severely decreased during ischemia, but recovered to the baseline values during reperfusion (data not shown). During reperfusion, functional parameters of control rats recovered to approximately baseline: heart rate was 96% ± 9% of baseline after 30 minutes of reperfusion (Fig 1), LV developed pressure was 85% ± 6% (Fig 2A), LV +dP/dt 75% ± 7% (Fig 2B), LV −dP/dt 77% ± 11% (Fig 2C), and cardiac work 73% ± 13% (Fig 3). In contrast, diabetic rat

Table 1. Characteristics of the Four Rat Groups

Characteristic	Control (n = 5)	Diabetic (n = 5)	Troglitazone-Treated Control (n = 5)	Troglitazone-Treated Diabetic (n = 5)
Body weight (g)	440 ± 19	232 ± 26†	450 ± 30	218 ± 14†
Heart weight (g)	1.58 ± 0.11	1.23 ± 0.09	1.65 ± 0.09	1.16 ± 0.04
Ratio of heart weight to body weight (10^{-4})	3.58 ± 0.10	5.39 ± 0.27†	3.69 ± 0.20	5.37 ± 0.25†
Plasma glucose (mmol/L)	7.1 ± 0.6	23.1 ± 1.5†	6.9 ± 0.3	23.8 ± 3.4†
Total cholesterol (mmol/L)	2.10 ± 0.39	6.30 ± 2.55*	2.10 ± 0.12	4.87 ± 1.54
Triglyceride (mmol/L)	0.90 ± 0.22	6.23 ± 1.93†	1.81 ± 0.09	2.31 ± 0.78‡
Serum insulin (mU/L)	30.5 ± 3.8	8.9 ± 4.9†	35.8 ± 7.3	8.0 ± 5.0†

NOTE. Values are the mean ± SEM.

*$P < .05$, †$P < .01$: *v* controls.

‡$P < .05$ *v* diabetics.

Table 2. Basal Values for Cardiac Function in the Four Rat Groups

Parameter	Control (n = 5)	Diabetic (n = 5)	Troglitazone-Treated Control (n = 5)	Troglitazone-Treated Diabetic (n = 5)
HR (bpm)	223 ± 12	162 ± 14†	214 ± 8	187 ± 10
LVDP (mm Hg)	123 ± 7	110 ± 7	113 ± 12	114 ± 4
LV +dP/dt (mm Hg/s)	6,310 ± 633	5,680 ± 596	5,675 ± 873	5,833 ± 253
LV −dP/dt (mm Hg/s)	4,180 ± 126	3,760 ± 312	3,975 ± 418	4,304 ± 319
CW (mm Hg · mL/min · g weight)	3,594 ± 392	2,141 ± 459*	2,882 ± 310	3,622 ± 343‡
CF (mL/min · g weight)	9.9 ± 0.6	9.4 ± 0.4	8.9 ± 1.2	12.7 ± 1.7

NOTE. Values are the mean ± SEM.

Abbreviations: HR, heart rate; CW, cardiac work; CF, coronary flow; DP, developed pressure.

*$P < .05$, †$P < .01$: *v* controls.

‡$P < .05$ *v* diabetics.

hearts showed an impaired recovery of heart rate (61% ± 14%, $P < .001$ *v* baseline), LV developed pressure (54% ± 17%, $P < .001$), LV +dP/dt (54% ± 10%, $P < .001$), LV − dP/dt (53% ± 14%, $P < .001$), and cardiac work (44% ± 14%, $P < .001$). Troglitazone treatment significantly improved the postreperfusion performance of diabetic rat hearts with respect to heart rate (92% ± 7%), LV developed pressure (94% ± 1%), LV +dP/dt (93% ± 3%), LV −dP/dt (94% ± 5%), and cardiac work (98% ± 4%).

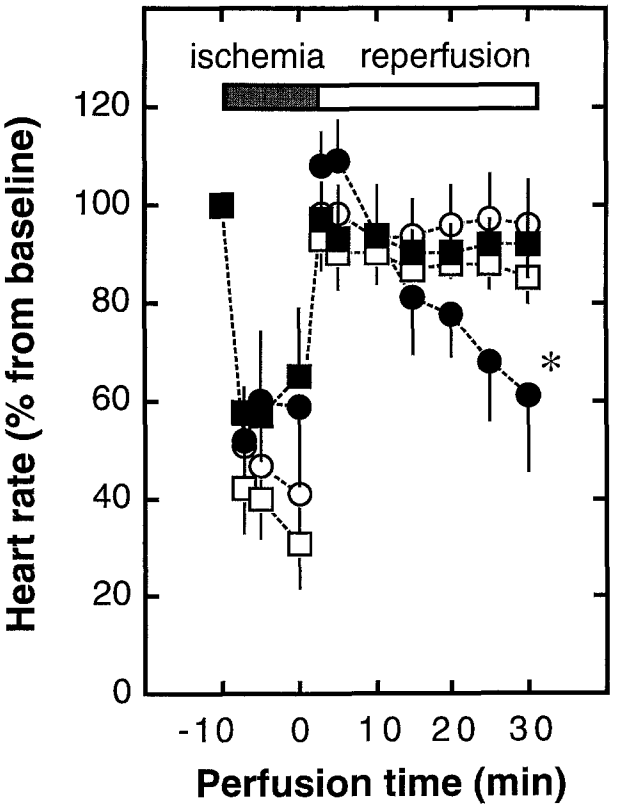

Fig 1. Percent change from baseline in heart rate during ischemia and reperfusion in perfused hearts isolated from control (○, n = 5), diabetic (●, n = 5), troglitazone-treated control (□, n = 5), and troglitazone-treated diabetic (■, n = 5) rats. Values are the mean ± SEM. *$P < .01$ *v* controls.

Histological Findings

Ultrastructural examination of control and troglitazone-treated control hearts similarly demonstrated normal arrays of sarcomere, myofibrils, and mitochondrial matrix (Fig 4A and C). At 14 weeks of age, diabetic rat hearts showed a disarray of sarcomere, loss or disorganization of mitochondrial matrix, cytoplasmic vacuolization, and invagination of nuclear membrane (Fig 4B). Troglitazone-treated diabetic animals showed almost normal alignment of sarcomere and relatively well-organized mitochondrial matrix, but vacuolization was seen, similar to that in the untreated diabetic rats (Fig 4D).

DISCUSSION

As described in our previous reports,[14,16,17] STZ-induced diabetes caused a reduction of basal cardiac function and impaired the postischemic functional recovery. Troglitazone treatment produced a recovery in the basal heart rate and cardiac work of diabetic rats to near-control values, and also improved the postischemic functional deficits of the heart rate, LV developed pressure, LV +dP/dt, LV −dP/dt, and cardiac work.

The microscopic findings demonstrated structural preservation of the heart of diabetic rats by troglitazone: normalized array of the sarcomere, myofibrils, and mitochondrial matrix. Jackson et al[3] reported that STZ treatment caused progression of ultrastructural damage in the rat heart, such as loss of contractile protein, vacuolization, myelin formation, myocytolysis, and contracture band formation, and that the alterations paralleled the depression of cardiac function. Insulin treatment has been reported to reverse such diabetes-induced structural[4] and functional[2] changes in the heart, suggesting that the changes were due to metabolic abnormalities induced by insulin depletion. In this study, troglitazone treatment did not affect the degree of hypoinsulinemia and hyperglycemia. This indicates that troglitazone restored the functional and structural deficits of diabetic hearts despite the diabetic state. Troglitazone did not alter cardiac performance in nondiabetic rats, suggesting that the effects of troglitazone were achieved through correction of diabetes-specific changes. Hyperlipidemia is a diabetes complication that was shown to be associated with cardiac dysfunction.[10,11] Correction of hyper-

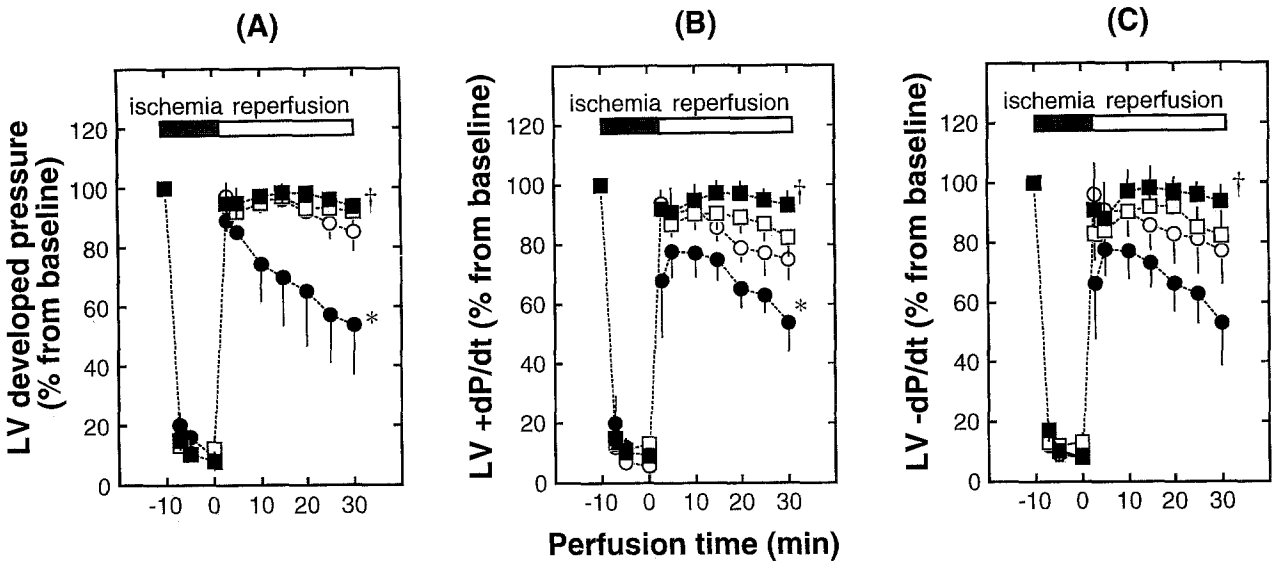

Fig 2. Percent change from baseline in LV developed pressure (A), LV +dP/dt (B), and LV −dP/dt (C) during ischemia and reperfusion in perfused hearts isolated from control (○, n = 5), diabetic (●, n = 5), troglitazone-treated control (□, n = 5), and troglitazone-treated diabetic (■, n = 5) rats. Values are the mean ± SEM. *$P < .01$ *v* controls; †$P < .01$ *v* diabetics.

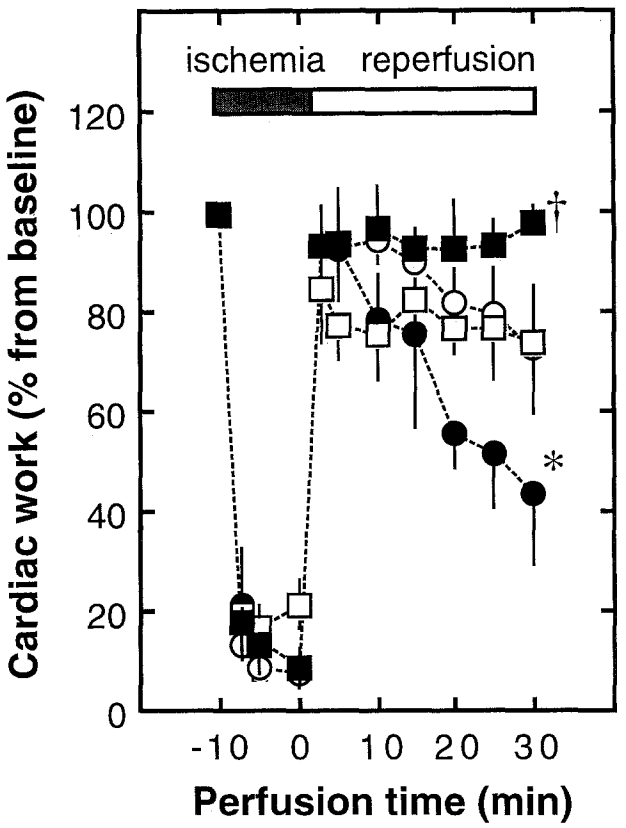

Fig 3. Percent change from baseline in cardiac work during ischemia and reperfusion in perfused hearts isolated from control (○, n = 5), diabetic (●, n = 5), troglitazone-treated control (□, n = 5), and troglitazone-treated diabetic (■, n = 5) rats. Values are the mean ± SEM. *$P < .01$ *v* controls; †$P < .01$ *v* diabetics.

lipidemia by probucol[10] and ω-3 fatty acid[11] was reported to improve cardiac performance, but such an improvement can be achieved without affecting the hyperlipidemic state.[14,16] The functional and structural recovery achieved with troglitazone via correction of hypertriglyceridemia appears to be limited, but the contribution of this mechanism should be determined.

The bases for the cardioprotective effects of troglitazone in diabetic hearts are not elucidated in this study. However, recent in vitro evidence may support the notion that troglitazone has in vivo effects on the heart of diabetic animals. First, Eckel et al[23] reported that troglitazone enhanced basal and insulin-stimulated glucose transport and increased expression of glucose transporters in rat cardiomyocytes. Earlier studies demonstrated that diabetes-induced functional deficits were related to decreased glucose transport, the normalization of which produced the functional recovery.[5] Clarification of the in vivo effects of troglitazone on glucose metabolism in the heart is needed. Second, pioglitazone and ciglitazone (other thiazolidinediones) reduce the inward Ca^{2+} current through the L-type Ca^{2+} channel in vascular smooth muscle[25,26]; troglitazone also attenuated the cytoplasmic Ca^{2+} concentration in pancreatic islets.[27] Abnormal Ca^{2+} handling in intracellular organelles[6-9] and the sarcolemmal L-type Ca^{2+} channel[15-17,28,29] is proposed to be a major mechanism of diabetic cardiomyopathy. We found that a 6-week treatment with nifedipine, an L-type Ca^{2+}-channel antagonist, improved postischemic recovery in the heart of diabetic rats,[16,17] and the recovery was correlated with a decrease in myocardial Ca^{2+} accumulation.[17] With regard to a possible condition of Ca^{2+} overload in diabetic cardiomyopathy, further studies are required to clarify the contribution of troglitazone to

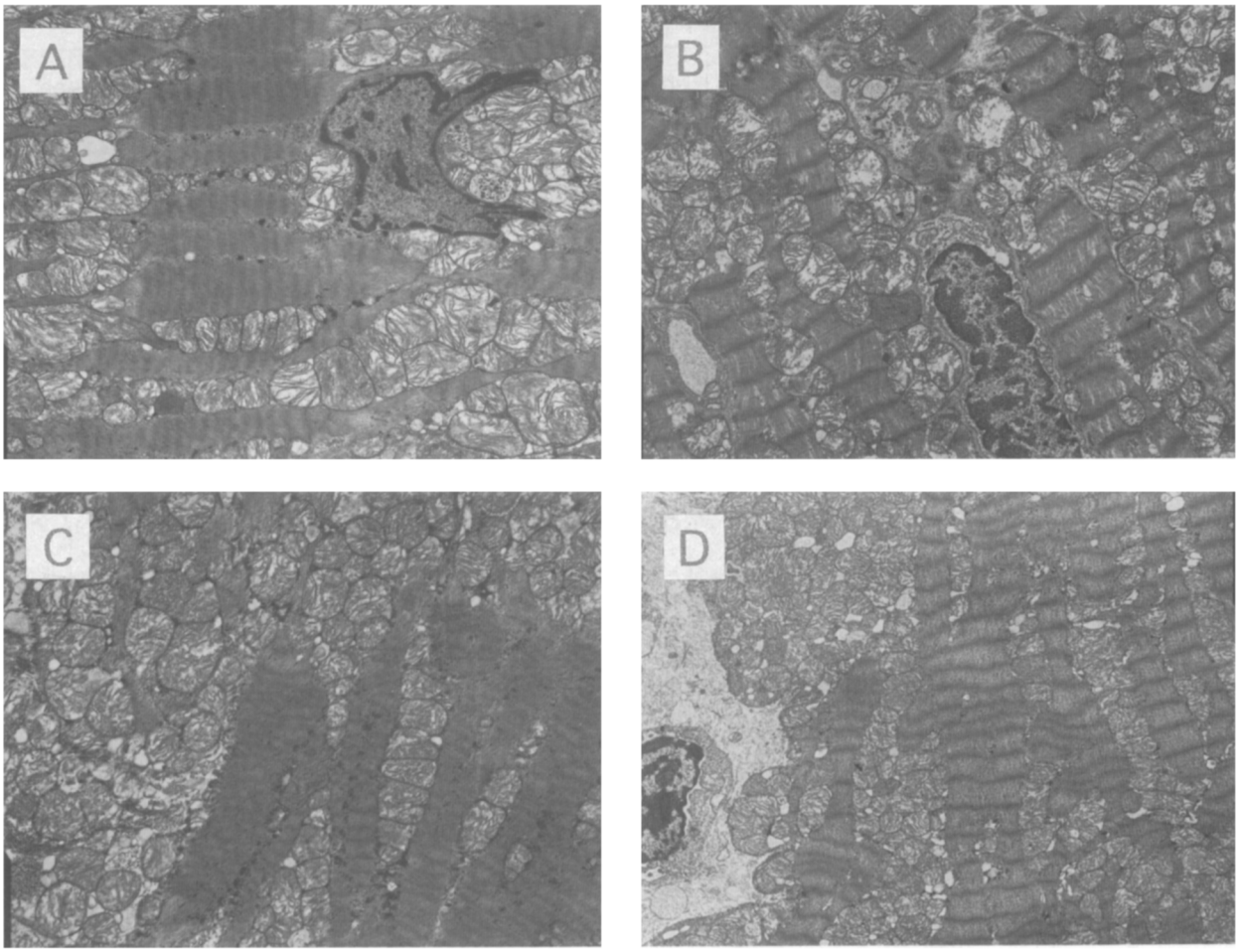

Fig 4. Electron microscopic findings in ventricular myocytes from control (A), diabetic (B), troglitazone-treated control (C), and troglitazone-treated diabetic (D) rats. Control and troglitazone-treated control hearts demonstrated normal arrays of sarcomere, myofibrils, and mitochondrial matrix. The diabetic heart showed disarrays of sarcomere, disorganization of mitochondrial matrix, cytoplasmic vacuolization, and invagination of nuclear membrane. Troglitazone-treated diabetic hearts showed almost normal alignment of sarcomere and relatively well-organized mitochondrial matrix. Original magnification ×5,000.

intracellular Ca^{2+} metabolism in the heart.[6,13-17,28,29] Third, troglitazone was shown to inhibit in vitro production of peroxide radical (ED_{50}, 3 μg/mL), superoxide radical (1.5 μg/mL), and hydrogen peroxide (4 μg/mL).[30] The diabetic state is a condition of excess oxidative stress,[31] which is thought to alter contractile pump activities in the heart.[10,11,32,33] Reduction of oxidative stress by free-radical scavengers is shown to improve cardiac performance.[34] If troglitazone could act in vivo as an antioxidant, it may reduce the free-radical–mediated tissue damage in the heart of diabetic animals.

In conclusion, our results suggest that troglitazone treatment has a cardioprotective effect against the basal and postischemic dysfunction and the ultrastructural damage induced by chronic diabetes. Further studies are required to establish the precise mechanisms of the effects of troglitazone on the heart of diabetic animals.

ACKNOWLEDGMENT

We are grateful to Dr K. Fukuda, Biological Research Laboratories, Sankyo, for skillful technical assistance in electron microscopic analysis; and to Dr F. Yasuzumi, Department of Anatomy, University of The Ryukyus, and Dr K. Murakami, Department of Internal Medicine, Suma Red Cross Hospital, for valuable advice. We also thank Sankyo for providing the troglitazone.

REFERENCES

1. Penpargkul S, Schaible T, Tipintsol T, et al: The effect of diabetes on performance and metabolism of rat hearts. Circ Res 47:911-921, 1981

2. Fein FS, Strobeck JE, Malhotra A, et al: Reversibility of diabetic cardiomyopathy with insulin in rats. Circ Res 49:1251-1261, 1981

3. Jackson CV, McGrath GM, Tahiliani AG, et al: A functional and ultrastructural analysis of experimental diabetic rat myocar-

dium. Manifestation of a cardiomyopathy. Diabetes 34:876-883, 1985

4. Thompson ED: Structural manifestations of diabetic cardiomyopathy in the rat and reversal by insulin treatment. Am J Anat 182:270-282, 1988

5. Rodrigues B, McNeill JH: The diabetic heart: Metabolic causes for the development of a cardiomyopathy. Cardiovasc Res 26:913-922, 1992

6. Makino N, Dhalla KS, Elimban V, et al: Sarcolemmal calcium transport in streptozotocin-induced diabetic cardiomyopathy in rats. Am J Physiol 253:E202-E207, 1987

7. Bouchard RA, Bose D: Influence of experimental diabetes on sarcoplasmic reticulum function in rat ventricular muscle. Am J Physiol 260:H341-H354, 1991

8. Pierce GN, Dhalla-NS: Mitochondrial abnormalities in diabetic cardiomyopathy. Can J Cardiol 1:48-54, 1985

9. Dillmann WH: Diabetes mellitus induces changes in cardiac myosin of the rat. Diabetes 29:579-582, 1980

10. Tada H, Oida K, Kutsumi Y, et al: Effects of probucol on impaired cardiac performance and lipid metabolism in streptozotocin-induced diabetic rats. J Cardiovasc Pharmacol 20:179-186, 1992

11. Black SC, Katz S, McNeill JH: Cardiac performance and plasma lipids of ω-3 fatty acid–treated streptozotocin-induced diabetic rats. Diabetes 38:969-974, 1988

12. Ramanadham S, Mongold JJ, Brownsey RW, et al: Oral vanadyl sulfate in treatment of diabetes mellitus in rats. Am J Physiol 257:H904-H911, 1989

13. Mozaffari MS, Allo S, Schaffer SW: The effect of sulfonylurea therapy on defective calcium movement associated with diabetic cardiomyopathy. Can J Physiol Pharmacol 67:1481-1486, 1989

14. Shimabukuro M, Nagamine F, Murakami K, et al: Chronic gliclazide treatment affects basal and post-ischemic cardiac function in diabetic rats. Gen Pharmacol 25:697-704, 1994

15. Afzal N, Ganguly PK, Dhalla KS, et al: Beneficial effects of verapamil in diabetic cardiomyopathy. Diabetes 37:936-942, 1988

16. Shimabukuro M, Shinzato T, Higa S, et al: Impaired mechanical response to calcium of diabetic rat hearts: Reversal by nifedipine treatment. J Cardiovasc Pharmacol 26:495-502, 1995

17. Higa S, Shimabukuro M, Shinzato T, et al: Nifedipine reduces calcium overload in isolated reperfused hearts of diabetic rats. Gen Pharmacol 26:1679-1686, 1995

18. Fujiwara T, Yoshioka S, Yoshioka T, et al: Characterization of new oral antidiabetic agent CS-045. Studies in KK and ob/ob mice and Zucker fatty rats. Diabetes 37:1549-1558, 1988

19. Lee MK, Miles PD, Khoursheed M, et al: Metabolic effects of troglitazone on fructose-induced insulin resistance in the rat. Diabetes 43:1435-1439, 1994

20. Iwamoto Y, Kuzuya T, Matsuda A, et al: Effects of new oral antidiabetic agent CS-045 on glucose tolerance and insulin secretion in patients with NIDDM. Diabetes Care 14:1083-1086, 1991

21. Nolan JJ, Ludvik B, Beerdsen P, et al: Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. N Engl J Med 331:1188-1193, 1994

22. Ciaraldi TP, Gilmore A, Olefsky JM, et al: In vitro studies on the action of CS-045, a new antidiabetic agent. Metabolism 39:1056-1062, 1990

23. Eckel J, Bähr M, Spelleken M, et al: Multiple direct effects of troglitazone on isolated ventricular cardiomyocytes. Diabetes 44:71A, 1995 (suppl 1, abstr)

24. Neely JR, Liebermeister H, Battersby EJ, et al: Effect of pressure development on oxygen consumption by isolated rat heart. Am J Physiol 212:804-814, 1967

25. Zhang F, Sowers JR, Ram JL, et al: Effects of pioglitazone on calcium channels in vascular smooth muscle. Hypertension 24:170-175, 1994

26. Pershadsingh HA, Szollosi J, Benson S, et al: Effects of ciglitazone on blood pressure and intracellular calcium metabolism. Hypertension 21:1020-1023, 1993

27. Niwa K, Horikoshi H, Kanno T: Troglitazone (CS-045) potentiates glucose-induced biphasic insulin secretion in the absence of potentiation of Ca^{2+} signaling in rat pancreatic islets. Biomed Res 16:345-351, 1995

28. Lee SL, Ostadalova I, Kolar F, et al: Alterations in Ca^{2+}-channels during the development of diabetic cardiomyopathy. Mol Cell Biochem 109:173-179, 1992

29. Kashiwagi A, Nishio Y, Ogawa T, et al: Increases in voltage-sensitive calcium channel of cardiac and skeletal muscle in streptozotocin-induced diabetic rats, in Nagano M, Mochizuki S, Dhalla NS (eds): Cardiovascular Disease in Diabetes. Boston, MA, Kluwer, 1992, pp 173-182

30. Dandona P, Khurana U, Aljada A, et al: Troglitazone as an antioxidant. Diabetes 44:57A, 1995 (suppl 1, abstr)

31. Collier A, Ailson R, Bradley H, et al: Free radical damage in type II diabetes. Diabetic Med 7:27-30, 1990

32. Hess ML, Okabe E, Kontos HA: Proton and free oxygen radical interaction with the calcium transport system on cardiac sarcoplasmic reticulum. J Moll Cell Cardiol 13:767-772, 1981

33. Kaneko M, Beamish RE, Dhalla NS: Depression of heart sarcolemmal Ca^{2+} pump activity by oxygen free radicals. Am J Physiol 256:H368-H374, 1989

34. Petty MA, Lukovic L, Grisar JM, et al: Myocardial protection by a cardioselective free radical scavenger. Eur J Pharmacol 255:215-222, 1994