Lipid Peroxidation in the Pancreas and Other Organs in Streptozotocin Diabetic Rats

Rie Tatsuki¹, Kumi Satoh¹, Atsuko Yamamoto¹, Katsuji Hoshi¹ and Kazuo Ichihara¹,²,*

¹Department of Pharmacology, Hokkaido College of Pharmacy, 7–1 Katsuraoka, Otaru 047–02, Japan
²Department of Clinical Pharmacology, Asahikawa Medical College, 4–5 Nishikagura, Asahikawa 078, Japan

Received June 23, 1997 Accepted August 5, 1997

ABSTRACT—We studied the relationship between changes in lipid peroxides and those in catalase activity in pancreases, livers and hearts of streptozotocin-induced diabetic rats. Animals were killed 2 or 7 weeks after saline or streptozotocin (32 mg/kg, i.v.) injection. The levels of blood glucose and plasma insulin in the 2-week streptozotocin-treated rats were 176.8 ± 20.5 mg/dl and 29.9 ± 3.2 µU/ml, respectively. In the pancreas, the lipid peroxide level significantly decreased and the catalase activity significantly increased 2 weeks after streptozotocin injection. These changes recovered after 7 weeks. In the heart, the lipid peroxide level significantly increased without any change of catalase activity 2 weeks after the initiation of diabetes. After 7 weeks, the catalase activity significantly increased and the lipid peroxide level returned to the control level. In the liver, there was no change in the lipid peroxides and catalase in the 2-week streptozotocin-treated rats, whereas the catalase activity significantly increased 7 weeks after the injection. It was suggested that the defense system in the pancreas to oxidative stress may be evoked in an early stage of streptozotocin-induced diabetes.

Keywords: Streptozotocin, Lipid peroxide, Catalase, Liver, Heart

Diabetes mellitus should be associated with increased oxidative stress because blood concentrations of lipid peroxides increase in experimental and clinical diabetes (1, 2). Oxidative stress means the production of highly reactive oxygen radicals that are toxic to the cells, particularly to the cell membrane in which these radicals interact with the lipid bilayer and produce lipid peroxides (3). Endogenous antioxidant enzymes (e.g., superoxide dismutase, catalase and glutathione peroxidase) are responsible for the detoxication of deleterious oxygen radicals (4, 5). In diabetes, catalase, in particular, is important to scavenge hydrogen peroxides that may be generated under diabetic conditions. Pieper et al. (6) have reported a significant increase in catalase activity, but not superoxide dismutase and glutathione peroxidase in diabetic vasculature. They have concluded that hydrogen peroxide is a major determinant of diabetic tissue damage. The increase in catalase activity under diabetic conditions may play an important role in protection against oxygen stress in several tissues.

Since streptozotocin was first reported to have a highly specific diabetogenic effect by Rakieten et al. (7), this agent has been widely used for induction of diabetes in animals as a model of insulin-dependent diabetes mellitus (8). Although the precise mechanism is not known, streptozotocin decreases insulin secretion from the β-cell of the pancreas. Streptozotocin is a nitrosourea that decomposes rapidly under physiological conditions to form a highly reactive carbonium ion which can alkylate DNA bases at various positions (9). In the case of the pancreas, the alkylation of DNA decreases transcription of the insulin gene, insulin formation, and then its secretion (9). Takasu et al. (10) have also demonstrated that streptozotocin generates some types of oxygen radicals that facilitate hydrogen peroxide generation, and the peroxides cause fragmentation of DNA. The lipid peroxidation resulting from the oxidative degradation of polyunsaturated fatty acids of the cell membrane (11, 12) may produce tissue damage and finally causes various diabetes-induced complications (13). The extent of tissue damage induced by oxygen radicals depends on the balance of oxygen radical formation and the endogenous antioxidant defense mechanism (14–16). Although there are many studies on lipid peroxidation and the activities
of antioxidant enzymes in experimental animals with diabetes, the results are still controversial (17–21). In the present study, therefore, we examined the effect of diabetes induced by streptozotocin on the tissue level of lipid peroxides and the activity of catalase, as a representative antioxidant enzyme, in the pancreas, heart and liver.

MATERIALS AND METHODS

This investigation conforms to the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

Animals and study design

Male Sprague–Dawley rats (6– to 8-week-old) weighing 180–200 g were fasted for 24 hr before inducing diabetes with streptozotocin. After anesthetization with sodium pentobarbital (30 mg/kg, i.p.), animals were injected with streptozotocin, either 50 or 32 mg/kg, into the left femoral vein. It is well-known that oxygen stress increases with aging (22). In preliminary experiments, we confirmed that the lipid peroxide level in the pancreas progressively increased as the animals grew older (data are not shown). Therefore, we carefully conceived an experimental design for determination of time course of tissue lipid peroxides up to 2 weeks. For example, animals that were sacrificed on the same day (1, 2, 7 and 24 hr) were injected with streptozotocin at the age of 8 weeks; those that were sacrificed 2 days after the injection were treated at the age of 7 weeks + 5 days; those that were sacrificed 3 days after the injection were injected at the age of 7 weeks + 4 days; and those that were sacrificed 2 weeks after the injection were administered streptozotocin at the age of 6 weeks. In this experimental design, all rats were sacrificed at the age of 8 weeks. However, animals sacrificed 7 weeks after the injection were administered streptozotocin at the age of 6 weeks. We cannot simply compare the result of lipid peroxide in animals sacrificed 2 weeks after the injection with those sacrificed 7 weeks after the injection.

Streptozotocin was dissolved in saline solution just before each injection. We tried to use streptozotocin dissolved in citrate buffer (pH 4.5) as many investigators have done. In our preliminary experiments, however, many animals died when the compound dissolved in citrate buffer was injected. Control animals received saline. The volume injected was fixed at 0.2 ml/100 g body weight. After the injection, all animals were housed and allowed access to food and water ad libitum.

Assays for blood glucose and insulin

Tail-vein blood samples were obtained from the 24-hr fasted rats for measurements of blood glucose and plasma insulin levels. The level of glucose in whole blood was determined by the glucose oxidase method of Dahlqvist (23). The level of insulin in plasma was determined by a radioimmunoassay method as the immunoreactive insulin with an insulin 125I RIA kit (Rat insulin 125I assay system; Amersham, Buckinghamshire, England). When a glucose tolerance test was performed, glucose solution at the concentration of 0.5 g/ml was given orally at 1 ml/100 g body weight (5 g/kg).

Determination of lipid peroxide level and catalase activity

Animals for determination of the level of lipid peroxides and the activity of catalase in the pancreas, liver, and heart were decapitated. After decapitation, the pancreas was immediately removed, pressed and frozen with clamps previously chilled in liquid nitrogen. The heart and liver were also removed for determination of the level of lipid peroxides. Prior to removal, the liver was washed with ice-cold saline introduced into the portal vein until the tissue color turned whitish. The heart was perfused by the Langendorff technique with aerated Krebs-Henseleit bicarbonate buffer for 5 min to remove residual blood. After these procedures, both tissues were pressed and frozen with clamps previously chilled in liquid nitrogen. The frozen tissues were stored at −80°C until assayed. The frozen samples were pulverized in a mortar with a pestle precooled with liquid nitrogen, and homogenized in 20 volumes of 50 mM Tris-HCl (pH 7.5) using a glass homogenizer with a Teflon pestle (24). The homogenate obtained was divided into two parts. An equal volume of medium containing 5 mM EDTA, 0.01% digitonin and 0.25% sodium cholate was added into one part of the homogenate and centrifuged at 12,000 × g for 30 min. The supernatant obtained was immediately used for assay of the catalase activity with an oxygen monitor (OHB-100; Otsuka Electronics Co., Osaka). The activity was estimated by the rate of O2 generation from the substrate of 10 mM H2O2 in 50 mM potassium phosphate buffer (pH 7.0) saturated with oxygen. Another part of the homogenate was used to determine the tissue level of lipid peroxides as thiobarbituric acid-reactive substances by the fluorimetric method described by Yagi (25). Briefly, the malondialdehyde formed from the breakdown of polyunsaturated fatty acids reacted with thiobarbituric acid to give a red product absorbing at 532 nm. Protein content in each sample was measured by the method of Lowry et al. (26).

Statistical analyses

Data are expressed as means ± S.E. The significance of differences between groups were evaluated using one-way analysis of variance followed by Dunnett’s t-test. Differ-
ences within groups were compared using the paired Student's t-test. A P value of less than 0.05 was considered statistically significant.

RESULTS

The levels of blood glucose and body weight of rats fasted for 24 hr were determined 2 weeks after injection of saline (control) or streptozotocin at either 32 or 50 mg/kg (Table 1). Streptozotocin injection significantly increased the blood glucose level, whereas it significantly decreased the body weight. The level of blood glucose in rats treated with 32 mg/kg of streptozotocin was significantly higher than that in the control rats, and it was significantly lower than that in rats treated with 50 mg/kg of streptozotocin. The body weight of rats treated with 32 mg/kg of streptozotocin was significantly lower than that of the control group, and it was significantly higher than that of rats treated with 50 mg/kg of streptozotocin. Animals injected with streptozotocin at the dose of 50 mg/kg showed considerable weight loss and a high mortality rate (approximately 33%). All animals given streptozotocin at the dose of 32 mg/kg survived and showed less hyperglycemia and weight loss. Therefore, streptozotocin at the dose of 32 mg/kg was employed for all subsequent experiments.

Figure 1 shows blood glucose and plasma insulin levels in rats before administration of streptozotocin; at 1, 2, 7, 24 and 48 hr; and 2 and 7 weeks later. There was a progressive increase in blood glucose after streptozotocin injection. The level of blood glucose significantly increased 2 hr after the injection. However, the glucose level obtained 7 hr after streptozotocin injection temporarily decreased and then significantly increased again. The increased level was sustained for at least 7 weeks. The transient decrease in glucose level at 7 hr was associated with a transient but significant increase in the plasma insulin level. Figure 2 shows the response of blood glucose and plasma insulin levels to a glucose tolerance test. Both control and streptozotocin-treated rats showed significant increases in the blood glucose level after glucose loading.

### Table 1. The level of blood glucose and body weight in fasted rats 2 weeks after streptozotocin injection

| Treatment     | n  | Blood glucose (mg/dl) | Body weight (g) |
|---------------|----|-----------------------|-----------------|
| Control       | 5  | 99.9 ± 2.7            | 243.6 ± 4.8     |
| STZ (50 mg/kg)| 5  | 449.2 ± 6.2*          | 160.0 ± 4.4*    |
| STZ (32 mg/kg)| 5  | 176.8 ± 20.5*         | 188.8 ± 8.1*    |

Rats were injected i.v. with saline or streptozotocin (STZ) at 50 mg/kg and 32 mg/kg. Animals were fasted overnight 2 weeks after the injection, and then the blood glucose level and body weight were measured. Data are means ± S.E. n = numbers of observations. *P < 0.01, compared with the control group. †P < 0.05, ‡P < 0.01, compared with the STZ (50 mg/kg) group.
A significantly enhanced increase in blood glucose in the streptozotocin-treated rats was observed as compared with that in the control rats. Glucose administration significantly increased plasma insulin level in the control group, while it did not change the insulin level in the streptozotocin-treated group.

In Fig. 3, changes in the level of lipid peroxides in the pancreas after streptozotocin injection are illustrated. The pancreatic tissue level of lipid peroxides did not change significantly by 24 hr, and then it decreased significantly. The level of lipid peroxides remained at low levels even 2 weeks after streptozotocin treatment. However, the level of lipid peroxides returned to its control level by 7 weeks after streptozotocin injection (Fig. 4). A significantly higher activity of catalase in the 2-week streptozotocin-treated rats was observed. There were no significant differences in catalase activity between the 7-week streptozotocin-treated and control rats. The levels of lipid peroxides and the activities of catalase in the liver and heart 2 and 7 weeks after streptozotocin injection are shown in Fig. 5. In the liver, there was no significant difference in lipid peroxides and catalase activity between control and 2-week streptozotocin-treated rats. Although the level of lipid peroxides was still unchanged, the activity of catalase was significantly increased 7 weeks after the streptozotocin injection. In the heart, after 2 weeks of streptozotocin treatment, the level of lipid peroxides was significantly increased without any change in the catalase activity. At 7 weeks after streptozotocin injection, the lipid peroxide level returned to the control level, whereas the catalase activity increased significantly.
Fig. 4. Changes in the level of lipid peroxide and the activity of catalase in pancreases 2 and 7 weeks after streptozotocin injection. The pancreas was removed from rats 2 weeks (upper panel) and 7 weeks (lower panel) after injection of saline (○) or streptozotocin at 32 mg/kg (□). Data are the means±S.E. of 3-5 (control) and 6-11 (streptozotocin) observations. STZ = streptozotocin, L.P. = lipid peroxides, MDA = malondialdehyde. *P<0.01, compared with the control group.

Fig. 5. Changes in the level of lipid peroxide and the activity of catalase in hearts and livers 2 and 7 weeks after streptozotocin injection. The tissues were removed from rats 2 weeks (upper panel) and 7 weeks (lower panel) after injection of saline (○) or streptozotocin at 32 mg/kg (□). Data are the means±S.E. of 3 (control) and 11 (streptozotocin) observations. STZ = streptozotocin, L.P. = lipid peroxides, MDA = malondialdehyde. *P<0.01, compared with the control group.
DISCUSSION

Streptozotocin-induced diabetes or hyperglycemia resulting from insulinopenia was reported to increase lipid peroxidation (10). In the present study, however, we found only a slight and insignificant increase in lipid peroxides in pancreases 7 hr after streptozotocin injection (Fig. 3), despite evidence of β-cell injury; e.g., transient but significant increases in plasma insulin and hypoglycemia (Fig. 2). The pancreatic levels of lipid peroxides significantly decreased within 48 hr, and the low levels were maintained for 2 weeks. According to the report of Pieper et al. (6), in streptozotocin-induced diabetes, a distal metabolite to hydrogen peroxide (e.g., hydroxyl radicals) may be an important mediator of tissue damage. We repeated the experiments in which the catalase activities were measured together with the lipid peroxide levels 2 weeks and 7 weeks after streptozotocin injection (Fig. 4). A marked induction of catalase in pancreases was found in the 2-week streptozotocin-treated rats, but not in livers and hearts. Because the activities of antioxidant enzymes in the pancreas are relatively lower than those in the other organs (27), radicals derived from streptozotocin or streptozotocin-induced diabetes may selectively attack the pancreas. When the organs such as the pancreas are attacked by oxygen stress, the defense mechanism could be evoked immediately. Starvation is one of the oxygen stresses that induce an increase in catalase activity in the pancreas (27). In nature, the catalase activity is high in the liver, medium in the heart, and low in the pancreas (27). The increase in lipid peroxide level due to streptozotocin was not observed in the liver, probably because the catalase activity is high enough to cancel the oxygen stress. On the other hand, the level of lipid peroxides in the pancreas was significantly decreased by streptozotocin, probably because of a marked induction of catalase (Fig. 4). In the heart, the catalase activity is not sufficient to interrupt the lipid peroxidation caused by streptozotocin, but is not low enough to induce the enzyme (Fig. 5).

In the rat at 7 weeks after streptozotocin injection, because the catalase was no longer induced in the pancreas, the level of lipid peroxides returned to the control level. In the liver and heart, the induction of catalase occurred when oxygen stress or diabetes was prolonged for 7 weeks. The level of lipid peroxides in these organs did not differ from the corresponding values of the control animals. Increase in the catalase activities in the liver and heart at 7 weeks may keep the lipid peroxide levels at the control level, although the production of lipid peroxide could be gradually increased during 7 weeks after streptozotocin injection.

The usual dosage of streptozotocin to induce diabetes is approximately 50–65 mg/kg (7, 10, 28, 29). However, the present result showed the high toxicity of streptozotocin at 50 mg/kg; i.e., marked weight loss and high mortality rate. Because Ganda et al. (30) have demonstrated a progressive increase in mean plasma glucose following administration of streptozotocin greater than 20 mg/kg, we reduced the dosage of streptozotocin from 50 to 32 mg/kg. At this dosage, streptozotocin still made the rat diabetic, although the blood glucose after 24 hr of fasting was lower than that obtained at 50 mg/kg. A transient hypoglycemia with plasma insulin elevation was also observed at 32 mg/kg of streptozotocin as reported at the higher dosages (28). The blood glucose level in these rats was markedly increased during a glucose tolerance test without any change in insulin levels as compared with that in the control rats. This status of diabetes is similar to the clinical status of patients with diabetes. Mukherjee et al. (29) have found a significant elevation of lipid peroxidation in the liver caused by streptozotocin within 24 hr. However, in the present study, the level of lipid peroxides in the liver did not increase up to 7 weeks after streptozotocin treatment (Fig. 5). The difference may be due to the difference in dosages; they used 65 mg/kg in their experiment, whereas 32 mg/kg was used in our experiment. Streptozotocin of 32 mg/kg exerts more mild effects than 65 mg/kg.

In conclusion, the defense system against oxygen stress in the pancreas quickly responds to streptozotocin treatment, resulting in an increase in catalase activity and a decrease in lipid peroxides. In the liver and heart, the defense system is slowly evoked. The catalase activity increased to scavenge the excess lipid peroxide produced by prolonged hyperglycemia.

REFERENCES

1. Godin DV, Wohaieb SA, Garnett ME and Goumeniouk AD: Antioxidant enzyme alterations in experimental and clinical diabetes. Mol Cell Biochem 84, 223–231 (1988)
2. Sato Y, Hotta N, Sakamoto N, Matsuoka S, Ohishi N and Yagi K: Lipid peroxide level in plasma of diabetic patients. Biochem Med 21, 104–107 (1979)
3. Haugaard N: Cellular mechanisms of oxygen toxicity. Physiol Rev 48, 311–373 (1968)
4. Del Maestro RF: An approach to free radicals in medicine and biology. Acta Physiol Scand 492, Suppl 153–168 (1980)
5. Michelson AM, Paget K, Dorsosay P and Bonneau JC: Clinical aspects of the dosage of erythropoetin. In Superoxide and Superoxide Dismutase, Edited by Michelson AM, McCord JM and Fridovich I, pp 467–499, Academic Press, New York (1977)
6. Pieper GM, Jordan M, Dondlinger LA, Adams MB and Raza AM: Peroxidative stress in diabetic blood vessels. Reversal by pancreatic islet transplantation. Diabetes 44, 884–889 (1995)
7. Rakieten N, Rakieten ML and Nadkarni MV: The diabetogenic action of streptozocin. Cancer Chemother Rep 29, 91–98
8 Rerup CC: Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol Rev 22, 485–518 (1970)
9 Okamoto H: Molecular basis of experimental diabetes: degeneration, oncogenesis and regeneration of pancreatic β-cells of islets of Langerhans. Bioessays 2, 15–21 (1985)
10 Takasu N, Komiya I, Asawa T, Nagasawa Y and Yamada T: Streptozocin- and alloxan-induced H2O2 generation and DNA fragmentation in pancreatic islets. Diabetes 40, 1141–1145 (1991)
11 Gardner HW: Oxygen radical chemistry of polyunsaturated fatty acids. Free Radic Biol Med 7, 65–86 (1989)
12 Porter NA: Chemistry of lipid peroxidation. Methods Enzymol 105, 273–282 (1984)
13 Baynes JW: Role of oxidative stress in development of complications in diabetes. Diabetes 40, 405–412 (1991)
14 Barnes PJ: Reactive oxygen species and airway inflammation. Free Radic Biol Med 9, 235–244 (1990)
15 Kanner J, German JB and Kinsella JE: Initiation of lipid peroxidation in biological systems. CRC Crit Rev Food Sci Nutr 25, 317–364 (1987)
16 Machlin LJ and Bendich A: Free radical tissue damage: Protective role of antioxidant nutrients.FASEB J 1, 441–445 (1987)
17 Lammi-Keefe CJ, Swan FB and Hegarty PVJ: Evidence for increased peroxidative activity in muscles from streptozocin-diabetic rats. Proc Soc Exp Biol Med 176, 27–31 (1984)
18 Loven DP, Schedl HP, Oberley LW, Wilson HD, Bruch L and Niehaus CL: Superoxide dismutase activity in the intestine of the streptozocin-diabetic rat. Endocrinology 111, 737–742 (1982)
19 Loven D, Schedl H, Wilson H, Daabees TT, Stegink LD, Diekus M and Oberley L: Effect of insulin and oral glutathione on glutathione levels and superoxide dismutase activities in organs of rats with streptozocin-induced diabetes. Diabetes 35, 503–507 (1986)
20 Matkovics B, Varga SI, Szabó L and Witas H: The effect of diabetes on the activities of the peroxide metabolism enzymes. Horm Metabol Res 14, 77–79 (1982)
21 Wohaieb SA and Godin DV: Alterations in free radical tissue-defense mechanisms in streptozocin-induced diabetes in rat. Diabetes 36, 1014–1018 (1987)
22 Hirai S, Okamoto K and Morimatsu M: Lipid peroxide in the aging process. In Lipid Peroxides in Biology and Medicine, Edited by Yagi K, pp 305–315, Academic Press, New York (1987)
23 Dahlqvist A: Determination of maltase and isomaltase activities with a glucose oxidase reagent. Biochem J 80, 547–551 (1961)
24 Ohkawa H, Ohishi N and Yagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95, 351–358 (1979)
25 Yagi K: Assay for blood plasma or serum. Methods Enzymol 105, 328–331 (1984)
26 Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193, 265–275 (1951)
27 Wohaieb SA and Godin DV: Starvation-related alterations in free radical tissue defense mechanisms in rats. Diabetes 36, 169–173 (1987)
28 Linder A: Streptozotocin. Arzneimittelforschung 22, 830–861 (1972)
29 Mukherjee B, Mukherjee JR and Chatterjee M: Lipid peroxidation, glutathione levels and changes in glutathione-related enzyme activities in streptozotocin-induced diabetic rats. Immunol Cell Biol 72, 109–114 (1994)
30 Ganda OP, Rossini AA and Like AA: Studies on streptozotocin diabetes. Diabetes 25, 595–603 (1976)