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Protective mechanism of glucose against alloxan-induced β-cell damage: Pivotal role of ATP

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Abbreviations: ATP, adenosine triphosphate; DNA, deoxyribonucleic acid; HEPES, (N-[2-hydroxyethyl]piperazine-N-[2-ethanesulfonic acid; ELISA, enzyme-linked immunosorbent assay; RIA, radioimmunoassay; BSA, bovine serum albumin; NAD^+, nicotinamide adenine dinucleotide; Tris, Tris[hydroxymethyl]aminomethane

Abstract

Glucose prevents the development of diabetes induced by alloxan. In the present study, the protective mechanism of glucose against alloxan-induced β-cell damage was investigated using HIT-T 15 cell, a Syrian hamster transformed β-cell line. Alloxan caused β-cell damages with DNA fragmentation, inhibition of glucose-stimulated insulin release, and decrease of cellular ATP level, but all of these β-cell damages by alloxan were prevented by the presence of 20 mM glucose. Oligomycin, a specific inhibitor of ATP synthase, completely abolished the protective effects of glucose against alloxan-inudced cell damage. Furthermore, treatment of nuclei isolated from HIT-T15 cells with ATP significantly prevented the DNA fragmentation induced by Ca^{2+}. The results indicate that ATP produced during glucose metabolism plays a pivotal role in the protection of glucose against alloxan-induced β-cell damage.

Keywords: glucose, ATP, alloxan, diabetes, DNA fragmentation

Introduction

Chemical compounds that selectively damage the pancreatic β-cells constitute a class of diabetogenic drugs (Fischer, 1985). Alloxan, a cyclic urea derivative, was reported as a potent diabetogenic agent (Dunn *et al.*, 1943), and has been widely used for the induction of experimental diabetes. Although the precise diabetogenic mechanism of alloxan has not been fully understood, evidences indicate that the pancreatic β-cell damage induced by alloxan is mediated through the generation of cytotoxic oxygen free radicals (Cohen & Heikkila, 1974; Yamamoto *et al.*, 1981; Takasu *et al.*, 1991). Okamoto (1985) has proposed that the primary target of reactive oxygen species produced from alloxan is DNA of pancreatic β-cells.

Our previous studies showed that pretreatment of rats with Ca^{2+}-antagonists such as lanthanum and verapamil completely prevented hyperglycemia induced by alloxan (Kim *et al.*, 1991), and alloxan caused the increase of cytosolic free Ca^{2+} in rat pancreatic β-cells (Kim *et al.*, 1994), suggesting that Ca^{2+} plays an important role in the diabetogenesis of alloxan.

Glucose is both metabolic fuel and physiologic stimulus for insulin secretion in pancreatic β-cells. Since the initial report of Sen & Bhattachaya (1952), it is well established that glucose prevents the development of alloxan-induced β-cell damage *in vivo* (Scheynius & Taljedal, 1971; Jansson & Sandler, 1988) and *in vitro* (Malaisse, 1982). This protection of glucose against alloxan-induced β-cell damage is mediated through the metabolism of glucose within the pancreatic β-cells.

HIT-T 15 cells, a Syrian hamster transformed β-cell line (Santerre *et al.*, 1981), retain the capacity of glucose-stimulated insulin release (Ashcroft *et al.*, 1986) and respond to glucose with increased insulin biosynthesis and preproinsulin messenger RNA levels (Gold *et al.*, 1988). The present study was undertaken to examine the protective mechanism of glucose against β-cell damage induced by alloxan in HIT-T 15 cells.

Our results showed that alloxan-induced β-cell damage is due to the DNA fragmentation by alloxan, which was significantly inhibited in the presence of glucose. The protective effect of glucose against alloxan-induced cell damage was completely abolished by treatment with oligomycin, a specific inhibitor of ATP synthase, and furthermore, ATP inhibited Ca^{2+}-induced DNA fragmentation. The results suggest that ATP produced through glucose metabolism within β-cells plays a pivotal role in the protection of glucose against alloxan-induced β-cell damage.

Materials and Methods

Cell culture

HIT-T 15 cells, a Syrian hamster clonal β-cell line, were obtained from Korea Cell Bank (College of Medicine, Seoul National University, Korea). HIT-T 15 cells were cultured in RPMI-1640 medium containing 11 mM glucose supplemented with 10% fetal calf serum, 100 units/ml of penicillin, 0.1 mg/ml streptomycin, and 2.5 μg/ml amphotericin B at 37°C in 5% CO_2 and 95% air.

Insulin secretion

HIT-T 15 cells were cultured in RPMI medium supplemented with 10% fetal calf serum for 3 days prior to secretion experiments. Cells at 70-80% confluence in 12-well dishes were used for experiments. The cells were preincubated for 1 h with Krebs-Ringer buffer (KRB) containing 119 mM NaCl, 4.74 mM KCl, 2.54 mM $CaCl_2$, 1.19 mM $MgSO_4$, 1.19 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM HEPES, and 0.1% bovine serum albumin at pH 7.4. Then cells were treated with alloxan for 30 min in the presence or absence of 20 mM glucose. At the end of experiments, cells were washed twice and incubated in fresh KRB containing 15 mM glucose for 30 min. The released insulin in medium was determined by radioimmunoassay (Coat-A-Count insulin kit, Diagnostic Products Cooperation, Los An-gles, CA).

DNA fragmentation

The cellular DNA fragmentation of HIT T-15 cells was determined using a Cellular DNA Fragmentation ELISA Kit (Boehringer Mannheim GmbH, Germany). HIT-T 15 cells were incubated with 10 mM BrdU overnight at 37°C, centrifuged at 250×g for 10 min, adjusted to 1×10^5 cells/ml in RPMI-1640 medium and plated in a 96-multiwell plate. After treatment with alloxan for 30 min in the presence or absence of 20 mM glucose, DNA fragmentation of cells was determined by ELISA kit using peroxidase-conjugated anti-BrdU antibody solution. The absorbance was measured at 450 nm against substrate solution as a blank.

Cell viability

Cell viability was determined by an exclusion method of Trypan blue dye (Rosengard & Cochrane, 1983). The cells were incubated briefly with trypan blue, and the number of cells excluding trypan blue out of cells were counted on a hemocytometer, and the percentage of viability was expressed as the number of live cells/total number of cells ×100.

Measurement of ATP

Cellular ATP was determined by the chemiluminescence method using luciferin-luciferase (Ludin, 1978). To measure cellular ATP, HIT-T 15 cells were treated with various drugs and then lysed with 100 mM glycine buffer (pH 7.4) containing 0.05% Triton X-100 (10^6 cells/100 μl). After brief centrifugation, 80 μl of the supernatant was added to 100 μl of luciferin-luciferase solution (10 mg/ml), and the chemiluminescence was read by Luminometer (Lumat, LB 9501, Netherland). A standard ATP was prepared just before use.

Isolation of nuclei

HIT-T 15 cells were detached by trypsin and homogenized with a glass-Teflon homogenizer in 0.25 M sucrose containing 50 mM Tris-HCl, 25 mM KCl and 5 mM $MgCl_2$ (pH 7.4). The nuclei were isolated from the homogenate by the method of Hogeboom (1955), and the resulting pellet of purified nuclei was resuspended in Ca^{2+}-free KRB.

Results

Alloxan induced experimental diabetes through the destruction of the insulin producing β-cells of the pancreas. In the present study, HIT-T 15 cells were used to investigate a protective mechanism of glucose against alloxan-induced diabetes.

To study the influence of alloxan on HIT-T 15 cells, cells were treated with various concentrations of alloxan. Cytotoxic effects of alloxan on HIT cells were determined by cell viability and DNA fragmentation. Cell viability was determined by trypan blue dye exclusion test and the DNA fragmentation was measured using ELISA method. As shown in Figure 1, alloxan caused the cell death in a dose-dependent manner, approximately 50% of the cells being dead by treatment with 2 mM alloxan. According to Okamoto's model (1985), the DNA fragmentation by alloxan is a critical step in the

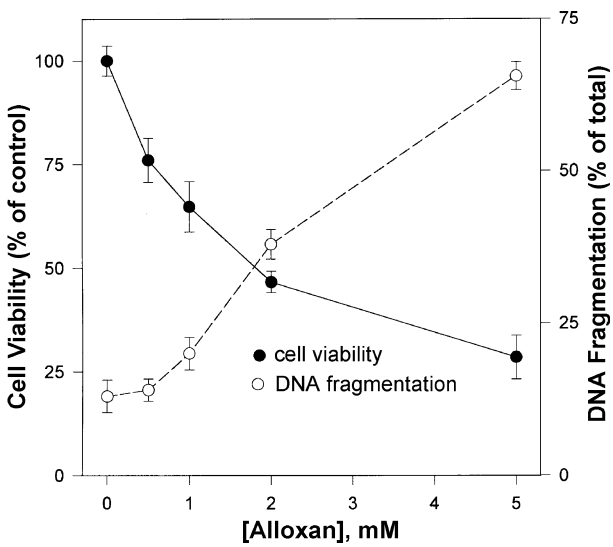

Figure 1. Effect of alloxan on HIT-T15 cell. HIT-T 15 cells were treated with various concentrations of alloxan at 37°C for 30 min. Cell viability and DNA fragmentation were determined by the ability to exclude trypan blue and ELISA method, respectively. Each value denotes the mean ± SE of five separate experiments.

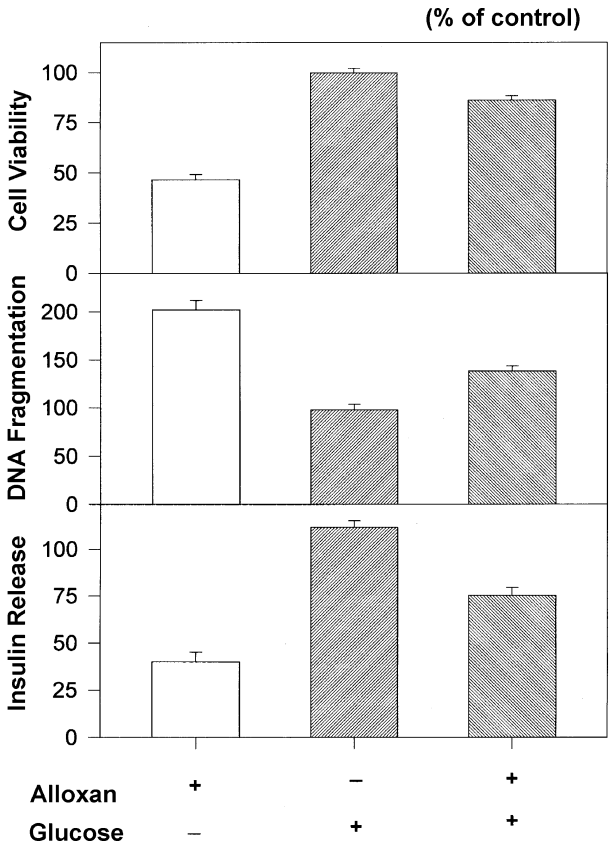

Figure 2. Protective effects of glucose against β-cell damage induced by alloxan. Cells were treated with 2 mM alloxan for 30 min in the absence or presence of 20 mM glucose. Results are mean ± SE of five experiments.

induction of alloxan-diabetes. Alloxan caused the increase of DNA fragmentation of HIT-T 15 cells dose-dependently, about 40% of total DNA was fragmented by treatment with 2 mM alloxan. This result indicates that alloxan induces HIT-T 15 cell damage with the increasing DNA fragmentation.

To investigate the protective mechanism of glucose against alloxan-induced cell damage, HIT-T 15 cells were treated with 2 mM of alloxan in the presence or absence of 20 mM glucose. The effect of glucose on cell death, DNA fragmentation and inhibition of glucose-stimulated insulin release induced by alloxan was evaluated. As shown in Figure 2, Alloxan caused the increase of DNA fragmentation and the inhibition of glucose-stimulated insulin secretion, resulting in cell death. Treatment of cells with 2 mM alloxan caused approximately 2-fold increase of DNA fragmentation and 65% inhibition of glucose-stimulated insulin secretion. However, these cytotoxic effects of alloxan were significantly abolished by treatment with 20 mM glucose.

ATP plays an important role in many cellular functions and the depletion of cellular ATP is known as a hallmark of the deterioration of cell metabolism. The effect of alloxan on cellular ATP level was studied. As shown in

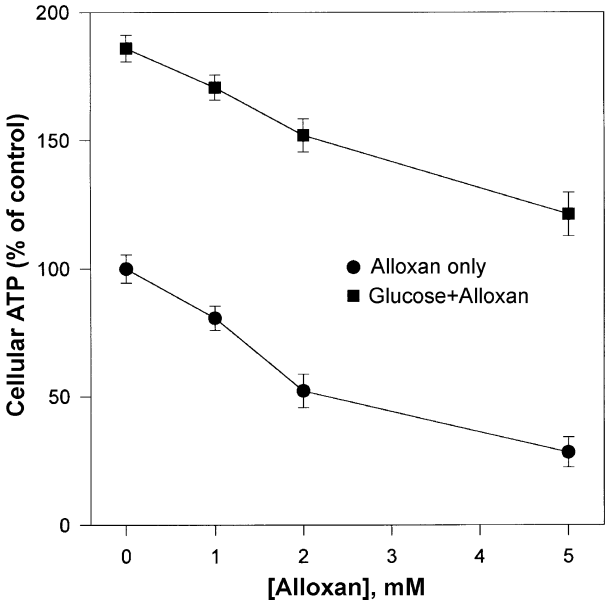

Figure 3. Effect of glucose on the depeletion of cellular ATP by alloxan in HIT-T 15 cells. Cells were treated with 2 mM alloxan for 30 min in the absence or presence of 20 mM glucose. The cellular ATP was determined by chemiluminescence method. Results are mean ± SE of five experiments.

Figure 3, alloxan caused the depletion of the cellular ATP in a dose dependent manner and approximately 50% of total ATP were depleted by treatment with 2 mM alloxan. However, the depletion of cellular ATP by alloxan was completely abolished in the presence of 20 mM glucose. Glucose, itself, caused the increase of the cellular ATP level (about 180% of control).

It is generally accepted that metabolism of glucose in the pancreatic β-cell is an important feature in the glucose-mediated protection against alloxan-diabetes. To verify whether the protective effects of glucose were mediated through ATP, oligomycin, a specific inhibitor of ATP synthase, was given to the cells. Table 1 shows that oligomycin caused the depletion of the cellular ATP

Table 1. Inhibitory effects of oligomycin on the protective action of glucose against alloxan-induced β-cell damage

Treatment	Cellular ATP	DNA Fragmentation	Cell Viability
		(% of control)	
Alloxan	51 ± 5.5	202 ± 9.6	46 ± 2.9
Glucose	153 ± 8.2	138 ± 5.4	86 ± 2.2
Oligomycin	74 ± 3.9	165 ± 4.5	65 ± 2.5
Glucose +Oligomycin	60 ± 3.8	180 ± 3.8	65 ± 2.1
Glucose +Oligomycin +Alloxan	27 ± 2.9	215 ± 8.9	38 ± 2.6

After preincubation of HIT-T 15 cells with 10 mg/ml oligomycin, cells were treated with 2 mM alloxan for 30 min in the absence or presence of 20 mM glucose. Results are mean ± SE of five experiments.

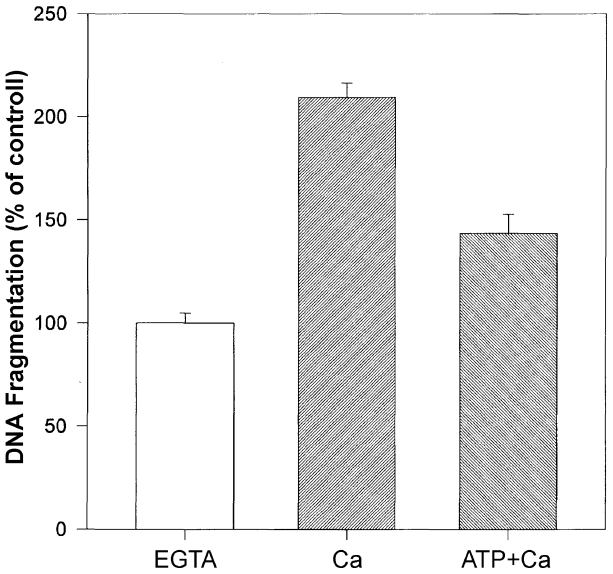

Figure 4. Effect of ATP on DNA fragmentation in the nuclei isolated from HIT-T 15 ells. HIT-T 15 cells were treated with 10 mM BrdU for 16 h at 37°C and nuclei were isolated from cells. Nuclei were incubated for 30 min in the incubation medium containing 5 mM EGTA or 2 mM $CaCl_2$ in the absence or presence of 1 mM ATP. The amount of DNA fragmentation in nuclei was measured by ELISA. Results are mean ± SE of five separate experiments.

level. Oligomycin completely blocked the protective effects of glucose against alloxan-induced cell damage, including the inhibition of DNA fragmentation and cell death. The results suggest that the protective effects of glucose against alloxan-induced cell damages may be mediated through ATP produced by glucose metabolism.

In our previous study (Park *et al*., 1995), treatment of rat pancreatic islet with Ca^{2+}-antagonist completely prevented alloxan-induced islet cell damage and the DNA strand breaks by alloxan. However, glucose did not abolish the generation of hydrogen peroxide and the increase of cytosolic free Ca^{2+} by alloxan, suggesting Ca^{2+} is participated in the DNA fragmentation. In order to investigate the effect of ATP on DNA strand breaks, the nuclei were isolated from HIT-T 15 cell homogenate. As shown in Figure 4, treatment with Ca^{2+} of nuclei isolated from HIT-T 15 cells caused the increase of DNA fragmentation and this DNA fragmentation by Ca^{2+} was significantly inhibited by ATP treatment.

Discussion

Alloxan induces diabetes in experimental animals through the selective damage of pancreatic β-cells. It is generally accepted that β-cell damage induced by alloxan occurs through the noxious oxygen free radicals (Cohen & Heikkila, 1974; Yamamoto *et al*., 1981; Takasu *et al*., 1991). However, the exact subcellular site for an initial attack by alloxan is still not clear. Evidences of damage to plasma membrane (Cooperstein & Watkins, 1978), mitochondria (Boquist *et al*., 1983) and nuclei (Yamamoto *et al*., 1981; Okamoto, 1985) are available. Okamoto (1985) proposed that reactive oxygen species produced from alloxan cause DNA strand breaks, and the damaged DNA activates nuclear poly(ADP-ribose) synthetase, which depletes the cellular pool of NAD^+, resulting in β-cell damage. In the previous studies, we reported that treatment of rat with Ca^{2+}-antagonists completely prevented hyperglycemia induced by alloxan (1991), and alloxan caused the increase of cytosolic free Ca^{2+} of rat pancreatic β-cells (1994). In addition, Ca^{2+}-antagonist did not prevent the hydrogen peroxide generation by alloxan. These results suggest that Ca^{2+} plays an important role between the generation of oxygen free radical and the DNA strand breaks by alloxan.

In the present study, HIT-T 15 cells were used to investigate the protective mechanism of glucose against alloxan-induced cell damage. HIT-T 15 cells have similar characteristics compared with normal pancreatic islets, in respect to the capacity of glucose-stimulated insulin secretion and the sensitivity to alloxan. Glucose significantly prevented the cytotoxic effects of alloxan including the increase of DNA fragmentation, inhibition of glucose-stimulated insulin release, and decrease of cellular ATP level, but did not affect the generation of hydrogen peroxide and increase of cytosolic free Ca^{2+} by alloxan.

There are many reports that glucose protected against alloxan-induced diabetes (Bhattacharya, 1952; 1954; Scheynius & Taljedal, 1971; Malaisse, 1982). Glucose did not affect the uptake of alloxan into the pancreatic β-cell (Weaver *et al*., 1978). Mannoheptulose that inhibits a glucose phosphorylation in islets (Malaisse *et al*., 1968), suppresses the protective action of glucose on the alloxan-induced alteration of islet function (Zawalich & Beidler, 1973), and the respective capacity of different hexoses to protect against alloxan was strictly superimposable to their capacity to stimulate glycolysis or insulin release in pancreatic islets (Zawalich & Beidler, 1973). Therefore, it has been generally accepted that glucose needs to be metabolized in islets in order to exert its protective action against alloxan. The major possible metabolites of glucose include ATP and NADPH. ATP plays an important role in many cellular functions, and NADPH is required for the glutathione redox cycle, which is an important pathway for peroxide detoxification (Ben-Yoseph *et al*., 1996). Averill-Bates & Przybytkowski (1994) reported that glucose can protect the ovarian cells of Chinese hamster from the damage by hydrogen peroxide, and Link (1988) reported that the absence of glucose can cause endothelial cells to be more susceptible to oxidative damage by hydrogen peroxide, suggesting the role of glucose on pentose phosphate pathway for detoxification of hydrogen peroxide via the glutathione redox cycle. However, in HIT-T 15 cells, glucose did not cause the significant reduction in the generation of

hydrogen peroxide by alloxan, suggesting that the protective effects of glucose against alloxan-induced β-cell damage may be mediated by another metabolites.

Alloxan caused the depletion of cellular ATP and this loss of ATP is believed to be a result of the lack of NAD^+ available and/or oxidative phosphorylation (Berger, 1985). Glucose completely inhibited the depletion of ATP by alloxan, and oligomycin, a specific inhibitor of ATP synthase, completely abolished the protective effects of glucose against alloxan-induced β-cell damage. The results indicate that ATP produced by glucose metabolism plays a pivotal role in the protection of glucose against alloxan-induced β-cell damage. In our previous study (Kim *et al*., 1994), verapamil, a Ca^{2+}-antagonist, abolished the toxic effect of alloxan, but did not affect the production of hydrogen peroxide by alloxan, suggesting that alloxan-derived oxygen radicals may induce the increase of Ca^{2+} influx, which results in secondary reactions ultimately leading to DNA strand breaks. To confirm this suggestion, the nuclei isolated from HIT-T 15 cell homogenate were treated with Ca^{2+} which caused the increase of DNA fragmentation and this DNA fragmentation was significantly inhibited in the presence of ATP (Figure 4). Jones *et al.* (1989) reported that the incubation of isolated rat liver nuclei with Ca^{2+} resulted in extensive DNA hydrolysis and glucocorticoid-induced apoptosis of thymocytes occurred by the activation of Ca^{2+}-dependent endonuclease (Duke *et al*., 1983; Cohen & Duke, 1984). The DNA fragmentation induced by alloxan may be mediated by Ca^{2+}-dependent endonuclease, the activity of which is affected by ATP.

The present results indicate that the glucose protects against alloxan-induced β-cell damage by inhibition of DNA fragmentation and this protective action of glucose is mediated through ATP produced during glucose metabolism.

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References

Ashcroft, S. J. H., Hammonds, P. and Harrison, D. E. (1986) Insulin secretory responses of a clonal cell line of simian virus 40-transfected B cells. *Diabetologia* 29: 727-733

Ben-Yoseph, O., Boxer, P. A. and Ross, B. D. (1996) Assessment of the role of the glutathione and pentose phosphate pathways in the protection of primary cere-brocortical cultures from oxidative stress. *J. Neurochemistry* 66: 2329-2337

Berger, N. A. (1985) Poly(ADP-ribose) in the cellular response to DNA damage. *Radiation Res.* 101: 4-15

Bhattacharya, G. (1954) On the protection against alloxan diabetes by hexoses. *Science* 120: 841-843

Boquist, L., Nelson, L. and Lorentzon, R. (1983) Uptake of labeled in mouse organs and mitochondria *in vivo* and *in vitro. Endocrinology* 113: 943-948

Cohen, G. and Heikkila, R. E. (1974) Generation of hydrogen peroxide, superoxide radical by 6-hydroxyl dopamine, dialuric acid and related agents. *J. Biol. Chem.* 49: 2447-2452

Cohen, J. J. and Duke, R. C. (1984) Glucocorticoid activation of a calcium-dependent endonuclease in thymocyte nuclei lead to death. *J. Immunol.* 132: 38-42

Cooperstein, S. J., Watkins, D. (1978) Effect of sulfhydryl binding reagents on islet tissue permeability: Protection and reversal by D-glucose and Phlorizin. *J. Pharmacol. Exp. Ther.* 204: 230-239

Duke, R. C., Chervenak, R. and Cohen, J. J. (1983) Endogenous endonuclease-induced DNA fragmentation: An early event in cell-mediated cytolysis. *Proc. Natl. Acad. Sci. USA* 80: 6361-6365

Dunn, J. S., Sheehan, H. L. and McLetchie, N. G. (1943) Necrosis of Langerhans produced experimentally. *Lancet* I: 484-487

Fischer, L. J. (1985) Drugs and chemicals that produce diabetes. *TIPS*. 6: 72-75

Gold, G., Walker M. D., Edwards, D. L. and Grodsky, G. M. (1988) Biosynthetic regulation of endogenous hamster insulin and exogenous rat insulin II intransfected HIT cells. *Diabetes* 37: 1509-1514

Hogeboom, G. H. (1955) Fractionation of cell components of animal tissues. *Methods of Enzymol.* 1: 16-19

Jansson, L. and Sandler, S. (1988) The protective effect of glucose, but not 3-O-methyl glucose, against alloxan-induced diabetes depends upon the route of hexose administration. *Arch. Int. Pharmacodyn.* 293: 257-264

Jones, D. P., McConkey, D. J., Nicotera, P. and Orrenius, S. (1989) Calcium-activated DNA fragmentation in rat liver nuclei. *J. Biol. Chem.* 264: 6398-6403

Kim, H. R., Rho, H. W., Park, B. H., Park, J. W., Kim, J. S., Kim, U. H. and Chung, M. Y. (1994) Role of Ca^{2+} in alloxan-induced pancreatic β-cell damage. *Biochim. Biophys. Acta* 1227: 87-91

Kim, H. R., Rho, H. W., Park, J. W., Kim, J. S., Kim, U. H. and Jhee, E. C. (1991) Protection of alloxan-induced β-cell damage of rat pancreas by Ca^{2+}-antagonists. *Kor. J. Biochemistry* 23: 141-146

Ludin, A (1978) Determination of creatine kinase isoenzyme in human serum by an immunological method using purified firefly luciferase. *Methods Enzymol.* 57: 56-65

Malaisse, W. J. (1982) Alloxan toxicity to the pancreatic B-cell. A new hypothesis. *Biochem. Pharmacol.* 31: 3527-3534

Malaisse, W. J., Lea, M. A. and Maiaisse-Lagae, F. (1968) The effect of mannoheptulose on the phosphorylation of glucose and the secretion of insulin by islets of Langerhans. *Metabolism*

17: 126-132

Okamoto, H. (1985) Molecular basis of experimental diabetes: Degeneration, Oncogenesis and regeneration of pancreatic B-cells of islet of Langerhans. *BioEssay* 2: 15-21

Park, B. H., Rho, H. W., Park, J. W., Cho, G. G, Kim, J. S., Chung, H. T. and Kim, H. R. (1995) Protective mechanism of glucose against alloxan-induced pancreatic β-cell damage. *Biochem. Biophys. Res. Commun.* 210: 1-6

Rerup, C. C. (1970) Drugs producing diabetes through damage of insulin secreting cells. *Pharmacol. Rev.* 22: 485-518

Rosengard, B. R. and Cochrane, D. E. (1983) Complement-mediated cytolysis: A quick, simple method for determining levels of immunoglobulin E bound to mast cells. *J. Histochem* 31: 441-444

Santerre, R. F., Cook, R. A., Crisel, R. M. D., Sharp, J. D., Schmidt, R. J., Williams D. C. and Wilson, C. P. (1981) Insulin synthesis in a cloned cell line of simian virus 40-transformed hamster pancreatic beta cells. *Proc. Natl. Acad. Sci. USA* 78: 4339-4343

Scheynius, A. and Taljedal, I. B. (1971) On the mechanism of glucose protection against alloxan toxicity. *Diabetologia* 7: 252-255

Sen, P. B. and Bhattacharya, G. (1952) Protection against alloxan diabetes by glucose. *Indian J. Physiol.* 6: 112-114

Sener, A., Levy, J. and Malaisse, W. J. (1976) Stimulus-secretion coupling of glucose-induced insulin release. Does glycolysis control calcium transport in the B-cell? *Biochem. J.* 156: 521-525

Takasu, N., Asawa, T., Komiya, I., Nagasawa, Y. and Yamada, T. (1991) Alloxan-induced DNA strand breaks in pancreatic islets: Evidence for H_2O_2 as an intermediate. *J. Biol. Chem.* 266: 2112-2114

Uchigata, Y., Yamamoto, H., Kawamura, A. and Okamoto, H. (1982) Protection by superoxide dismutase, catalase, and poly(ADP-ribose) synthetase inhibitors against alloxan- and streptozotocin-induced islet DNA strand breaks and against inhibition of proinsulin synthesis. *J. Biol. Chem.* 257: 6984-6088

Weaver, D. C., McDaniel, M. L. and Lacy, P. E. (1978) Alloxan uptake by isolated rat islets of Langerhans. *Endocrinology.* 102: 1847-1855

Yamamoto, H., Uchigata, Y. and Okamoto, H. (1981) DNA strand breaks in pancreatic islets by *in vivo* administration of alloxan or streptozotocin. *Biochem. Biophys. Res. Commun.* 103: 1014-1020.

Zawalich, W. S and Beidler, L. M. (1973) Glucose and alloxan interactions in the pancreatic islets. *Am. J. Physiol.* 224: 963-966.