Protective mechanism of glucose against streptozotocin-induced free radical generation in pancreatic islets *in vivo* and *in vitro*

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The present study was undertaken to investigate the role of glucose and its mechanism of action in protecting β-cells *in vivo* and *in vitro* against cytotoxic effect of the diabetogenic compound streptozotocin (STZ). It was found that none of the mice injected with glucose prior to STZ treatment became hyperglycemic for 15 days whereas in only STZ treated group 7 out of 8 mice became hyperglycemic within 8–10 days. *In vitro* studies were carried out in RINm5F cells and isolated islets from Balb/c male mice. It was observed that concomitant exposure of RIN and isolated islets to glucose and STZ protect them from injury as evidenced by the reduction in lipid peroxidation and DNA damage, coupled with decreased death of cells. This mode of action of glucose is of significance in promoting β-cell health prior to diabetogenic insults.

THE diabetogenic agent streptozotocin (STZ) is selectively toxic to insulin-secreting β-cells of the pancreatic islets[1]. Recently it has been shown that STZ-induced diabetes could be prevented by treatment with D-glucose and 5-thio-D-glucose[2]. Further, it has been documented that alloxan-induced cytotoxicity can also be reduced by glucose[3]. However, the mechanism of action of glucose in attenuating STZ or alloxan induced β-cell injury has not been elucidated[2,3]. Present studies were therefore undertaken to ascertain the mode of action of STZ through free radical generation and its possible scavenging by glucose treatment employing *in vivo* and *in vitro* studies.

A rat insulinoma cell line (RINm5F) was a generous gift from the University of Calgary, Canada which was used in all the experiments. All reagents and chemicals were purchased from Sigma Chemical Co., USA unless otherwise specified.

Six to eight-week old Balb/c male mice were injected intra peritoneally with glucose (100 mg/kg body weight) and immediately subjected to a single dose of STZ (200 mg/kg body weight dissolved in ice cold citrate buffer pH 4.5 prior to injection). Only glucose treated and only STZ treated animals were also included in the same experiments as positive and negative controls. Each group contained 7–8 mice. Plasma glucose levels (mg/dl) were estimated once a week for 15 days to ascertain the hyperglycemic status in different groups of mice.

Pancreatic islets were isolated from Balb/c male mice pancreata by collagenase digestion following the method described by Gotoh *et al.*[4]. Briefly, pancreata were distended by injecting 0.1% collagenase in Hank's balanced salt solution (HBSS) through common bile duct. Islets were separated from the digestion mixture on Ficoll gradient. Fixed number of islets were hand picked under the dissecting microscope and were incubated at 37°C in a CO_2 incubator in Roswell Park Memorial Institute (RPMI-1640) supplemented with 10% foetal calf serum (FCS).

Rat insulinoma (RIN) cells were cultured in 25 cm^2 tissue culture flasks (Nunc, Denmark) and grown in RPMI-1640 medium (Gibco, USA) supplemented with heat inactivated 10% FCS, in a humidified atmosphere of 5% CO_2 at 37°C. On attaining 75–80% confluency the cells were washed with RPMI-1640 medium and treated with 1,5 and 10 mM STZ/H_2O_2 dissolved in citrate buffer (pH 4.5) and in L-15 medium with or without 16 mM glucose. The culture flasks were incubated at 37°C for 1 h. The cells were washed with phosphate buffered saline (PBS, pH 7.4) and levels of malondialdehyde (MDA) were estimated.

After incubation with the reagents described, MDA levels were measured by the thiobarbituric acid (TBA) method as reported previously[5] and expressed in terms of MDA formed per mg protein of the cell suspension. Cell protein concentration was measured by the method as described by Lowry[6] using bovine serum albumin as standard. Cell viability was determined by the Trypan blue dye exclusion test and MTT assay[7].

The amount of apoptosis was determined using the stain propidium iodide (PI) which intercalates into double stranded nucleic acids that can be excited by 488 nm lasers[8]. Cells in suspension (optionally stained for surface immuno-fluorescence) are fixed with methanol and stained with propidium iodide. Since PI also intercalates RNA, RNAse was added, incubated in the dark at room temperature for 30 min and the fluorescence intensity was measured with a FACScan (Becton Dickinson, San Jose, CA).

One way ANOVA followed by Student–Newman–Keul's method was used to compare the significance of the differences between data. All data are expressed as means ± SD.

It was observed that none of the mice in uninjected controls, citrate buffer injected controls, glucose treated group and glucose + STZ treated group became hyperglycemic for the period studied (15 days). The only STZ treated mice became hyperglycemic within 8 to 10 days after injection (Table 1). It appears from the data that injection of glucose prior to STZ treatment prevents the incidence of hyperglycemia probably by protecting the islets/β -cells against the STZ induced cytotoxicity.

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RIN cells/isolated islets treated with STZ alone exhibited highest level of MDA as opposed to untreated and only D-glucose (16 mM) treated controls. However, the MDA levels were found to be reduced substantially in glucose and STZ-treated cultures (37.2% in RIN cells and 52.7% in islets) as compared to those obtained in only STZ-treated cultures (100%) (Figure 1).

RIN cells treated with STZ alone (67.56%) exhibited lowest level of viability as opposed to untreated (100%) and STZ + 16 mM D-glucose (96.096%) treated controls (Figure 2).

RIN cells treated with STZ alone exhibited highest level of DNA damage (% Gated, 8.16) as opposed to untreated (% Gated, 6.24) and STZ + D-glucose (16 mM) treated controls (% Gated, 6.32) (Figure 3).

In the present investigation we have demonstrated that glucose treatment prior to STZ injections in Balb/c mice leads to prevention of hyperglycemia and subsequent diabetes in all the mice (Table 1), indicating beneficial role of glucose by protecting β-cells from the cytotoxic

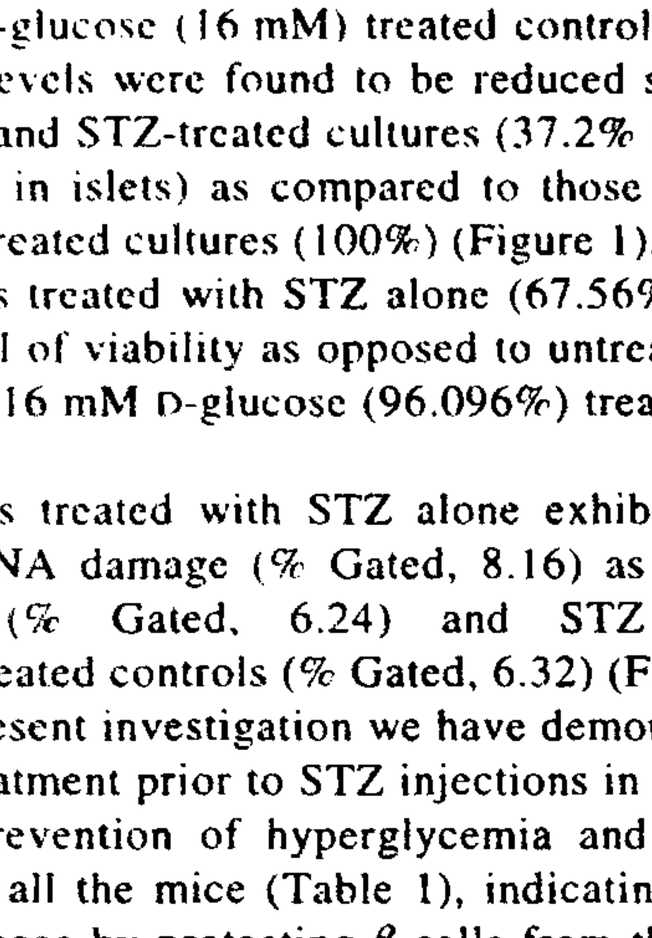

Figure 1. Effect of D-glucose on STZ-induced lipid peroxidation in RIN cells and isolated islets. RIN cells and isolated islets were treated with 16 mM concentration of D-glucose prior to STZ treatment. Lipid peroxidation was estimated and expressed in terms of nmole MDA/mg protein. Values represent an average of triplicates ± SD. ◆ Significantly different from control ($P < 0.05$). ■ Significantly different from STZ-treated cells ($P < 0.05$).

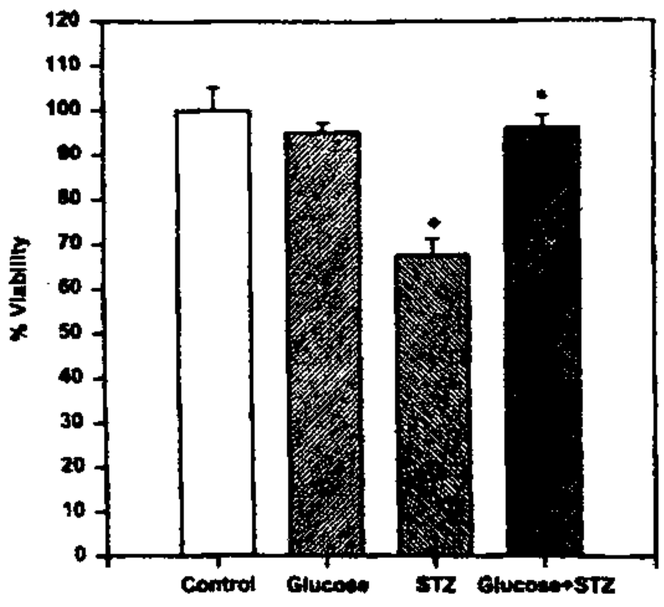

Figure 2. Effect of D-glucose on STZ-induced cell viability in RIN cells. RIN cells were treated with 16 mM concentration of D-glucose prior to STZ (1 mM) treatment. Viability was determined by MTT assay and expressed as % viability. Values represent an average of triplicates ± SD. ◆ Significantly different from control ($P < 0.05$). ■ Significantly different from STZ-treated cells ($P < 0.05$).

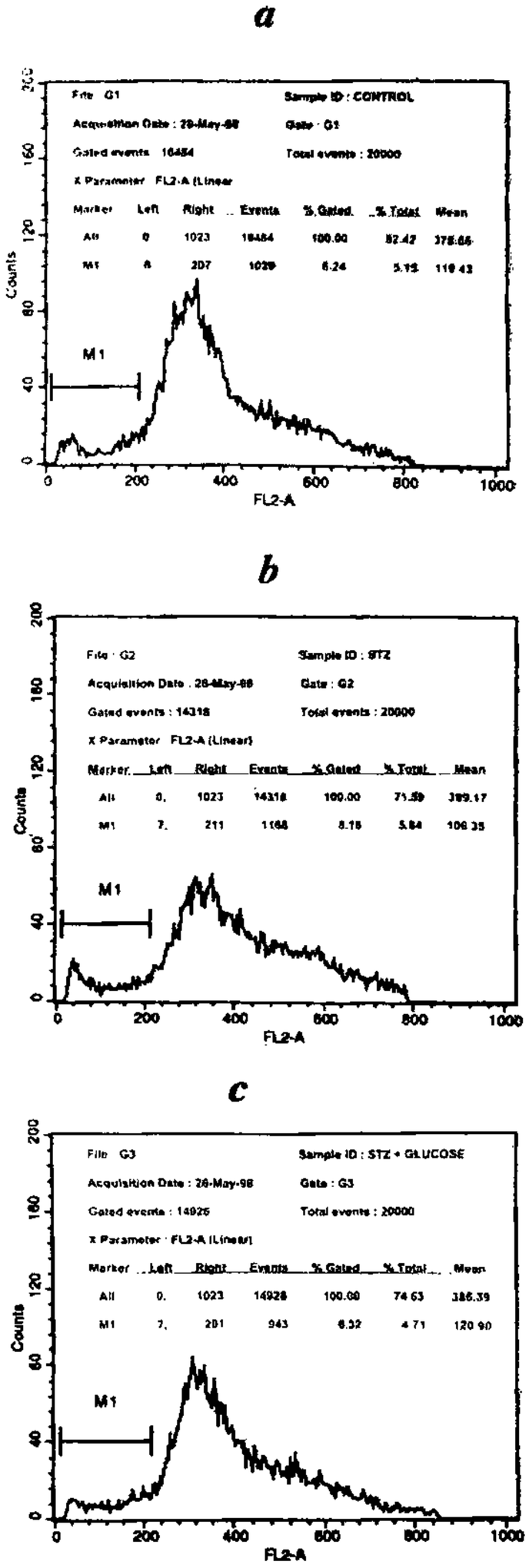

Figure 3. Analysis of apoptosis in cells treated with STZ. Relative amount of apoptosis was measured. After 24 h incubation without or with glucose, treated cells in suspension (optionally stained for surface immuno-fluorescence) were fixed with methanol and stained with propidium iodide. Since PI also intercalates RNA, RNAse was added, incubated in the dark at room temperature for 30 min and the fluorescence intensity was measured.

Table 1. Blood glucose status on day 15

Group	Blood glucose on 15th PI day (mg/dl of plasma)	Hyperglycemic	Normoglycemic
Control	91 ± 11	0/8	8/8
Glucose treated	108 ± 17	0/8	7/7
Citrate buffer	118 ± 15	0/7	7/7
STZ	360 ± 22	7/8	1/8
Glucose + STZ	110 ± 19	0/7	7/7

action of STZ. On the other hand, only STZ treatment resulted in the development of diabetes in 7 out of 8 mice. In addition to this our *in vitro* results show that incubation of rat insulinoma cells (RINm5F) and isolated islets with STZ for 1 h at 37°C induces high levels of MDA formation (Figure 1), and DNA damage (Figure 3). Perhaps this is the first report on lipid peroxidation in RIN cells induced by STZ. It has been suggested that cytotoxic action of STZ and alloxan on RIN cells are mediated by the generation of hydroxyl free radicals and DNA strand breaks[9]. Presence of D-glucose at the time of STZ action seems to play an important role in attenuating the toxic effect of STZ as revealed by low levels of MDA obtained in STZ along with glucose treated cultures as opposed to STZ treated ones alone (37.2% in RIN cells and 52.7% in islets). Our results not only support those obtained by Wang *et al.*[2], wherein they have shown the protective role of D-glucose and other sugars in preventing STZ-induced diabetes in mice but also explain the mechanism of action of glucose via reduction of lipid peroxidation. Moreover, our FACS analysis data clearly demonstrates prevention of DNA damage obtained by glucose treatment. Our viability studies employing MTT assay also provides substantial evidence for the protection offered by glucose against STZ-induced cytotoxicity.

All these aspects taken together strongly suggest a protective role of glucose permitting survival of islets *in vivo* as well as *in vitro* after concomitant exposure to glucose and STZ.

Such protection has been offered by 20 mM glucose against cytotoxicity of interleukin (IL-1) and tumour necrosis factor (TNF) *in vitro*[10].

Even though several mechanisms have been postulated for the action of glucose in attenuating STZ-induced damage, the exact role of glucose in such situations has not been pinpointed[11,12]. The underlying mechanism behind all these beneficial effects of glucose could be attributed to reduction in lipid peroxidation, DNA damage, and increment in cell viability as revealed by our present data. The study further implies that in a well-fed state the islets are activated which makes them rather resistant to diabetogenic insults, whereas undernourishment during pregnancy or adulthood makes the islets vulnerable to diabetogenic insult and thereby increase the incidence of diabetes[13].

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Disease and stress-induced mortality of corals in Indian reefs and observations on bleaching of corals in the Andamans

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A study was carried out in the Lakshadweep and Andaman islands and the Gulf of Kutch to assess the health of corals in Indian reefs. Disease, predation and stress were the major factors of coral mortality. Death caused by diseases – the black band disease (BBD), the white band disease (WBD) – necrotic lesions, and bleaching was observed in Kavaratti and Kadamat islands of Lakshadweep. The predatory starfish, *Acanthaster planci*, grazing on coral polyps was also noticed in these reefs. Large-scale silt deposition in the intertidal zone of Paga, Boria, Vadinar and Mangunda reefs in the gulf of Kutch buried the coral colonies and appeared to be the main cause of coral mortality. A severe incidence of coral bleaching was observed during July 1998 in some reefs in the Andamans. While more than 85% of corals near Ross island and Marina Park exhibited partial bleaching, up to 10% were totally bleached.

SEVERAL diseases and stress reactions in scleractinian corals from various parts of the world have been reported[1,2]. Many of these diseases are microbially

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