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Major species differences between humans and rodents in the susceptibility to pancreatic β-cell injury

(nitric oxide/streptozotocin/alloxan/insulin-dependent diabetes mellitus)

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ABSTRACT **The ability of β cells to endure assaults may be relevant in the development of insulin-dependent diabetes mellitus. This study examines the susceptibility of human pancreatic islets to agents that are cytotoxic for rodent β cells—i.e., sodium nitroprusside (NP, a nitric oxide donor), streptozotocin (SZ), or alloxan. After 5–8 days in tissue culture, human or rodent islets were exposed for 14 h to NP (50–200 μM) or for 30 min to SZ or alloxan (1–3 mM). Glucose oxidation by human islets was not reduced by NP, but there was a dose-dependent inhibition in rat (40–90% inhibition; $P < 0.001$) and mouse (10–60% inhibition; $P < 0.05$) islet glucose oxidation. Glucose (16.7 mM)-induced insulin release by human islets was not impaired after a 30-min exposure to SZ or alloxan, at concentrations that inhibited insulin release from rat (30–80% inhibition; $P < 0.001$) or mouse (10–70% inhibition; $P < 0.05$) islets. The viability of human β cells purified by flow cytometry was not affected by SZ or alloxan (5 mM), as judged 1 or 4 days after a 10-min exposure and subsequent culture; these conditions were cytotoxic for rat β cells, with 65–95% ($P < 0.01$) dead β cells after 4 days. Human islets transplanted under the kidney capsule of nude mice were not affected by *in vivo* alloxan exposure, as suggested by preserved graft morphology and insulin content, whereas the endogenous β cells of the transplanted mice were severely damaged (80% decrease in pancreatic insulin content and morphological signs of β-cell destruction). Thus human β cells are resistant to NP, SZ, or alloxan at concentrations that decrease survival and function of rat or mouse β cells. These marked interspecies differences emphasize the relevance of repair and/or defense mechanisms in β-cell destruction and raise the possibility that such differences may also be present among individuals of the same species.**

The clinical outbreak of insulin-dependent diabetes mellitus (IDDM) is probably preceded by a long prediabetic period (1), characterized by a delicate balance between autoimmune-induced β-cell damage (1, 2) and β-cell repair (3). Among the potential mediators of β-cell damage, cytokines and cytokine-induced nitric oxide (NO) production have received special attention (4–8). It has been suggested that these agents damage rat islets *in vitro* (4–8), but their effects on cultured human islets are less pronounced, despite production of similar amounts of NO (9). These observations raise the question whether species differences exist in the susceptibility of pancreatic β cells to injury. To assess this possibility, we exposed cultured human, rat, or mouse pancreatic islets to sodium nitroprusside (NP; an NO donor) (10), streptozotocin (SZ; an alkylating agent) (11), or alloxan (a generator of oxygen free radicals) (12) and evaluated the effects of these toxins on islet function. The *in vitro* cytotoxicity of alloxan and SZ was measured on purified human or rat β cells, by using a cell viability test after 1–4 days of subsequent culture (13). Finally, the *in vivo* toxicity of alloxan was compared in nude mice that had received a graft of either human or mouse pancreatic islets under the kidney capsule.

MATERIALS AND METHODS

Islet Isolation. Ten human pancreata were excised from organ donors and transported to the Central Unit of the β-Cell Transplant, Brussels, and the islets were isolated as described (14). The age of the donors (mean ± SEM) was 26 ± 3 years (range, 9–42 years). Aliquots of the islet-enriched fraction were examined by electron microscopy ($n = 10$), which indicated 5 ± 1% dead cells and 1 ± 1% acinar cells in the preparations. The prevalence of insulin- and glucagon-positive cells was evaluated by light microscopical examination of immunocytochemically stained islets (15), revealing 57 ± 4% insulin-positive cells and 10 ± 1% glucagon-positive cells. The islet insulin content was 1.60 ± 0.27 ng of insulin per ng of DNA. Human or Wistar rat pancreatic β cells were purified in Brussels by autofluorescence-activated cell sorting in a FACS-IV (Becton Dickinson) (15, 16) and tested locally in the cytotoxicity studies. The isolation of adult rat (Sprague–Dawley) and mouse (NMRI) islets in Uppsala has been described (17). NMRI islets cultured as in the present series of experiments present a prevalence of 77% insulin-positive cells (18).

***In Vitro* Tests.** After isolation, human islets were cultured in Brussels in Ham's F-10 medium containing 6.1 mM glucose and supplemented with 0.5% bovine serum albumin, penicillin (0.08 mg/ml), and streptomycin (0.1 mg/ml). After 2–8 days (4.8 ± 0.6 days), the islets were sent by air to Uppsala. There they were cultured free-floating in groups of 100–150 islets per dish in RPMI 1640 medium containing 5.6 mM glucose, 10% (vol/vol) fetal calf serum (FCS), benzylpenicillin (100 units/ml), and streptomycin (0.1 mg/ml), with medium changed every 2 days (14). Rat or mouse islets isolated in Uppsala were cultured similarly (14, 17), except for the presence of 11 mM glucose in the RPMI medium (17). This laboratory has shown that functional preservation in RPMI 1640 medium is optimal at 5.6 mM glucose for human islets (14) and at 11 mM glucose for rodent islets (17). After 5–6 days in culture, the islets were exposed for 30 min to SZ or alloxan (19, 20) and then tested for glucose-induced insulin release (see below). For NP, the exposure time was prolonged to 14 h (culture conditions as above). After this period,

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Abbreviations: NO, nitric oxide; SZ, streptozotocin; NP, sodium nitroprusside; IDDM, insulin-dependent diabetes mellitus; FCS, fetal calf serum.

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medium was collected for nitrite determination (9) and the islets were retrieved for assessment of glucose-induced insulin release, content of insulin and DNA, and glucose oxidation (14). In brief, insulin release was studied in triplicate groups of 7–10 islets by a 1-h incubation with 1.7 mM glucose. The incubation medium, Krebs–Ringer/bicarbonate buffer (KRBH), was then gently removed and replaced by medium containing 16.7 mM glucose. In the experiments in which islets were exposed to alloxan, the insulin-release experiments were performed for only 30 min in the presence of 16.7 mM glucose (experimental conditions as above). This was done to avoid passive leakage of insulin, which starts 60–120 min after alloxan exposure (20).

Purified human or rat β cells were exposed to alloxan or SZ for 10 min at 30°C (13), washed, and then plated in polylysine-coated microtiter cups filled with Ham's F-10 medium containing 1% bovine serum albumin (see above). After 1–4 days in Ham's F-10 medium with or without 2% FCS, the number of surviving cells was determined by a vital staining technique (13).

***In Vivo* Tests.** Duplicate groups of 150–200 cultured human or 100 mouse pancreatic islets were suspended in Hanks' salt solution and implanted under the left renal capsule space (one group at the top and one at the bottom) (21) of avertin-anesthetized normoglycemic nude (*nu/nu*) BALB/c mice (Bomholtgaard, Ry, Denmark). Two weeks after transplantation, the grafted animals were injected i.v. with alloxan (80 mg/kg body weight) or saline. One week later blood was collected for glucose determination, the mice were killed, and the graft and pancreas were harvested for insulin extraction (top graft) or insulin immunostaining (bottom graft) (21). In one series of experiments, the graft-containing kidney was removed 2 weeks after transplantation and the glycemia followed over 3 days.

Statistical Analysis. Data are presented as the mean ± SEM, and groups of data were compared using Student's paired or unpaired *t* test. In all experimental series, each islet preparation (i.e., islets obtained from one donor) was considered as one individual observation. When experiments were performed in triplicate, a mean was calculated and considered as one observation.

RESULTS

Exposure of rodent or human pancreatic islets to SZ, alloxan, or NP did not modify islet DNA or insulin content (data not shown). When pooling all data from the various control groups, the DNA content (ng per 10 islets; mean ± SEM) was, respectively, 341 ± 28 (n = 17), 334 ± 28 (n = 17), or 287 ± 30 (n = 16) for rat, mouse, or human pancreatic islets. The insulin content (ng per 10 islets; mean ± SEM) was, respectively, 720 ± 50 (n = 17), 870 ± 62 (n = 17), or 413 ± 62 (n = 16) for rat, mouse, or human pancreatic islets. Exposure of rat or mouse pancreatic islets to SZ (1–3 mM) did not affect basal insulin release, but the toxin dose-dependently decreased glucose-induced insulin release (Table 1). At the lower concentration of SZ (1 mM), rat islets were more affected (80% inhibition) than mouse islets (43% inhibition). Control human pancreatic islets presented a 10-fold increase in insulin release in response to glucose (Table 1), which was not inhibited by 3 or 6 mM SZ. Only at 12 mM SZ was there a significant (50%) inhibition of human islet insulin release, similar to that observed in rodent islets exposed to 1 mM SZ.

When rat pancreatic islets were incubated with alloxan, the drug dose-dependently inhibited insulin release from ≥1.0 mM (30–70% inhibition; Table 2). Mouse islets were also suppressed by alloxan, but only from ≥2.0 mM (40–60% inhibition; Table 2). In contrast, human pancreatic islets were not inhibited by any alloxan concentration tested.

Table 1. Effects of SZ on glucose-induced insulin release by rat, mouse, or human pancreatic islets

Species	SZ, mM	Insulin release, ng per 10 islets per h: 1.7 mM glucose	16.7 mM glucose	Increase ratio (16.7/1.7)
Rat	0	6.7 ± 1.4 (6)	68.9 ± 3.7	12.6 ± 3.0
	1.0	5.4 ± 1.3 (6)	14.1 ± 4.2‡	4.4 ± 2.0*
	3.0	5.0 ± 1.5 (6)	12.0 ± 3.2‡	3.4 ± 0.9*
Mouse	0	3.4 ± 0.6 (6)	57.4 ± 10.2	19.0 ± 2.6
	1.0	4.3 ± 0.8 (6)	31.8 ± 11.1	7.7 ± 1.5†
	3.0	2.9 ± 0.4 (6)	17.7 ± 5.9†	6.5 ± 1.6†
Human	0	1.6 ± 0.2 (5)	16.5 ± 3.9	10.4 ± 1.4
	3.0	2.2 ± 0.5 (5)	18.1 ± 4.7	8.5 ± 1.4
	6.0	1.9 ± 0.2 (5)	17.8 ± 4.7	10.9 ± 4.1
	12.0	1.3 ± 0.2 (5)	9.3 ± 2.9†	6.9 ± 1.7*

Groups of islets were isolated and exposed to various SZ concentrations. For insulin release experiments, islets were incubated in triplicate groups of 10 in KRBH buffer containing 1.7 mM glucose at 37°C. After 60 min, the medium was removed and the islets were incubated for another 60 min in medium containing 16.7 mM glucose. The increase ratio was calculated by dividing the insulin release observed at 16.7 mM glucose by that observed at 1.7 mM glucose in each experiment. The results are the mean ± SEM of the number of experiments indicated in the parentheses. *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$, when compared with respective controls (paired *t* test).

In a third series of experiments, rodent or human pancreatic islets were cultured for 14 h in the presence of NP, a NO generator. NP generated similar amounts of NO in these preparations, as evaluated by medium nitrite concentration (Table 3). Measurement of glucose-induced insulin release was hampered by leakage of insulin from rodent islets that had been damaged by the highest NP concentrations. Indeed, rat or mouse islets treated with 100 or 200 μM NP showed morphological signs of islet cell damage, such as extrusion of islet necrotic material and accumulation of cell debris in the culture medium, high basal insulin values, and lack of response to 16.7 mM glucose (data not shown). Thus, while rat and mouse pancreatic islets presented a 50–70% decrease in the ratio between glucose-stimulated and basal insulin release at 100 μM NP ($P < 0.05$ for rat and $P = 0.07$ for mouse) and an 80–90% decrease at 200 μM NP ($P < 0.05$), human islets showed a preserved ratio at 100 μM NP and only a 50% decrease ($P < 0.05$) at 200 μM NP (Table 3). Moreover, morphological signs of islet damage were not present in human islets. To further evaluate this issue, islet glucose oxidation rates were determined in rodent or human islets

Table 2. Effects of alloxan on glucose-induced insulin release by rat, mouse, or human pancreatic islets

Species	Alloxan, mM	Insulin release, ng per 10 islets per 30 min
Rat	0	75.0 ± 11.5 (6)
	1.0	50.2 ± 9.0 (6)*
	2.0	29.5 ± 5.9 (6)†
	3.0	20.0 ± 5.9 (6)†
Mouse	0	64.5 ± 10.7 (5)
	1.0	55.4 ± 7.0 (5)
	2.0	38.1 ± 5.3 (5)*
	3.0	23.9 ± 4.1 (5)*
Human	0	15.5 ± 2.5 (5)
	1.0	16.0 ± 4.0 (5)
	2.0	20.8 ± 6.1 (5)
	3.0	21.0 ± 3.7 (5)

Groups of islets were isolated and exposed to various alloxan concentrations. For insulin release experiments, islets were incubated in triplicate groups of 10 for 30 min in KRBH buffer containing 16.7 mM glucose at 37°C. *, $P < 0.05$; †, $P < 0.01$ when compared with respective controls (paired *t* test).

Table 3. Effects of NP on glucose oxidation rates and insulin release of rat, mouse, or human pancreatic islets

Species	NP, μM	Medium nitrite, pmol per islet per 14 h	Glucose oxidation, pmol per 10 islets per 90 min	Insulin release (increase ratio) (16.7/1.7)
Rat	0	5 ± 1 (3)	464 ± 77 (5)	12.2 ± 2.5 (5)
	50	65 ± 1 (3)‡	276 ± 51 (5)†	14.3 ± 2.2 (5)
	100	77 ± 5 (3)‡	131 ± 25 (5)†	3.3 ± 0.9 (5)*
	200	112 ± 5 (3)‡	47 ± 15 (6)†	1.1 ± 0.2 (5)†
Mouse	0	2 ± 0.2 (3)	399 ± 45 (5)	11.6 ± 2.9 (5)
	50	80 ± 2 (3)‡	344 ± 27 (5)	10.3 ± 1.3 (5)
	100	104 ± 9 (3)‡	180 ± 29 (5)†	5.4 ± 1.1 (5)
	200	125 ± 17 (3)†	134 ± 37 (5)*	2.5 ± 0.7 (5)*
Human	0	2 ± 1 (3)	123 ± 8 (4)	6.3 ± 1.0 (6)
	50	65 ± 8 (3)†	127 ± 9 (4)	4.6 ± 0.8 (6)
	100	65 ± 16 (3)*	134 ± 12 (4)	5.5 ± 1.5 (6)
	200	120 ± 25 (3)†	117 ± 12 (4)	3.1 ± 0.6 (6)†

Groups of islets were isolated and exposed for 14 h to various concentrations of NP. For glucose oxidation experiments, islets were incubated in triplicate groups of 10–20 for 90 min in KRBH buffer (without albumin) supplemented with D-[U-^{14}C]glucose and 16.7 mM nonradioactive glucose. For insulin release experiments, triplicate groups of 10 islets were sequentially exposed to 1.7 and 16.7 mM glucose (data not shown; experiments performed as in Table 1), and the increase ratio was calculated as described in Table 1. The results are the mean ± SEM of the number of experiments indicated in the parenthesis. *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$, when compared with respective controls—paired t test (glucose oxidation and increase ratio) or unpaired t test (medium nitrite).

exposed to NP for 14 h (Table 3). In good agreement with the observations described above, NP (50–200 μM) induced a 40–90% decrease in rat islet glucose oxidation. For mouse islets, inhibition of glucose metabolism (50–60% decrease) was only apparent at 100 or 200 μM NP, whereas human islet glucose oxidation was not affected by any of the tested NP concentrations.

The survival of human β cells was not affected by alloxan or SZ (Table 4), whereas rat β cells underwent a dose-dependent increase in cell death (50–90%) over the subsequent 1–4 days of culture. Even when rat β cells were cultured in the presence of FCS, a condition that reduces cell damage (Z.L. and D.G.P., unpublished data), there was still a marked decline in cell viability after alloxan or SZ exposure.

To test whether the higher resistance of human islets to tested toxins was also present under *in vivo* conditions, human pancreatic islets were grafted under the kidney capsule of normoglycemic nude mice. After 2 weeks, the animals were injected with a diabetogenic dose of alloxan (80 mg/kg), but none of the treated animals developed hyperglycemia during a 2-week observation period (Table 5). Alloxan induced a 75% decrease in mouse pancreatic insulin content ($P < 0.01$; paired t test), but there was no decrease in the human islet graft insulin content (Table 5). The apparent increase in insulin content of the human islet graft exposed to alloxan is due to a single high value (177 μg per graft). If this value (and the corresponding control) is excluded from the mean, the results are similar for saline-exposed (14.7 ± 7.2 μg per graft; $n = 4$) and alloxan-exposed (14.2 ± 4.7 μg per graft; $n = 4$) grafts. Immunostaining indicated a marked reduction in the insulin-positive cells of the mouse pancreata, but not in the human islet graft (data not shown). When islets isolated from NMRI mice were grafted under the kidney capsule of nude mice and the animals subsequently were injected with alloxan (80 mg/kg; experimental conditions as in Table 5), there was a 80% decrease in insulin content in the mouse pancreata. The mouse islet grafts were totally destroyed and contained no insulin, and the animals became diabetic (data not shown). Thus, human islets grafted into nude mice are not destroyed by an alloxan injection, which severely damages mouse islets. Moreover, the human islet graft is probably responsible for maintenance of normoglycemia in these animals. To test this possibility, three alloxan-injected nude mice bearing human islet grafts were nephrectomized, with consequent loss of the graft. Blood glucose before nephrectomy was 3 mmol/liter, but 3 days after removal of the graft blood glucose concentration increased to >25 mM in all animals.

Table 4. Effects of alloxan or SZ on the survival of rat or human isolated β cells

Species	Test condition	FCS	Toxicity, % dead cells: 1-day culture	4-day culture
Rat	Alloxan (5 mM)	−	80 ± 5 (4)†	>95†
	SZ (5 mM)	−	50 ± 7 (4)†	65 ± 8†
Rat	Alloxan (1 mM)	+	8 ± 4 (5)	23 ± 3*
	SZ (5 mM)	+	39 ± 5 (5)*	44 ± 5*
Human	Alloxan (5 mM)	−	<5 (3)	<5
	SZ (5 mM)	−	<5 (3)	<5

Rat or human β cells purified by flow cytometry were exposed to alloxan or SZ. After drug exposure, cells were cultured for 1 or 4 days with or without 2% FCS. The percentage of living cells was determined after vital staining with neutral red. Toxicity is expressed as percent dead cells and is listed as the mean ± SEM for the number of experiments indicated in the parenthesis. *, $P < 0.05$; †, $P < 0.01$, when compared with respective controls (paired t test).

DISCUSSION

The present study compares the functional and structural consequences of the exposure of human or rodent pancreatic islets to agents with known β-cell toxicity in rodents, namely, SZ (an alkylating agent; ref. 11), alloxan (a generator of oxygen free radicals; ref. 12), and NP (a NO donor; ref. 10).

Table 5. Effects of alloxan injection [80 mg/kg (body weight)] in nude mice bearing a human islet graft on the insulin contents of the graft and the pancreas

Treatment	Mouse pancreatic insulin content, ng/mg (net weight)	Human islet graft insulin content, μg per graft	Blood glucose, mmol/liter
Saline	175 ± 63 (5)	18 ± 6	3.5 ± 0.4
Alloxan	27 ± 7 (5)*	47 ± 33	3.0 ± 0.1

Human islets were transplanted under the kidney capsule of nude mice and the animals were injected with alloxan. One week after the alloxan injection, blood glucose levels were determined. The animals were then killed, and the graft and pancreas were retrieved for insulin determination. Results are the mean ± SEM of the number of experiments indicated in the parenthesis. *, $P < 0.05$, when compared with saline group (unpaired t test).

In the three conditions, human islets were less sensitive than mouse and rat pancreatic islets. This observation extends a previous finding, wherein human islets appeared more resistant than rodent islets to the deleterious effects of cytokines (9). Experiments on purified β-cell preparations indicated that SZ or alloxan did not induce cell death in human preparations at concentrations that were toxic for rat β cells. These latter findings were based on human or rat β cells that were isolated and cultured in the same laboratory (Brussels), under similar conditions. It is therefore unlikely that the observed differences between human and rodent pancreatic islets are explained only by different isolation, transport, and/or culture procedures.

The above described studies are compatible with the knowledge that SZ is not diabetogenic in humans, whereas it readily induces overt diabetes in rodents (11, 22). It was also previously reported that human fetal islets grafted into nude mice were not destroyed by injections of SZ, in spite of adequate uptake of the drug by the human tissue (23). In our transplanted animals, human islet grafts retained well-preserved morphology and insulin content after alloxan injection, whereas the mouse endogenous β cells were destroyed. The *in vitro* resistance of human β cells to SZ and alloxan is thus also present *in vivo*. This reinforces the view that the observed interspecies difference in the susceptibility to β-cell injury is a true biological phenomenon. In this context, it is noteworthy that other human cell types seem to posses an increased capacity for DNA repair compared to rodent cells (24, 25). The reasons for these species differences remain to be clarified.

In conclusion, the present observations emphasize three main points. (*i*) Models for human IDDM pathogenesis, including the potential role for interleukin 1β (4) and NO (7, 8), are to a large extent based on studies performed in rat pancreatic islets. Considering that rat β cells are particularly sensitive to various noxious agents (present data), we suggest that great caution must be exercised when observations obtained in rat islets are extrapolated to human IDDM. (*ii*) It is well known that the time between the appearance of islet autoimmunity and clinical onset of IDDM is much longer in humans than in mice (NOD mice) and in particular rats (BB rats). Since the degree of resistance to various toxins (present data) seems to follow a similar pattern (i.e., human > mouse > rat), perhaps an increased resistance to injury may contribute to a longer prediabetic period in humans. (*iii*) The presently described interspecies difference in β-cell resistance to injury raises the possibility that a similar variation may exist also among individuals of the same species. It has been previously proposed that differences in β-cell susceptibility to injury may contribute to different susceptibility to IDDM (3, 20). If this is indeed the case, clarification of the mechanisms behind human islet resistance to injury, as compared to mouse or rat pancreatic islets, may point to relevant defence mechanisms that should be evaluated when studying human populations at risk for IDDM.

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