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DNA damage and cytotoxicity in pancreatic β-cells expressing human CYP2E1

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Abstract

Epidemiological studies have identified nitrosamines as a risk factor for Type I (insulin dependent) diabetes mellitus. These compounds require bioactivation by cytochrome P450 2E1 (CYP2E1) for exertion of their toxic effects. Two mammalian insulin secreting pancreatic β-cell lines BRIN BD11h2E1 and INS-1h2E1, which express human full length *CYP2E1* cDNA, were used to elucidate the role of CYP2E1-mediated nitrosamine bioactivation in pancreatic β-cell dysfunction and destruction. These cell lines were shown to metabolise dimethylnitrosamine to produce formaldehyde at rates of 3.41 ± 0.24 and 3.65 ± 0.26 nmol/min mg microsomal protein, respectively. Following incubation with various concentrations of the nitrosamines dimethylnitrosamine, *N*-nitrosopyrrolidine and 1-nitrospiperidine, all of which are bioactivated by CYP2E1, cytotoxicity and DNA damage were assessed using either the neutral red assay or comet assay respectively. Exposure of CYP2E1 expressing cells to nitrosamines resulted in significant dose-dependent decreases in cell viability, which were not seen in cells which did not express CYP2E1. Following culture with nitrosamine concentrations as low as 2.5 mM 1-nitrosopiperidine, cell viability was significantly lower in BRIN BD11h2E1 and INS-1h2E1 cell lines in comparison to the BRIN BD11 and INS-1 parental cell lines (72.5 ± 4.96 and 66.4 ± 3.09% in BRIN BD11h2E1 and INS-1h2E1 versus 109.0 ± 3.40 and 100.0 ± 3.25% in BRIN BD11 and INS-1 respectively, $P < 0.001$). The highest dose of any of the nitrosamines tested failed to significantly reduce cell viability in the cells which lacked CYP2E1. Expression of CYP2E1 did not cause any change in the basal level of DNA damage in any of the cell lines. However, 16 h exposure to various nitrosamines resulted in significant dose-dependent DNA damage in the BRIN BD11h2E1 and INS-1h2E1 cells compared to their respective non CYP2E1-expressing parental controls, e.g. DNA damage increased from 34.38 ± 1.25 to 44.01 ± 1.56% DNA in comet tail in BRIN BD11h2E1 cells incubated with 10 or 40 mM *N*-nitrosopyrrolidine, respectively ($P < 0.001$). Similar treatment of the BRIN BD11 and INS-1 cell lines did not result in a significant increase in DNA damage (20.33 ± 1.0 and 22.4 ± 0.98% DNA in comet tail). The pancreatic β-cell is richly vascularised and expresses CYP2E1. This study suggests that expression of human CYP2E1 in pancreatic β-cells make them highly susceptible to cytotoxicity and DNA damage by nitrosamines and other agents bioactivated by CYP2E1.

Keywords: DNA damage; Cytotoxicity; Nitrosamines; β-Cells; CYP2E1; Diabetes mellitus

1. Introduction

The cytochrome P450 monooxygenases have been identified as a major source of free radicals [1], which contribute to oxidative stress observed in Type 1 (insulin dependent) diabetes mellitus, the most severe form of diabetes [2]. The cytochrome P4502E1 isoform has been found to generate higher amounts of various species of ROS than other P450 forms [3,4] and has been shown to be constitutively expressed and inducible within the pancreatic islets of Langerhans [5,6].

Following onset of Type I diabetes mellitus modulation of the cytochrome P450 enzymes has been observed in the liver, with the CYP2E1 isoform being elevated in animals [7–9] and humans [10,11] suggesting that diabetic patients may be more susceptible to the toxicity of xenobiotics metabolised by this isoform. A marked increase in hepatic CYP2E1 activity in obese Type II (non insulin-dependent) diabetic patients has also been observed [12]. Coupled with

Abbreviations: CYP2E1, cytochrome P450 2E1; DMN, dimethylnitrosamine; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; NPIP, 1-nitrosopiperidine; NPYR, *N*-nitrosopyrrolidine; ROS, reactive oxygen species

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the fact that pancreatic islets have low levels of scavenging antioxidant enzymes [13–15], it would appear that pancreatic β-cells are particularly susceptible to cellular biomolecule damage due to attack by the bio-reactive products of CYP2E1 metabolism. It has been well documented that certain nitrosamines are bioactivated by CYP2E1 via a pathway of α-hydroxylation to yield a variety of radicals which can react with various cellular targets [16]. If DNA is attacked strand breakage may result. These breaks may induce DNA repair, in part via the activation of poly(ADP-ribose)synthetase which uses cellular NAD as a substrate. If cellular NAD becomes depleted, cellular activities may be inhibited, including insulin biosynthesis, with cytotoxicity and β-cell death ensuing [17]. The reactive intermediates of nitrosamine metabolism also have the ability to alkylate nucleophilic sites of DNA [18] resulting in alkali labile adducts, which can lead to the formation of abasic sites and DNA strand breaks.

Exposure to nitrosamines may result from a variety of sources such as cooked meats, tobacco smoke and cosmetics [19]. Such exposure has been shown to transform explants of human pancreas, producing transformed foci which grew as tumours in nude mice [20]. Epidemiological studies have proposed that diabetics have a higher cancer incidence [21–23]. The increase in pancreatic cancer and chronic pancreatitis in developed countries during the last 40 years suggests that xenobiotics may be implicated in the aetiology of these diseases [24,25].

In this study we have investigated the role of human CYP2E1 expression in pancreatic β-cell dysfunction and destruction. DNA damage and cytotoxicity in response to various concentrations of short chain nitrosamines: dimethylnitrosamine, *N*-nitrosopyrrolidine and 1-nitrosopiperidine, were measured in two mammalian pancreatic insulin-secreting β-cell lines genetically engineered for stable human CYP2E1 expression, BRIN BD11h2E1 and INS-1h2E1, which have previously been characterised and found to express an active and inducible CYP2E1 enzyme [26].

2. Materials and methods

2.1. Materials

Tissue culture reagents were purchased from Life Technologies. Tissue culture 24-well plates were purchased from Falcon. Nitrosamines, 2-mercaptoethanol, neutral red, low melting point and normal agarose, ethidium bromide and general reagents were purchased from Sigma.

2.2. Cell culture

The parental BRIN BD11 cell line, a glucose-responsive insulin-secreting cell line created by electrofusion of NEDH rat islet cells with the RIN 5 mF cell line [27], were cultured in RPMI supplemented with 10% (v/v) foetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C with 5% CO_2. INS-1 parental cells, a second insulin-secreting pancreatic cell line [28] were cultured in the same medium supplemented with 50 mM 2-mercaptoethanol and 50 mM HEPES/pyruvate and identical culture conditions. BRIN BD11h2E1 and INS-1h2E1 cells, which were generated by cotransfection of pSVh2El vector containing a full length human *CYP2E1* cDNA and the selectable pCIneo vector (Promega, UK), and BRIN BD11 NEO and INS-1 NEO cells, generated by transfection with the pCIneo vector alone, were grown in the same medium and under identical culture conditions as parental controls but were supplemented with 0.2 mg/ml G418. BRIN BD11h2E1 and INS-1h2E1 cells have previously been shown to have specific CYP2E1 activities of 0.341 ± 0.08 and 0.633 ± 0.10 nmol/min mg microsomal protein, respectively, as determined by *p*-nitrophenol hydroxylation [26].

2.3. Dimethylmtrosamine N-demethylase activity

Activity of dimethylnitrosamine *N*-demethylase was determined to assess the ability of the CYP2E1 expressing cells, BRIN BD11h2E1 and INS-1h2E1 to metabolically activate a xenobiotic compound. Microsomal preparations [29] were diluted 1:10 (v/v) in incubation mixture containing 15 mM $MgCl_2$, 6 mM dimethylnitrosamine and 5 mM phosphate buffer (pH 7.4). Following a 5 min preincubation at 37 °C in a shaking water bath, the reaction was initiated by adding 1 mM NADPH. The mixture was incubated for a further 10 min and the reaction stopped by adding 0.5 ml ice-cold 12.5% (v/v) TCA and mixing. All tubes were incubated on ice for a further 5 min before centrifugation at 1000 × *g* for 15 min. Supernatant was diluted 1:1 (v/v) with freshly prepared NASH reagent (4 mM ammonium acetate containing 0.4% (v/v) acetyl acetone) and heated in a shaking water bath at 58 °C for 10 min. Following 5 min cooling, absorbance of each sample was read at 412 nm in a Pharmacia NovaSpecII. A standard curve was constructed and used to determine the concentration of formaldehyde produced.

2.4. 16 h incubation with nitrosamines

BRIN BD11h2E1, INS-1h2E1 and their respective parental and neo control cells were harvested with the aid of trypsin and seeded at a density of 1×10^5 cells per well, for the comet assay, or 2×10^6 cells per well, for the neutral red assay, in 24 well plates.

Medium was supplemented with various concentrations of dimethylnitrosamine, *N*-nitrosopyrrolidine and 1-Nitrosopiperidine which were added to test wells. Control wells contained RPMI with no nitrosamines added. Nitrosamine concentrations used for the neutral red assay were: 2.5, 5, 10, 20 and 40 mM dimethymitrosamine; 5, 10, 20, 40 and 80 mM *N*-nitrosopyrrolidine; and 2.5, 5 and 10 mM

1-nitrosopiperidine. As a positive control for the neutral red assay, 2.5, 5, 10 and 20 mM streptozotocin was added to the culture medium as streptozotocin does not require CYP2E1 bioactivation to exert its toxicity. The nitrosamine concentrations used for the comet assay were: 10 and 40 mM dimethylnitrosamine, 10 and 40 mM *N*-nitrosopyrrolidine, 2.5 and 10 mM 1-nitrosopiperidine. Incubations were carried out for 16 h at 37 °C and 5% CO_2 for both assays. Cells were then used in either the neutral red assay or the alkaline comet assay. As a positive control for the comet assay, cells were treated with medium supplemented with 150 μM hydrogen peroxide for 5 min on ice. Hydrogen peroxide does not require CYP2E1 transformation to exert its toxicity.

2.5. Neutral red assay

Following 16 h incubation with test solution, the cells were incubated with neutral red dye to assess cytotoxicity. Viable cells actively transport this dye across their cell membrane, therefore upon subsequent lysis, absorbance can be used as a measure of cell viability. Cells were incubated with test chemicals as indicated above. Following incubation neutral red assay was carried as described by [30]. Essentially, 1 ml of freshly prepared neutral red solution (50 μg/ml) prewarmed to 37 °C was added to each well and all plates returned to the incubator for 2 h. The cells were washed three times with HBSS with the last wash lasting for 40 min. Following draining of the plates, 1 ml of glacial acetic acid solution (1% (v/v) glacial acetic acid, 50% (v/v) absolute ethanol in dH_2O) was added to each well and plates were shaken on an Orbital plate shaker for 15 min to release all of the dye from the cells. Samples were transferred to cuvettes and absorbance read at 546 nm on a Pharmacia NovaSpecII. Results were calculated by dividing sample absorbance by control absorbance and are expressed as mean percentage cell viability ± standard error of the mean (S.E.M). Sample number was eight, four from each of two independent experiments.

2.6. Alkaline comet assay

The comet assay or single cell gel electrophoresis assay is a simple and rapid method for detection of single strand DNA breaks and alkali-labile sites which can result from attack by free radicals such as the reactive intermediates of nitrosamine metabolism. Single cells are embedded in agarose and lysed in denaturing buffer. They are then subjected to electrophoresis and stained with an intercalating dye and examined by fluorescence microscopy. Damaged DNA can migrate through the agarose on application of an electric current to form the comet 'tail', while undamaged DNA stays in the 'head' of the comet as it is unable to migrate through the agarose gel [31]. The comet assay was carried out as described by [31].

Cells were harvested by trypsinisation following overnight exposure to nitrosamines and resuspended in 1% (w/v) low melting point agarose at 37 °C. The cell suspension was applied to a frosted slide which was already prepared with 1% (w/v) normal melting point agarose spread onto the frosted slide. The slides were immersed in freshly prepared chilled lysis solution (2.5 mM NaCl, 100 mM EDTA and 10 mM Tris, pH 10 with 1% (v/v) Triton-X 100) for 1 h at 4 °C. The slides were placed in a horizontal electrophoresis tank and just covered with fresh electrophoresis buffer (0.3 mM NaOH, 1 mM EDTA, pH 13) and were incubated for 20 min to allow DNA to unwind and expression of alkali-labile damage. Electrophoresis was then carried out at 25 V and 300 mA for 20 min. The slides were washed in three changes of neutralising buffer (0.4 mM Tris, pH 7.5) to neutralise the alkali. Finally, the gels were stained by adding ethidium bromide (20 μg/ml). Slides were stored in a humidified light-proof box for up to 12 h at 4 °C before analysis.

Comet analysis was facilitated by a Nikon Ophtiphot II compound microscope using an epifluorescent light source and Nikon × 40 Fluor lens giving a final magnification of 400×. The microscope was equipped with a CCD Camera allowing computer analysis using Komet 3.0 software (Kinetic Imaging Ltd., Liverpool, UK). Microsoft Excel 5.0 was used for statistical analysis of densiometric and geometric parameters of the comets. Percentage of DNA present in the comet tail (% DNA in comet tail) was used as a measure of DNA damage. Fifty cells per slide were analysed and results are presented as the mean % DNA in comet tail ± S.E.M. of 100 cells from two independent experiments.

2.7. Statistical analysis

All results are presented as mean ± standard error of the mean (S.E.M.) for a given number of observations (n). Data groups were compared using the unpaired Student's t-test. Differences were considered to be significant at the 95% confidence level ($P < 0.05$).

3. Results

3.1. Dimethylnitrosamine N-demethylase activity

BRIN BD11h2E1 and INS-1h2E1 cell lines were both shown to be capable of metabolising dimethylnitrosamine to produce formaldehyde at the rate of 3.41 ± 0.244 and 3.65 ± 0.263 nmol/min mg microsomal protein respectively (Fig. 1A and B). These values represent a 1.5-fold ($P < 0.05$) and 1.6-fold ($P < 0.01$) increase in this activity compared to parental BRIN BD11 and INS-1 cells.

3.2. Neutral red assay

There were no significant differences in % cell viability between the parental BRIN BD11 cell line and the respec-

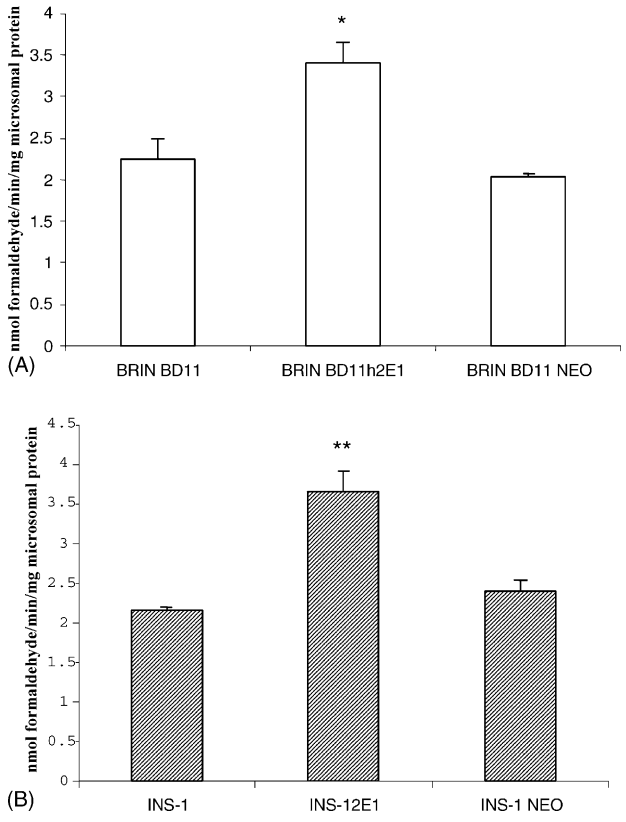

Fig. 1. Dimethylnitrosamine *N*-demethylase activity in (A) parental BRIN BD11 and transfected BRIN BD11h2E1 and BRIN BD11 NEO cell lines (white bars) and (B) parental INS-1 and transfected INS-1h2El and INS-1 NEO cell lines (striped bars). Demethylase activity was calculated with reference to formaldehyde standards. Results are presented as mean ± S.E.M. of three independent experiments ($n = 3$). 2E induced rat liver microsomes produced 4.1 nmol formaldehyde/min mg microsomal protein. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to parental cell line.

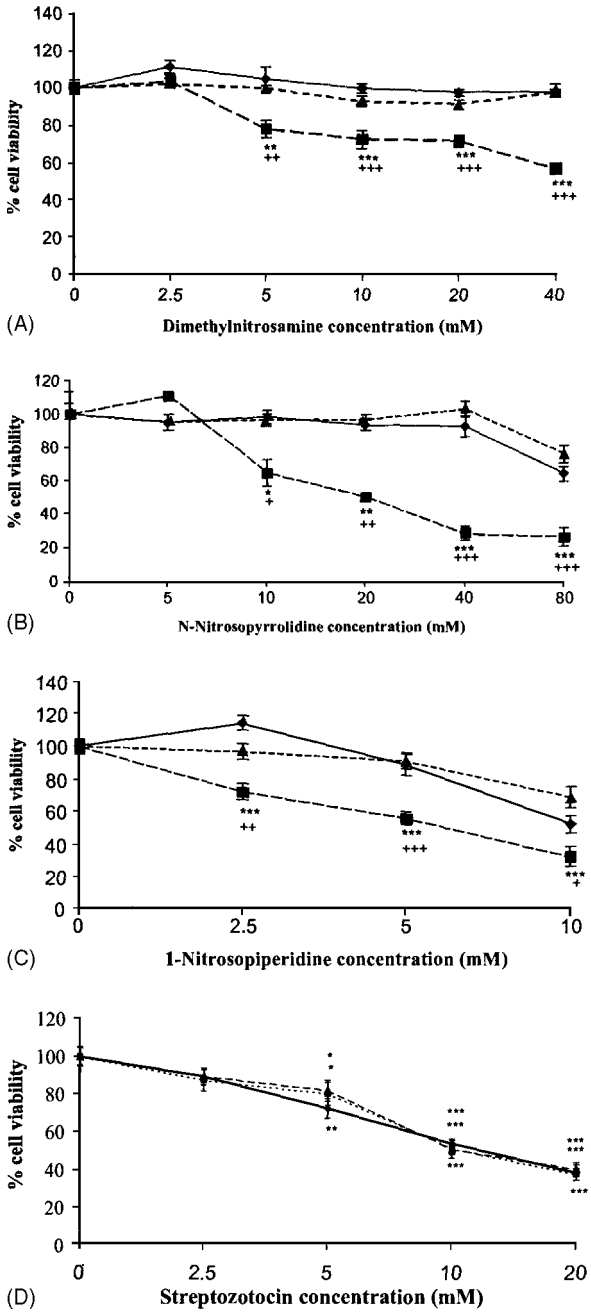

Fig. 2. Cell viability in parental BRIN BD11 (solid line) and transfected BRIN BD11h2El (long dash line) and BRIN BD11 NEO (short dash line) cell lines in response to (A) dimethylnitrosamine, (B) *N*-nitrosopyrrolidine, (C) 1-nitrosopiperidine and (D) streptozotocin. Cell viability was measured using the neutral red assay and results are expressed as mean ± S.E.M. ($n = 8$, four from two independent experiments). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to same cell line cultured without test chemical (stars above trend line indicate significance levels for BRIN BD11h2E1 and BRIN BD11 NEO cell lines). $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ compared to parental cell line.

tive BRIN BD11 NEO control cell line (Fig. 2A–C), or between INS-1 and INS-1 NEO (Fig. 3A–C) following exposure to the various nitrosamines. Indeed, even at the highest nitrosamine concentrations tested the viability of the parental and neo control cells was not significantly reduced. However, following incubation with nitrosamine concentrations as low as 2.5 mM 1-nitrosopiperidine the viability of BRIN BD11h2E1 cell line was significantly lower than for both the untreated BRIN BD11h2E1 control and the parental and neo controls treated with 2.5 mM 1-nitrosopiperidine (72.5 ± 4.96, $P < 0.01$, 109.0 ± 4.5 and 91.1 ± 5.03%, respectively, $P < 0.001$). Cytotoxicity in BRIN BD11h2E1 became significantly greater than that of the parental and neo controls at the 5 mM level for dimethylnitrosamine ($P < 0.01$) and 10 mM for *N*-nitrosopyrrolidine ($P < 0.05$). In the INS-1h2E1 cell line, incubation with 2.5 mM 1-nitrosopiperidine caused significantly greater cytotoxicity than in the INS-1 or INS-1 NEO cell lines (66.4 ± 3.39, 100 ± 1.93 and 98.32 ± 1.14%, $P < 0.001$). Levels of cytotoxicity became significant at the 10 mM level for dimethylnitrosamine ($P < 0.01$) and 5 mM for *N*-nitrosopyrrolidine ($P < 0.001$) in comparison to parental INS-1 cells. There were no significant differences in cytotoxicity caused by incubation with streptozotocin between the parental and transfected cell lines.

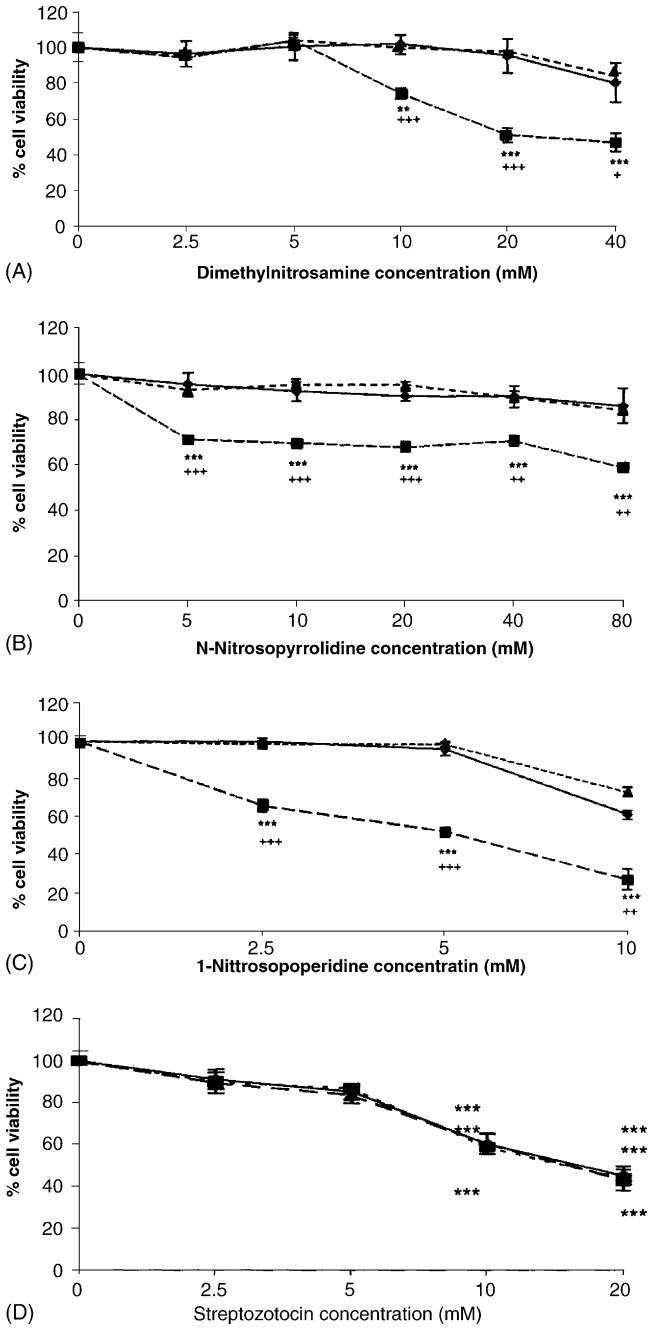

Fig. 3. Cell viability in parental INS-1 (solid line) and transfected INS-1h2E1 (long dash line) and INS-1 NEO (short dash line) cell lines following overnight treatment with (A) dimethylnitrosamine, (B) *N*-nitrosopyrrolidine, (C) 1-nitrosopiperidine and (D) streptozotocin. Cell viability was assessed using the neutral red assay and results are presented as mean ± S.E.M. ($n = 8$, four from two independent experiments). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to same cell line cultured without test chemical (stars above trend line indicate significance levels for INS-1h2E1 and INS-1 NEO cell lines). $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ compared to parental cell line.

3.3. Alkaline comet assay

Basal levels of DNA damage remained unchanged from parental controls in the BRIN BD11h2E1, INS-1h2E1, BRIN BD11 NEO and INS-1 NEO transfected cell lines

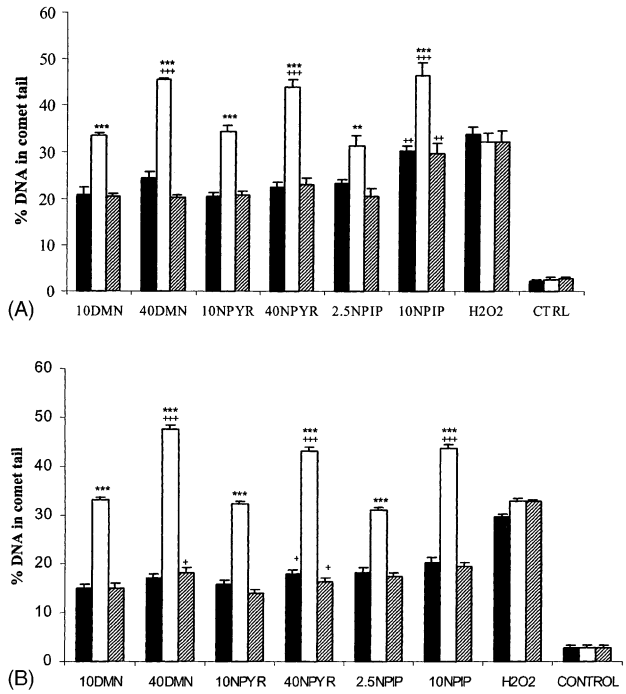

Fig. 4. DNA damage in (A) parental BRIN BD11 (black bars) and transfected BRIN BD11h2E1 (white bars), and BRIN BD11 NEO (striped bars) cell lines and (B) parental INS-1 (black bars), transfected INS-1h2E1 (white bars) and INS-1 NEO (striped bars) cell lines following 16 h exposure to various test chemicals (10DMN: 10 mM dimethylnitrosamine; 40DMN: 40 mM dimethymitrosamine; 10NPYR: 10 mM *N*-nitrosopyrrolidine; 40NPYR: 40 mM *N*-nitrosopyrrolidine; 2.5NPIP: 2.5 mM 1-nitrosopiperidine; 10NPIP: 10 mM 1-nitrosopiperidine; H2O2: 150 μM hydrogen peroxide, CONTROL: cultured without test chemical). The alkaline comet assay was carried out to assess DNA damage. Results are presented as the mean % DNA in comet tail of 100 cells (50 from each of two independent experiments) ± S.E.M. $^{**}P < 0.01$, $^{***}P < 0.001$ compared to parental cell line exposed to same test chemical concentration. $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ compared to same cell line exposed to a lower nitrosamine concentration.

(Fig. 4A and B). Similarly, there were no significant differences in DNA damage in parental or transfected cell lines when exposed to hydrogen peroxide, which is not bioactivated by CYP2E1. However, the BRIN BD11h2E1 and INS-1h2E1 cells showed significantly more DNA damage, expressed as % DNA in comet tail, than the parental and neo controls when similarly treated. Levels of DNA damage were found to increase in a dose-dependent manner following nitrosamine treatment of the BRIN BD11h2E1 cell line. No dose-dependent increase in DNA damage was seen in parental BRIN BD11 between 10 and 40 mM *N*-nitrosopyrrolidine (20.33 ± 1.0 and 22.5 ± 1.0%). However, there was a significant increase in DNA damage in both parental BRIN BD11 and neo control BRIN BD11 NEO cells when the nitrosamine concentration was raised to 10 mM 1-nitrosopiperidine (BRIN BD11: 23.2 ± 1.0 and 30.0 ± 1.3%, $P < 0.01$; BRIN BD11 NEO: 20.5 ± 1.6% and 29.7 ± 2.0%, $P < 0.01$). INS-1h2E1 cells also showed a nitrosamine concentration-dependent increase in DNA damage. When incubated with 2.5 mM 1-nitrosopiperidine, DNA damage was

30.9 ± 0.5%, but upon exposure to a concentration of 10 mM 1-nitrosopiperidine DNA damage was significantly increased to 43.6 ± 0.9%, $P < 0.001$. No such increase was seen in the parental or neo controls incubated with these concentrations. However, the increase in DNA damage just reached significance at 40 mM *N*-nitrosopyrrolidine compared to 10 mM *N*-nitrosopyrrolidine for both parental INS-1 cells and INS-1 NEO control cells (Fig. 4B; 17.9 ± 0.8% compared to 15.7 ± 0.8%, $P < 0.05$ in parental INS-1, 14.02 ± 0.74% compared to 16.32 ± 0.78%, $P < 0.05$ in INS-1 NEO).

4. Discussion

Studies have shown that diabetic animals are profoundly more effective in the metabolic activation of nitrosamines [32,33], a major group of chemical carcinogens. It is well recognised that nitrosamines require metabolic activation for the generation of reactive species which can elicit cytotoxic or genotoxic damage in cells [34–37]. It has also been established that levels of the CYP2E1 isoform are increased in both streptozotocin-induced and spontaneous diabetes [9,38,39], providing an explanation for the increased activation of nitrosamines. Indeed, BRIN BD11h2E1 and INS-1h2El cell lines are both more efficient in the metabolism of dimethylnitrosamine via the α-hydroxylation pathway and producing formaldehyde than parental, BRIN BD11 and INS-1, and BRIN BD11 NEO and INS-1 NEO controls.

The BRIN BD11h2E1 and INS-1h2E1 cell lines generated by transfection of human *CYP2E1* cDNA into the parental insulin-secreting cell lines are excellent tools for investigating CYP2E1 mediated xenobiotic metabolism and its role in pancreatic β-cell dysfunction and destruction. This is due to the fact that these cell lines are defined for human CYP2E1 as the parental cells and neo control cells do not express CYP2E1 [26]. The present study investigates the consequences of CYP2E1-mediated nitrosamine metabolism in pancreatic β-cells. Dimethylnitrosamine, *N*-nitrosopyrrolidine and 1-nitrospiperidine exhibited dose-dependent cytotoxicity in BRIN BD11h2E1 and INS-1h2El cells but not in their respective parental or neo control cell lines at the concentrations tested in this study. Even at the highest concentrations of any of the nitrosamines used there was no significant decrease in parental or neo cell viability. Therefore the increased cytotoxicity in the CYP2E1 expressing cell lines may be attributable to CYP2E1-mediated nitrosamine metabolism. Basal levels of DNA damage remained unaltered in BRIN BD11h2E1 and INS-1h2E1 cell lines in comparison to their respective parental and neo controls suggesting that CYP2E1 expression per se does not increase DNA damage. However, overnight exposure to each of the nitrosamines tested resulted in greater DNA damage in the CYP2E1 expressing cell lines than in the parental or neo controls. As this damage cannot be due solely to the presence of CYP2E1 in these cells, the damage may be a result of CYP2E1-mediated bioactivation which is characterised by generation of high levels of electrophilic species which can attack cellular biomolecules such as DNA. DNA damage may ensue and if this is not repaired, mutation may result. If a mutation occurs in a gene which codes for a factor which plays a role in the regulation of cell division, e.g. oncogenes or tumour suppressor genes, cellular transformation and carcinogenesis may result [40]. Indeed expression of rat *CYP2E1* cDNA in the human fibroblast GM2E1 cell line has shown that reactive oxygen species may contribute to the carcinogenic effects of nitrosamines [41]. Indeed, the generation of hydroxyl radicals and DNA strand breaks is a common feature of the mechanism leading to pancreatic β-cell dysfunction observed in Type 2 (non insulin-dependent) diabetes mellitus and destruction seen in Type 1 (insulin-dependent) diabetes mellitus [17,42]. Systemic oxidative stress is present upon early onset of Type 1 diabetes and is increased by early adulthood [2]. Type 1 diabetic patients have been shown to have higher levels of DNA damage in lymphocytes [43–45] and whole blood [46]. An increase in CYP2E1 has also been observed in lymphocytes from poorly controlled Type I diabetic patients [47]. Work within this laboratory has shown that Type 1 diabetes is also associated with altered in vivo antioxidant status. Patients who had been diagnosed for more than 17 years showed decreases in the antioxidant enzyme glutathione peroxidase in blood plasma, and also exhibited lower levels of the antioxidants vitamin A and uric acid while there was an increase in caeroplasmin levels [48]. Another study revealed decreases in the activity level of superoxide dismutase and glutathione peroxidase in the plasma of well controlled IDDM patients compared to control subjects. Inherently low levels of antioxidant enzymes in pancreatic β-cells [13–15,48] means that the β-cell is at high risk of attack from free radical species and resultant oxidative stress. Indeed INS-1 cells are protected from oxidative injury by overexpression of antioxidants [49]. Studies on Type II diabetic animal models suggest that the progressive reduction of islet β-cells is associated with oxidative stress [50,51].

The source of free radicals involved in DNA damage and increased oxidative stress observed in Type 1 diabetes mellitus still remains to be elucidated. Although these studies indicate that CYP2E1 mediated metabolism of xenobiotics is associated with increased DNA damage and cytotoxicity in pancreatic β-cells, other mechanisms leading to increased oxidative stress cannot be ruled out.

However, as the specific CYP2E1 isoform has been shown to be present and inducible within the richly vascularised pancreatic islets of Langerhans [5,24] this may make these cells vulnerable to toxic xenobiotics entering the body. The cytochrome P450 enzymes have been identified as a major source of free radicals [1] and CYP2E1, in

particular, has been shown to exhibit a high propensity for generating reactive oxygen species [52]. In conclusion, it is possible that xenobiotic metabolism by P450 isoforms expressed in the pancreas could contribute to cytotoxicity and DNA damage associated with the pancreatic β-cell dysfunction observed in Type 2 diabetes mellitus and destruction of these cells in Type 1 diabetes mellitus.

Acknowledgments

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