Original Article

Protection of INS-1 Cells From Free Fatty Acid–Induced Apoptosis by Targeting hOGG1 to Mitochondria

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Chronic exposure to elevated levels of free fatty acids (FFAs) impairs pancreatic β-cell function and contributes to the decline of insulin secretion in type 2 diabetes. Previously, we reported that FFAs caused increased nitric oxide (NO) production, which damaged mitochondrial DNA (mtDNA) and ultimately led to apoptosis in INS-1 cells. To firmly establish the link between FFA-generated mtDNA damage and apoptosis, we stably transfected INS-1 cells with an expression vector containing the gene for the DNA repair enzyme human 8-oxoguanine DNA glycosylase/apurinic lyase (hOGG1) downstream of the mitochondrial targeting sequence (MTS) from manganese superoxide dismutase. Successful integration of MTS-OGG1 into the INS-1 cellular genome was confirmed by Southern blot analysis. Western blots and enzyme activity assays revealed that hOGG1 was targeted to mitochondria and the recombinant enzyme was active. MTS-OGG1 cells showed a significant decrease in FFA-induced mtDNA damage compared with vector-only transfectants. Additionally, hOGG1 overexpression in mitochondria decreased FFA-induced inhibition of ATP production and protected INS-1 cells from apoptosis. These results indicate that mtDNA damage plays a pivotal role in FFA-induced β-cell dysfunction and apoptosis. Therefore, targeting DNA repair enzymes into β-cell mitochondria could be a potential therapeutic strategy for preventing or delaying the onset of type 2 diabetes symptoms. *Diabetes* 55:1022–1028, 2006

Chronic elevation of cellular free fatty acids (FFAs) is associated with both insulin resistance and type 2 diabetes (1,2). Results obtained from a variety of different laboratories suggest that the accumulation of lipid into islet tissue is deleterious to normal β-cell function and that this elevation in FFA content ultimately leads to β-cell failure and death through a process termed "lipotoxicity." Exposure of β-cells to high concentrations of FFAs has been correlated with impaired insulin secretion and changes in the expression of genes involved in the lipogenic and fat oxidation pathways and is considered to be an important factor in the pathogenesis of type 2 diabetes (3,4). Moreover, FFAs have been shown to cause β-cell death by both apoptotic and necrotic mechanisms (5,6). Although the exact mechanisms involved in FFA-induced apoptosis and necrosis remain to be clarified, it has been suggested that the β-cell dysfunction and death observed in type 2 diabetes may involve exaggerated activation of the inducible form of nitric oxide synthase (iNOS) by FFAs with the consequent generation of excess nitric oxide (NO) (7).

Although NO plays a prominent role in regulating many biological functions, a growing body of evidence indicates that at high concentrations, it can also be cytotoxic and mutagenic (8). However, the cellular targets with which NO interacts have not been fully identified. We hypothesized that one of these targets might be mitochondrial DNA (mtDNA). Recently, we reported that FFAs (2:1, oleate:palmitate) caused a rise in NO production that damaged mtDNA and ultimately led to apoptosis in INS-1 cells (9). Moreover, previous work from our laboratory and others has established that in other cellular systems, damage to mtDNA is an early event leading to apoptosis and that protection against this damage through the use of recombinant DNA repair enzymes targeted to mitochondria blocks the induction of programmed cell death (10–12). In the present study, we examined whether targeting the DNA repair enzyme, human 8-oxoguanine DNA glycosylase/apurinic lyase (hOGG1) to mitochondria of INS-1 cells can prevent FFA-induced mtDNA damage and thus protect against the FFA-induced apoptosis. This enzyme is a glycosylase/apurinic lyase that has glycosylase activity for the mutagenic 8-oxoguanine and lyase activity for abasic sites and 5′-deoxyribose phosphate. This enzyme was selected for these studies because we previously found that it had a protective effect against toxicity caused by NO (13). Our results reveal that overexpression of hOGG1 in mitochondria of INS-1 cells has a protective effect against FFA-induced mtDNA damage and significantly reduced FFA-induced apoptosis.

RESEARCH DESIGN AND METHODS

Cell culture, transfection, and treatment. INS-1 cells (a gift from C.B. Wollheim [University of Geneva, Switzerland]) (14) were grown in RPMI 1640 medium containing 10 mmol/l HEPES, 10% fetal bovine serum (Hyclone, Logan, UT), 50 μmol/l 2-mercaptoethanol, 50 μg/ml gentamicine sulfate, and 1 μmol/l pyruvate (Sigma, St. Louis, MO). For transfection, cells were transfected with either pcDNA3.neo/mitochondrial targeting sequence (MTS)-OGG1 (10,15) or empty vector (pcDNA3.neo) DNA (Invitrogen). Transfection was conducted using Fugene-6 reagent (Roche Molecular Biochemicals, Mannheim, Germany). MTS-OGG1– or vector-transfected cells were selected by culturing the cells in 800 μg/ml G418 (Invitrogen). Antibiotic-resistant cells

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ELISA, enzyme-linked immunosorbent assay; FFA, free fatty acid; hOGG1, human 8-oxoguanine DNA glycosylase/apurinic lyase; iNOS, inducible nitric oxide synthase; MTS, mitochondrial targeting sequence.

were removed from the culture vessel by trypsinization and exposed to single-cell sorting using forward and side light scatter. Individual cells were plated into wells in a 96-well culture vessel and maintained in RPMI 1640 medium containing 800 μg/ml G418. Cells in wells containing colonies were passaged for further study. For experiments, cells were seeded into appropriate culture dishes 2–3 days before experiments. Long-chain FFAs (2:1, oleate:palmitate; Sigma) were dissolved in 50% ethanol and exposed to INS-1 cells for times ranging from 6 to 48 h. BSA at a final concentration of 2% was added to the culture medium during FFA treatment.

Insulin secretion. Immunoreactive insulin release into the culture medium was determined using the back-titration method originally described by Wright et al. (16) using guinea pig anti-insulin serum (Linco) and rat insulin standards.

NO release assay. Wild-type and MTS-OGG1– and vector-only–transfected INS-1 cells were exposed to FFAs for 6 h. Control cells received drug diluent only. After treatment, aliquots of media were collected, and nitrite production was evaluated using the Griess reaction (17). Nitrite levels were measured in FFA-supplemented medium because it has been shown that FFAs in the medium increase the background in these reactions (6). Therefore, all samples were adjusted to contain the same amount of FFAs. Sodium nitrite was used as a standard.

Quantitative and neutral Southern blots. MTS-OGG1– and vector-only–transfected cells were exposed to FFAs as described above. DNA isolation and quantitative Southern blots were performed as previously described (18,19). Briefly, cells were lysed in 10 mmol/l Tris-HCl (pH 8.0), 1 mmol/l EDTA (pH 8.0), 0.5% SDS, and 0.3 mg/ml proteinase K overnight at 37°C. High–molecular weight DNA was extracted with phenol, treated with RNase, and digested to completion with *Bam*HI. Digested samples were precipitated, resuspended in TE buffer, and precisely quantified using a Hoefer TKO 100 minifluorometer. Samples containing 5 μg DNA were heated at 70°C for 15 min and then cooled to room temperature. A sodium hydroxide solution was then added to a final concentration of 0.1 N, and samples were incubated for 15 min at 37°C. Gel electrophoresis and vacuum transfer were carried out as described previously (18,19). Membranes were hybridized with a denatured PCR-generated mitochondrial probe (9,20), washed, and autoradiographed. The resultant band images were scanned from films using DeskScan II scanning software (Hewlett-Packard, San Diego, CA) and then analyzed using Molecular Analyst (Bio-Rad, Hercules, CA) software. Break frequency was determined using the Poisson expression ($s = -\ln P_o$, where s is the number of breaks per fragment and P_o is the fraction of fragments free of breaks). Neutral Southern blots were performed similarly, except that there was no alkaline pretreatment, and NaOH was not included in the loading dye, the agarose gel, or the electrophoresis buffer. To confirm integration of the transfected MTS-OGG1 sequence, DNA samples were digested with *Eco*RI and *Xho*I, and hybridization was performed using a ^{32}P-labeled MTS-OGG1 fragment.

Preparation of cellular fractions and Western blot analysis. Mitochondrial and cytosolic protein fractions were isolated as described previously (9–11). Total cellular fractions were isolated by lysing the cellular pellets in a buffer containing 50 mmol/l Tris, pH 7.4, 150 mmol/l NaCl, 1% NP-40, 10% glycerol, and 5 μl/ml of a mixture of protease inhibitors (Sigma). The protein concentration was determined using the Bio-Rad protein dye microassay. SDS-PAGE and transfer of separated proteins to polyvinylidine fluoride membrane were performed as previously described (9–11), with minor modifications. Blocking and antibody immunoblotting were performed in 5% nonfat dry milk and Tris-buffered saline (TBS) with 0.1% Tween 20. TBS with 0.1% Tween 20 and TBS were used for washing. Anti-hOGG1 antibodies were from Novus Biologicals (Littleton, CO), anti-cytochrome c monoclonal antibody was purchased from PharMingen (San Diego, CA), anti-actin antibodies were from Sigma, monoclonal anti-iNOS antibody was from BD Biosciences, and polyclonal anti-insulin and anti-GLUT2 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The complexes formed were detected with horseradish peroxidase–conjugated anti-mouse IgG or anti-rabbit IgG antibodies (Promega, Madison, WI) using chemiluminescent reagents (SuperSignal; Pierce, Rockford, IL).

OGG1 activity assays. 8-oxoguanine–containing duplex DNA was prepared and specific reaction assays were performed as previously described (18,21). Equal amounts of proteins (10–15 μg) from mitochondrial fractions isolated from MTS-OGG1– and vector-transfected cells were used. For control reactions, 5 units pure formamidopyrimidine DNA glycosylase enzyme (Trevigen, Gaithersburg, MD) was added instead of cell lysates. Reaction mixtures were incubated for 6 h at 37°C. After PAGE, wet gels were autoradiographed at −70°C. The resultant band images were scanned from films using DeskScan II scanning software (Hewlett-Packard) and analyzed using Molecular Analyst (Bio-Rad) software.

Cell viability. For viability studies, MTS-OGG1– or vector-only–transfected cells were treated with 2 mmol/l FFAs for 24 and 48 h. Control cultures were exposed to drug diluent only. Twenty-four and 48 h later, trypan blue was added and viable cells were counted using light microscopy. Data are expressed as a percentage of untreated control.

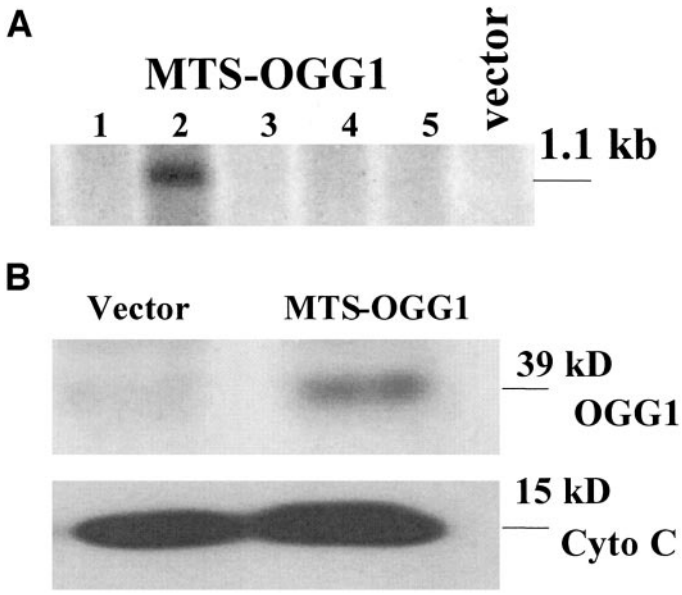

FIG. 1. Incorporation of the MTS-OGG1 insert and targeted expression of hOGG1 in mitochondria from INS-1 cells. *A*: Southern blot analysis of total DNA isolated from five INS-1/MTS-OGG1–transfected clones and a clone transfected with vector alone using the MTS-OGG1 sequence as a probe. *B*: Western blot analysis using hOGG1 antiserum. Immunodetection of cytochrome c was performed to assure mitochondrial localization. Data represent results from three separate experiments.

DNA fragmentation assay. The presence of fragmented nuclear DNA in the cytoplasmic fraction of cell lysates was assessed by measuring DNA associated with nucleosomal histones using a specific two-site enzyme-linked immunosorbent assay (ELISA) with an anti-histone primary antibody and a secondary anti-DNA antibody (Roche Diagnostics, Indianapolis, IN). Briefly, the cells were treated with FFAs for 9 h, washed twice with PBS, and incubated with lysis buffer for 20 min at room temperature. After centrifugation to remove nuclei and cellular debris, the supernatants were diluted 1:5 with lysis buffer, and each sample was analyzed by ELISA. The intensity of the color that developed was determined by measuring the absorbance at 405 nm, whereas that at 490 nm was used as a blank (reference wavelength). All experiments were repeated three times independently.

ATP levels. Cells were initially treated with FFAs for 9 h. To determine the total cellular ATP level, an ATP bioluminescence assay kit (Roche Molecular Biochemicals) was used. This kit uses a well-established technique for the measurement of extremely low concentrations of ATP (22). The emitted light is linearly related to the ATP concentration and is measured using a luminometer.

Statistical analysis. Data are expressed as means ± SE. For comparisons of two groups, significance was determined using unpaired Student's t test. For multiple comparisons, one- or two-way ANOVA followed by Bonferroni analysis was used. Statistical significance was determined at the 0.05 level.

RESULTS

Generation of MTS-OGG1–transfected INS-1 cells and hOGG1 expression in mitochondria of INS-1 cells. INS-1 cells were stably transfected with either pcDNA3.neo/MTS-OGG1 (10,15) or empty vector (pcDNA3.neo) DNA. After isolation of individual clones, total DNA was isolated from cells transfected with MTS-OGG1 or vector only, and Southern blot analysis was performed. The presence of the insert was detected by hybridization with a 1.1-kb probe containing the full MTS-OGG1 sequence. One particular clone (clone 2) showed a high level of incorporation of the full-length insert (Fig. 1*A*); therefore, this clone, designated INS-1/MTS-OGG1–2, was chosen for subsequent study. The other four MTS-OGG1 transfectants probably contained the MTS-OGG1

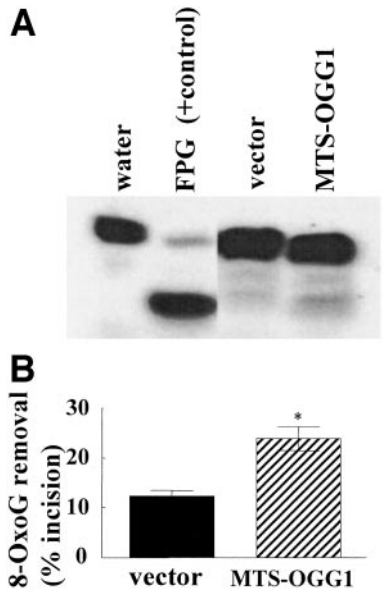

FIG. 2. Recombinant hOGG1 is active in mitochondria from INS-1 cells. *A*: An increase of 9-mer cleavage product was observed in lanes containing extracts from MTS-OGG1 cells, indicating that OGG1 activity was significantly higher in these cells. *B*: The average results ± SE from three separate experiments are shown. *$P < 0.05$.

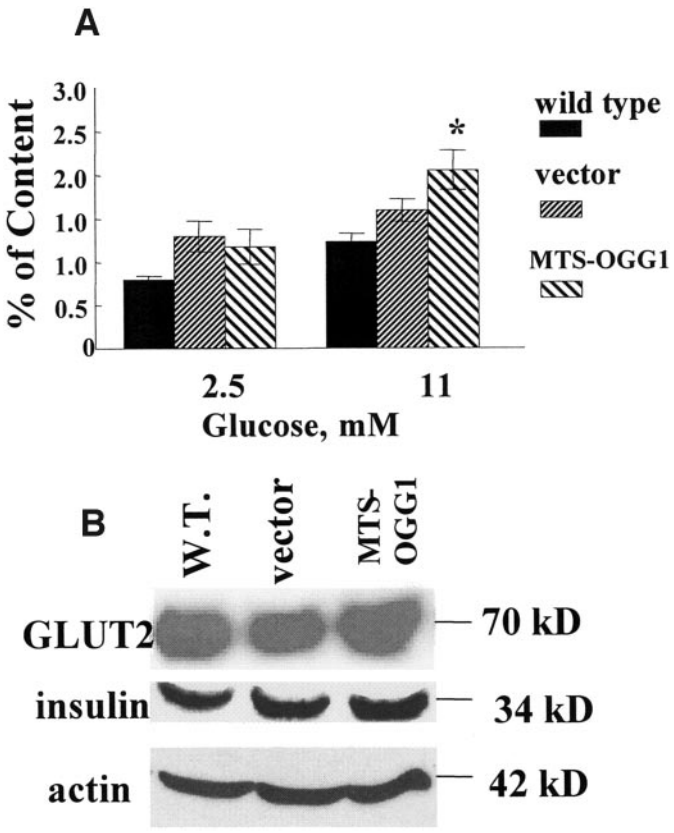

FIG. 3. Insulin secretion and insulin and GLUT2 gene expression in wild-type and transfected INS-1 cells. *A*: Glucose-stimulated insulin secretion in wild-type (W. T.), vector, and MTS-OGG1 INS-1 cells. Data are means ± SE ($n = 4$ or 5). An asterisk indicates a significant difference from wild type ($P < 0.05$, one-way ANOVA). *B*: A Western blot representative of three independent experiments of insulin and GLUT2 gene expression in wild-type and transfected INS-1 cells. Equal loading was confirmed using actin antibody.

insert below the level detectable by Southern blot analysis or fragments shorter than the full-length insert. To show that the recombinant hOGG1 enzyme made by the selected clone was targeted to mitochondria, mitochondrial extracts were prepared from INS-1/MTS-OGG1–2 and a vector-only–transfected clone. Mitochondria were isolated by differential centrifugation, and Western blots were performed using a polyclonal antibody to human OGG1. Even loading was confirmed by Ponceau staining of the membrane after transfer. The INS-1/MTS-OGG1–2 clone showed an increase in OGG1 protein (39 kDa) when compared with vector-transfected cells (Fig. 1*B*). To determine whether the recombinant hOGG1 was functional in mitochondria, OGG1 activity assays were performed. A labeled 24-bp duplex oligonucleotide with 8-oxoguanine placed at the 10th position on one strand was incubated with mitochondrial extracts isolated from MTS-OGG1– and vector-transfected cells. As a positive control, the bacterial glycosylase/apurinic lyase formamidopyrimidine DNA glycosylase (Trevigen) was used (Fig. 2*A*). As shown in Fig. 2*B*, the mitochondrial extracts isolated from MTS-OGG1 clones were about twofold better able to cleave the DNA substrate than vector-only–transfected cells ($P < 0.05$). Thus, these data reveal that the recombinant hOGG1 protein, which is targeted to mitochondria, is functional in INS-1 cells.

Insulin secretion and insulin and GLUT2 gene expression in wild-type and transfected INS-1 cells. To establish whether transfection INS-1 cells with either the vector or vector containing MTS-OGG1 altered their function, insulin secretory studies were performed. Cultures were given normal culture medium containing 5.5 mmol/l glucose for 24 h. At this point, the medium was withdrawn, and the cultures were given Hanks' balanced salt solution without glucose for 20 min. The Hanks' balanced salt solution was removed, and the cultures were replenished with Krebs-Ringer's bicarbonate solution containing either 2.5 or 11 mmol/l glucose. After 45 min, the medium was removed and frozen for determination of immunoreactive insulin concentrations. As can be seen in Fig. 3*A*, there was not a significant difference in the insulin released between the three groups at the low concentration of glucose. However, at the 11 mmol/l glucose concentration, the cells transfected with MTS-OGG1 released significantly more insulin. To determine whether the increased release of insulin seen in the MTS-OGG1–containing cells was correlated with increased expression of the glucose transporter, GLUT2, or the insulin genes, Western blot analysis was performed on wild-type cells and those containing either vector or vector with MTS-OGG1. The results showed that all three types of cells expressed GLUT2 and insulin equally (Fig. 3*B*).

FFAs induce iNOS expression and NO production in INS-1 cells. Previously, it has been shown that FFAs induce NO production in β-cells and INS-1 cells (7,9). However, several other studies have reported that FFAs neither induced iNOS expression nor increased NO production in rat islets, β-cells, and INS-1 (6,23–25). Thus, in these studies, to evaluate whether NO is produced, nitrite levels in the culture medium and iNOS protein expression were examined in both wild-type and transfected INS-1 cells. Although we found that the addition of FFAs to cell-free medium also caused a relatively high background, nitrite production in all three cell types was significantly greater after FFA exposure (Fig. 4*A*). Moreover, as shown by Western blot analysis in Fig. 4*B*, iNOS expression in all

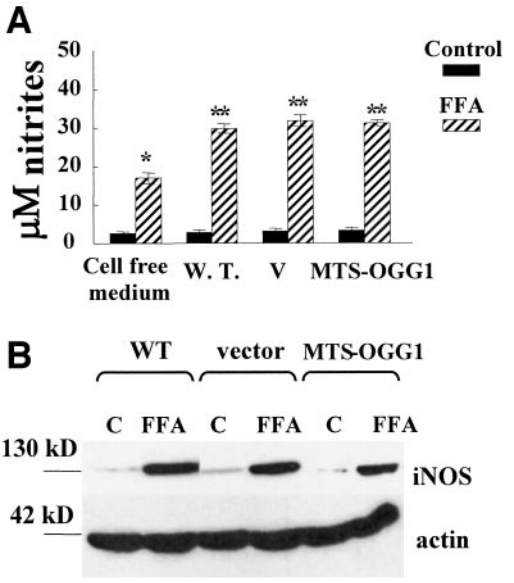

FIG. 4. FFA-induced NO production and iNOS expression in INS-1 cells. *A*: Nitrite production by wild-type (W. T.), vector, and MTS-OGG1 INS-1 cells exposed for 6 h to 2 mmol/l FFAs. Data are means ± SE (n = 3). *P < 0.05 vs. cell-free medium without FFAs, **P < 0.05 vs. control condition and cell-free medium plus FFAs (two-way ANOVA). *B*: Representative immunoblot of iNOS protein expression after 6-h treatment with 2 mmol/l FFAs. C, untreated control. Actin antibody was used to show equal loading. Figure represents three separate experiments.

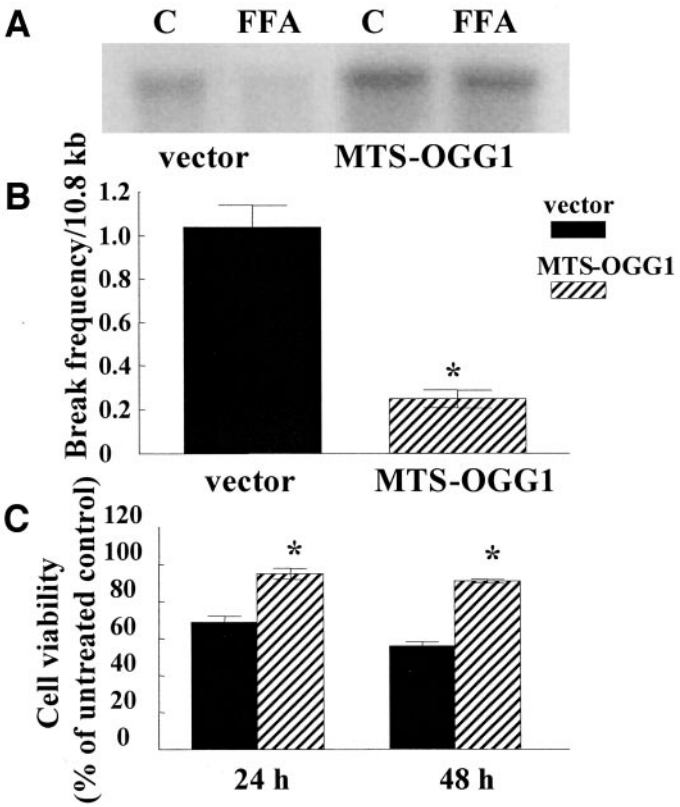

FIG. 5. Overexpression of hOGG1 in mitochondria from INS-1 cells prevents FFA-induced mtDNA damage and increases cell survival. *A*: A representative autoradiograph of three independent quantitative alkaline Southern blots (C, untreated control; FFA, treated with 2 mmol/l FFA cells) is shown. *B*: Break frequency/10.8-kb mtDNA fragment. *C*: Cell viability after 24- or 48-h exposure to FFAs. The average results ± SE are shown (n = 3). *P < 0.05.

cell types also was induced after 6 h of exposure to 2 mmol/l FFAs.

hOGG1 expression in mitochondria protects mtDNA from FFA-induced damage and enhances cell survival. Recent findings from our laboratory have shown that FFAs caused a rise in NO that damages mtDNA and ultimately leads to apoptosis in INS-1 cells (9). In the present study, experiments for evaluation of mtDNA damage after FFA exposure were performed in which MTS-OGG1– and vector-transfected INS-1 cells were exposed to 2 mmol/l FFAs for 6 h. Control cells were incubated with drug diluent only. Cells were lysed, total DNA was isolated, and quantitative Southern hybridizations were performed using a mtDNA-specific probe. The results revealed a significant decrease in FFA-induced mtDNA damage in MTS-OGG1–transfected INS-1 cells compared with cells containing only vector (Fig. 5*A*). The break frequency per 10.8-kb mtDNA restriction fragment is shown in Fig. 5*B*. To determine whether the increase in mtDNA repair enhanced cellular survival after FFA exposure, we performed a viability evaluation using trypan blue dye exclusion. MTS-OGG1– and vector-transfected cells were treated with 2 mmol/l FFAs for 24 and 48 h, and the percentage of viable cells was determined. The results showed that a significantly greater percentage of the MTS-OGG1 cells were able to exclude the dye after 24 and 48 h (Fig. 5*C*).

hOGG1 expression in mitochondria protects INS-1 cells from FFA-induced DNA fragmentation and prevents cytochrome c release from mitochondria. To access whether the protective effect of hOGG1 overexpression on mtDNA integrity was correlated with inhibition of FFA-induced apoptosis, an ELISA assay for DNA fragmentation was used (Fig. 6*A*). We found that DNA fragmentation was significantly decreased in MTS-OGG1 cultures compared with vector-containing cultures 9 h after treatment with FFAs. To evaluate whether the initiation of apoptosis was through a mechanism mediated by mitochondria, changes in the localization of cytochrome c after exposure of cells to FFAs were determined. Treatment with FFAs caused release of cytochrome c from mitochondria into the cytoplasm as determined by Western blot analysis of cytosolic protein fractions (Fig. 6*B*). There was a noticeable decrease in intensity of the band in the cytosolic fractions isolated from MTS-OGG1 transfectants, indicating that targeting of hOGG1 in mitochondria is able to reduce cytochrome c release from these organelles and diminish the initiation of apoptosis through mitochondrial signaling.

hOGG1 overexpression in mitochondria partially restores FFA-induced inhibition of ATP production. MTS-OGG1– or vector-transfected cells were grown in 24-well culture plates, treated with FFAs for 9 h, and washed twice with PBS; and ATP production was measured. Control cultures were exposed to diluent only for the same amount of time. As shown in Fig. 7, MTS-OGG1–transfected cells significantly restored ATP production by 9 h after incubation with FFAs.

DISCUSSION

Chronic exposure of β-cells to FFAs results in elevated basal insulin secretion with a concomitant suppression of glucose-stimulated insulin secretion increased oxidative stress, which consists of an increase in both reactive oxygen and nitrogen species, the inhibition of insulin

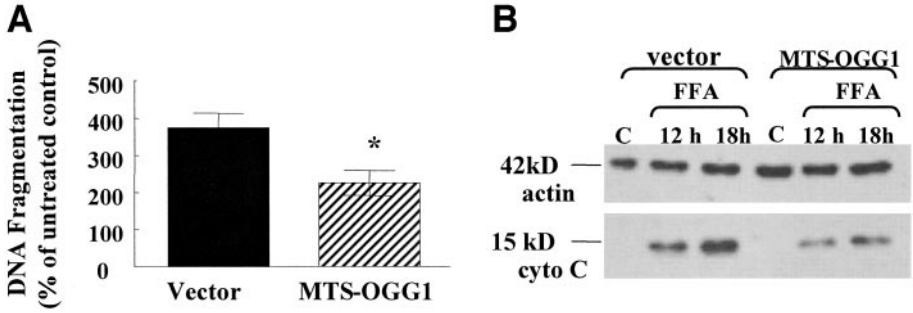

FIG. 6. The effect of hOGG1 overexpression in mitochondria on DNA fragmentation and cytochrome c release after exposure to FFAs. *A*: Protective effect of hOOG1 was assessed by an ELISA assay 9 h after FFA treatment. Data are means ± SE (n = 3). *P < 0.05. *B*: Western blots were performed on the cytosolic protein fractions from untreated control cells (C) and cells treated with 2 mmol/l FFAs for the indicated time points (12 and 18 h) using cytochrome c antiserum. Equal loading was confirmed using actin antibody. Representative of three experiments.

biosynthesis, and ultimately the induction of β-cell death (1,26,27). Several hypotheses have been proposed to explain how conditions resulting from lipotoxicity induce β-cell dysfunction (26,28,29); however, the mechanisms that cause this effect have not been fully elucidated. The purpose of the present study was to explore the hypothesis that NO-induced mtDNA damage generated after exposure to FFAs initiates a cascade of processes that ultimately lead to mitochondrial dysfunction and apoptosis in INS-1 cells. To test this hypothesis, INS-1 cells were stably transfected with either an expression vector containing the gene for the DNA repair enzyme hOGG1 downstream of the MTS from manganese superoxide dismutase (MTS-OGG1) or an empty vector in an effort to modulate mtDNA repair. INS-1 cells were selected for these studies because it has previously been shown that factors that are known to cause apoptosis in β-cells, such as FFAs and cytokines, also cause apoptosis in these cells (9,24,25,30). Therefore, we felt that they serve as an appropriate surrogate for β-cells when mechanisms involved in apoptosis are studied. The mixture of oleate and palmitate was selected because this was the mixture used by Shimabukuro et al. (7) when they reported on the role of NO in lipotoxicity. It is likely that the degree of toxicity could be greater if we used palmitate alone, because saturated fatty acids like palmitate have been found to cause marked apoptosis (5,31–33). Conversely, unsaturated fatty acids, such as oleate, are less cytotoxic and may even exert some protection against the proapoptictic effects generated by saturated fatty acids (31,33–35). It has been reported that INS-1 cells do not show an increase in iNOS expression or NO production after exposure to FFAs (25). This was not the case in our studies with these cells. We found that exposure to FFAs increased the expression of iNOS and caused an elevation in the levels of NO. Moreover, previously, we used the technically sophisticated technique of ligation-mediated PCR to identify the type of lesion caused by FFAs in mtDNA (9). The results showed that the lesions appear to be caused by deamination of guanines and adenines through the exposure to N_2O_3, which is formed by the reaction of NO with molecular oxygen.

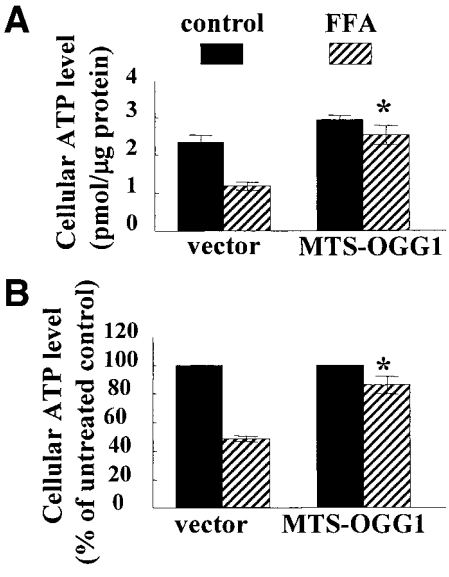

FIG. 7. ATP content in transfected INS-1 cells after FFA exposure. Cells were treated with 2 mmol/l FFAs for 9 h, and ATP production was measured. *A*: Total intracellular ATP content expressed as picomoles per microgram of protein. Data are means ± SE (n = 3). *P < 0.05. *B*: Values are expressed as means ± SE relative to untreated control cells (n = 3). *P < 0.05.

Recombinant hOGG1 was selected for these experiments because we had previously found that mtDNA repair and cellular survival can be enhanced by targeting this recombinant enzyme to mitochondria in HeLa cells (15,18). Furthermore, experiments targeting hOGG1 to mitochondria have been conducted in oligodendrocytes (10) and pulmonary artery endothelial cells (12). These studies have all confirmed that hOGG1, when expressed in mitochondria, enhances mtDNA repair and protects cells from oxidant-mediated death. Additionally, we recently have shown that targeting hOGG1 to mitochondria in oligodendrocytes decreases the sensitivity of mtDNA to damage caused by exposure to cytokines and protects these cells against apoptosis (11). Although these studies have predominantly looked at oxidant-mediated damage, we also have found that conditional expression of hOGG1 in mitochondria improves mitochondrial repair of NO-induced DNA damage in HeLa cells and protects these cells from apoptosis induced by NO generated by the decomposition of PAPA/NONOATE (13). These findings led us to hypothesize that this recombinant enzyme could provide protection against mtDNA damage produced by a physiologically relevant toxic agent to the β-cell, FFAs, which have been reported to induce the production of NO (9). The results of these studies showed that transgenic MTS-OGG1 INS-1 cells, which express hOGG1 in mitochondria, significantly protect their mtDNA from FFA-induced damage compared with vector-only–containing controls. Furthermore, these cells are markedly resistant to FFA-induced apoptosis. The significant reduction seen in cytochrome c relocation into the cytosol indicates that the protection against apoptosis worked through a mitochondrially mediated mechanism.

In previous work, which formed the background for the present studies, we determined that exposure of INS-1 cells to FFAs caused a reproducible amount of lesions in mtDNA (9). Also, it was discovered that the mtDNA damage was produced by NO generated through the

apparent activation of iNOS. Ligation-mediated PCR analysis showed that NO produced after exposure to FFAs reacted with molecular oxygen to form nitrous anhydride, N_2O_3, an effective nitrosating agent, which reacts with the primary amino group in adenine or guanine to form hypoxanthine and xanthine, respectively. These base adducts are unstable and are easily hydrolyzed at normal physiological temperature to form apurinic sites (36–38). Besides being a block to DNA replication and transcription, apurinic sites also may be toxic by virtue of their lability at adjacent phosphodiester bonds, which when broken lead to the formation of single-strand breaks (39). When present in template DNA used by DNA polymerases, apurinic sites are noninstructional and potentially mutagenic, because the incorporation of specific bases opposite an apurinic site varies depending on the DNA polymerase used (37,40). Therefore, these studies suggest that it is the buildup of these potentially toxic, NO-induced lesions in mtDNA that initiate apoptosis after exposure to FFAs. Thus, the question arises of how this recombinant DNA glycosylase/apurinic lyase protects against this damage? We believe that the lyase activity of hOOG1 is responsible for the protective effect observed from the targeting of this enzyme to mitochondria. There are several reasons for this belief. First, if an apurinic site is generated, there is no substrate for the glycosylase activity. The second reason is the mix of enzymes involved in base excision repair. These enzymes have been characterized by Pinz and Bogenhagen (41) and are composed of an apurinic-endonuclease, which cleaves the phosphate backbone on the 5′ side of the abasic site, γ-polymerase, which attaches the new base to the 3′OH group and has lyase activity to remove the 5′-deoxyribose phosphate and a ligase, which reseals the phosphate backbone. However, in contrast to β-polymerase, the base excision repair polymerase in the nucleus, which has strong lyase activity, γ-polymerase has very weak lyase activity (42). This renders mitochondria more sensitive to the excessive generation of abasic sites, which occurs when N_2O_3 is formed by the reaction of NO with O_2. By increasing the lyase activity through the targeting of recombinant hOGG1 to mitochondria, the buildup of this toxic intermediate can be blocked. It has recently been reported that the lyase activity of hOGG1 can be inhibited by high Mg^{2+} concentrations (43). Although this effect may have important consequences on the action of this enzyme in the nucleus, we feel that the effects of Mg^{2+} will be minimal in the matrix of mitochondria. In the study showing inhibition of lyase activity in hOGG1, Mg^{2+} concentrations of 5 mmol/l were required for this effect. However, it has recently been shown that the concentration of Mg^{2+} in mitochondria is ~1.2 mmol/l, and much of this Mg^{2+} is bound to ATP (44). Therefore, it is unlikely that Mg^{2+} concentrations in mitochondria will adversely affect hOGG1 lyase activity.

In summary, we believe that the findings reported here have demonstrated that targeting hOGG1 to mitochondria is a viable strategy for protecting β-cell against FFA-induced apoptosis. However, we also believe that this work has broader implications for the field of diabetes. Because we have found that this enzyme protects against apoptosis generated by oxidant damage to mtDNA in other cell types, we believe that it or similar recombinant enzymes can be used to protect β-cells against the apoptosis that is caused by cytokine-generated cell death and during the stress of transplantation.

ACKNOWLEDGMENTS

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