Original Article

3,4-Dideoxyglucosone-3-ene Induces Apoptosis in Renal Tubular Epithelial Cells

Pilar Justo,[1] Ana Belén Sanz,[1] Jesús Egido,[1,2,3] and Alberto Ortiz[1,2,3]

Diabetes complications are caused by hyperglycemia. Hyperglycemia results in increased concentrations of glucose degradation products. The study of peritoneal dialysis solution biocompatibility has highlighted the adverse effects of glucose degradation products. Recently, 3,4-dideoxyglucosone-3-ene (3,4-DGE) has been identified as the most toxic glucose degradation product in peritoneal dialysis fluids. Its role in renal pathophysiology has not been addressed. 3,4-DGE induces apoptosis in murine renal tubular epithelial cells in a dose- and time-dependent manner. Peak apoptosis is observed after 72 h of culture. The lethal concentration range is 25–50 μmol/l. 3,4-DGE results in Bax oligomerization, release of cytochrome c from mitochondria, activation of caspases-9 and -3, and Bid proteolysis. Apoptosis induced by 3,4-DGE is caspase dependent and could be prevented by the broad-spectrum caspase inhibitor zVAD-fmk (Z-Val-Ala-DL-Asp-fluoromethylketone) and by specific inhibitors of caspases-2, -8, and -9. However, caspase inhibition did not prevent eventual cell death. In contrast, antagonism of Bax by a Ku-70–derived peptide or antisense oligonucleotides prevented both apoptosis and cell death. In conclusion, 3,4-DGE promotes apoptosis of cultured renal parenchymal cells by a Bax- and caspase-dependent mechanism. A role for 3,4-DGE in diabetes complications in the kidney and in the modulation of residual renal function in peritoneal dialysis should be further explored. *Diabetes* **54:2424–2429, 2005**

K idney disease is one of the major complications of diabetes. Although vascular and glomerular injury have been considered the main features of diabetic kidney disease, tubular atrophy is also found (1). In addition, early signs of tubular dysfunc-

tion have been reported in diabetes (2). Moreover, diabetic kidneys are particularly prone to acute tubular necrosis in diverse clinical situations, such as post–cardiac surgery (3). Hyperglycemia per se is an independent risk factor for acute tubular necrosis under these circumstances (3). Tubular cell death is the main morphologic feature of acute tubular necrosis (4). In particular, apoptotic cell death is prominent in human acute tubular necrosis (5). Apoptosis is an active form of cell death that, if dysregulated, can promote disease (6). Apoptosis participates in the loss of tubular cells during acute tubular necrosis (4,5) and chronic tubular atrophy (7), including during the course of diabetic nephropathy (8).

Apoptosis is characterized by the activation of intracellular lethal molecules. Caspases are a family of intracellular cysteine proteases that behave as activators and effectors of apoptosis, and they play a central role in the process (6). *Bcl*II-related proteins comprise a family of positive and negative regulators of apoptosis (6). Among them, Bax is central to apoptotic pathways that implicate the mitochondria. Apoptosis is usually a response to an adverse microenvironment. Glucose is one of the microenvironmental factors that may induce apoptosis. A high glucose concentration per se promotes apoptosis in a variety of cell types, including renal tubular epithelium (8–10). Bax protein was upregulated in tubular epithelium exposed to high glucose (8). In addition, apoptosis induced by high glucose in the blastocyst requires Bax and does not occur in Bax knockout mice (10). More recently, attention has been drawn to factors related to high glucose concentrations but different from glucose itself. Several aspects of the bioincompatibility of peritoneal dialysis solutions containing high glucose concentrations are not directly related to glucose. Rather, a role for glucose degradation products has been demonstrated for processes such as the apoptotic death of leukocytes (11). Indeed, glucose degradation products from peritoneal dialysis solutions have systemic effects such as modulation of appetite and increased serum concentrations of advanced glycation end products (12–14). Most of the glucose degradation products identified so far (e.g., methylglyoxal, acetaldehyde, formaldehyde, and 3-deoxyglucosone [3-DG]) are not toxic to peritoneal cells at clinically relevant concentrations (15,16). 3,4-dideoxyglucosone-3-ene (3,4-DGE) was recently identified as the most cytotoxic glucose degradation product in peritoneal dialysis fluids (17). The concentration of several of these glucose degradation products, such as 3-DG, is increased
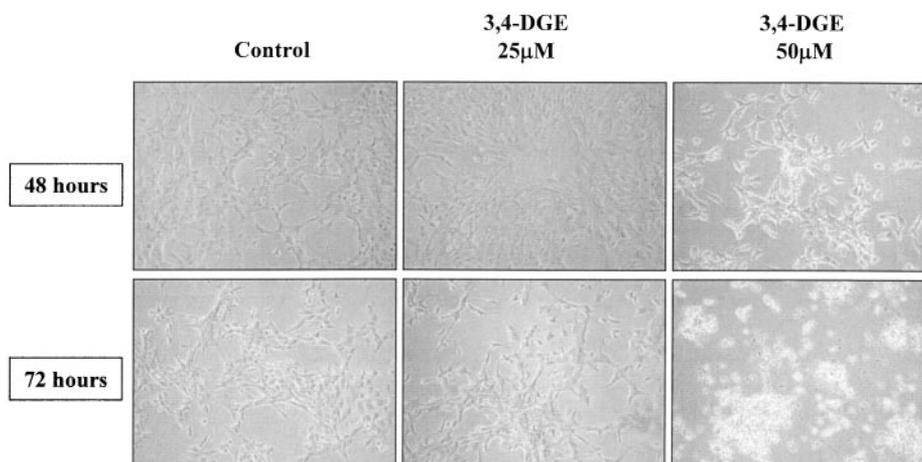
FIG. 1. 3,4-DGE induces tubular cell death. Tubular cells were cultured in the presence of 3,4-DGE for different time periods. Cytotoxicity is most evident at the 50 μmol/l concentration, and it is observed from 48 h of incubation. Phase contrast microscopic photographs are shown (original magnification ×200).

in serum and cells from diabetic patients (18–20). 3-DG is in equilibrium with 3,4-DGE because the latter is formed by dehydration of 3-DG (21). We have now explored the biological effects of 3,4-DGE in cultured renal tubular epithelial cells.

RESEARCH DESIGN AND METHODS

MCT cells are a cultured line of proximal tubular epithelial cells harvested originally from the renal cortex of SJL mice (22). The cells were maintained in culture in RPMI 1640 (Gibco, Grand Island, NY), 10% heat-inactivated FCS, 2 mmol/l glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37°C in 5% CO_2 (23). For cell death experiments, cells were plated and then grown for 24 h in RPMI with 10% FCS. Then, the media was replaced with fresh serum-free RPMI (RPMI with 0% FCS), unless otherwise stated. RPMI with 0% FCS contained the same supplements as the serum-supplemented RPMI, with the exception of the FCS.

3,4-DGE and caspase and Bax inhibitors. 3,4-DGE was generously provided by Anders Weislander (Gambro, Lundia, Sweden) and was directly dissolved in cell culture medium (24). Endotoxin levels were below the detection limit as assessed by a limulus amebocyte assay (Sigma).

The following caspase inhibitory peptides were used: the caspase-3 inhibitor Z-Asp(OMe)-Glu(OMe)-Val-DL-Asp(OMe)-fluoromethylketone (DEVD-fmk) and the pancaspase inhibitor Z-Val-Ala-DL-Asp-fluoromethylketone (zVAD-fmk) from Bachem (Bubendorf, Switzerland); and the caspase-8 inhibitor Z-Ile-Glu(OMe)-Thr-Asp(OMe)-fluoromethylketone (IETD-fmk), the caspase-9 inhibitor Z-Leu-Glu(OMe)-His-Asp(OMe)-fluoromethylketone (LEHD-fmk), and the caspase-2 inhibitor benzyloxycarbonyl-Val-Asp-Val-Ala-Asp-fluoromethyl-ketone (z-VDVAD-fmk) from Calbiochem (San Diego, CA). The caspase inhibitory peptides were used at concentrations previously shown to protect from apoptosis in MCT cells and other cell systems (23). DEVD-fmk, zVAD-fmk, IETD-fmk, LEHD-fmk, and z-VDVAD-fmk were dissolved in DMSO. Final concentration of DMSO was 0.05%, and it did not influence MCT cell apoptosis. Peptides (200 μmol/l) or vehicle were added to the cell cultures 3 h before addition of 3,4-DGE. Cells were cultured in the presence of stimuli for an additional 24–72 h.

Bax inhibitor peptide P5 and a negative control peptide were from Tocris (Ellisville, MO). P5 is a cell-permeable synthetic peptide inhibitor of Bax translocation to mitochondria. The peptide was designed from Ku-70, a protein that suppresses the mitochondrial translocation of Bax, and it inhibits Bax-mediated apoptosis (25). Cells were treated with 50 μmol/l 3,4-DGE for 72 h in the presence or absence of Bax inhibitor peptide P5 (200 μmol/l) or negative control (200 μmol/l).

Assessment of cell death and apoptosis. For quantification of cell death, 10,000 cells were seeded in 12-well plates (Becton Dickinson, Franklin Lakes, NJ) under the experimental conditions. Apoptosis was quantified by flow cytometry assessment of DNA content in pooled attached and detached cells, permeabilized, and stained with propidium iodide (23). The percentage of cells with decreased DNA staining (A_0), comprising apoptotic cells with fragmented nuclei, was counted. To assess the typical nuclear changes seen in apoptosis, cells were fixed and stained with propidium iodide (23). After fixation, propidium iodide stains both live and dead cells.

Western blot. Western blot was carried put according to previously described techniques (23,26). Primary antibodies were rabbit polyclonal anti–caspase-9 (1:1,000; Cell Signaling, Hertfordshire, U.K.), rabbit polyclonal anti–cleaved caspase-3 (1:1,000; Cell Signaling), or rabbit polyclonal anti-Bid (1:3,000; gift of S.J. Korsmeyer, Howard Hughes Medical Institute). Blots were then probed with anti-tubulin antibody, and levels of expression were corrected for minor differences in loading (23,26).

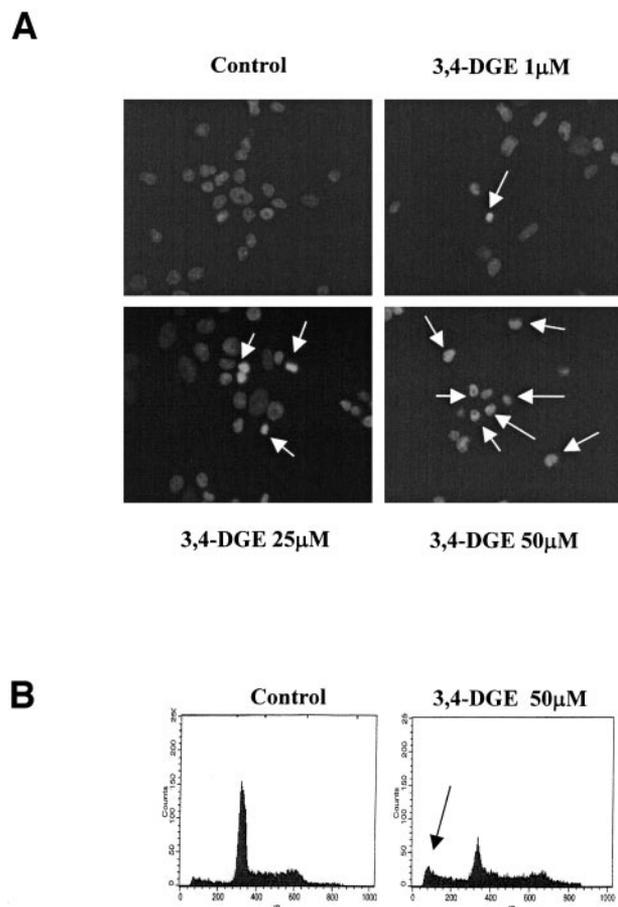**FIG. 2. 3,4-DGE promotes tubular cell apoptosis. *A*: Morphological evidence of apoptosis (fragmented, shrunk, bright nuclei indicated by arrows) in permeabilized cells stained with propidium iodide in the presence of RNase A (original magnification ×200). *B*: Presence of hypodiploid apoptotic cells (flow cytometry of DNA content indicated by an arrow) among cells exposed to 3,4-DGE for 72 h.**

TABLE 1
3,4-DGE–induced apoptosis was time dependent

	Control	50 μmol/l 3,4-DGE
24 h	1.6 ± 1.01	3.3 ± 0.7
48 h	1.7 ± 1.2	7.9 ± 1.9*
72 h	2.7 ± 1.06	22.1 ± 2.3†

Data are the means ± SD of four independent experiments (% apoptosis). Quantification of flow cytometry results (percentage of hypodiploid cells): time course. *$P < 0.05$, †$P < 0.01$ vs. control.

Confocal microscopy. Cells were plated onto Labtek slides in RPMI with 10% FCS. After 24 h the media was changed to RPMI with 0% FCS, and then cells were incubated for 24 h with 3,4-DGE. Staining with rabbit polyclonal anti-Bax antibody or rabbit polyclonal anti–cytochrome c antibody (1:100; Santa Cruz Biotechnology) was carried out as previously described (23). Cell nuclei were counterstained with propidium iodide.
Caspase-3 activity assay. Caspase-3 activity was measured after the manufacturer's instructions (Biomol, Plymouth, PA) (23). The assay is based on the cleavage of a DEVD-pNA peptide, yielding colored free p-nitroaniline.
Bax antisense oligonucleotide. We used a phosphorothionate-modified antisense oligodeoxynucleotide 5′-TGCTCCCCGGACCCGTCCAT-3′ directed against the translation initiation region of mouse Bax mRNA and a control scrambled oligodeoxynucleotide 5′-TCGTCCGGCCACCGCTCACT-3′, which has little complementarity with Bax mRNA but the same composition as the antisense oligodeoxynucleotide (Metabion, Martinsried, Germany) (23,27). Cells were treated with 50 μmol/l 3,4-DGE for 72 h in the presence or absence of Bax antisense oligodeoxynucleotide (20 μg/ml) or scramble oligodeoxynucleotide (20 μg/ml) (27).
Statistics. Results are expressed as the means ± SD. Significance at the $P < 0.05$ level was assessed by the nonparametric Mann-Whitney test for two groups of data and Kruskal-Wallis for three of more groups by means of the SigmaStat statistical software (Jandel, San Rafael, CA).

RESULTS

3,4-DGE promotes tubular epithelial cell apoptosis.
3,4-DGE induces renal tubular epithelial cell death in a dose- and time-dependent manner (Fig. 1). Morphologic studies in cells permeabilized and stained with propidium iodide demonstrated the presence of shrunk, bright, pyknotic nuclei characteristic of apoptosis among cells exposed to 3,4-DGE (Fig. 2A). Flow cytometry confirmed an increase in the number of hypodiploid apoptotic cells (Fig. 2B). 3,4-DGE–induced apoptosis was time and dose dependent (Tables 1 and 2). An increased rate of apoptosis was already evident at 48 h and peaked at 72 h (Table 1).
Bax oligomerization and cytochrome c release from mitochondria. Next, we studied the molecular mechanisms of apoptosis induced by 3,4-DGE. In the mitochondrial pathway for apoptosis, Bax oligomerizes at mitochondria and promotes the release of caspase activators, such as cytochrome c (6). We observed that 3,4-DGE induced Bax oligomerization that preceded the nuclear apoptotic morphology (Fig. 3A). In addition, cytochrome c was released from mitochondria (Fig. 3B).
Caspases are mediators of tubular cell apoptosis induced by 3,4-DGE. Release of cytochrome c from mitochondria promotes activation of caspase-9 (6).

FIG. 3. Bax oligomerization and release of cytochrome c from mitochondria in 3,4-DGE–induced tubular cell apoptosis. *A*: Bax oligomerizes at mitochondria. Note the formation of aggregates in a cell whose nucleus does not yet display apoptotic features (indicated by an arrow). *B*: Cytochrome c was released from mitochondria in cells exposed to 3,4-DGE. Note the punctuate pattern in control cells and the diffuse cytoplasmic pattern in an apoptotic cell (indicated by an arrow). Cells were treated with 50 μmol/l 3,4-DGE for 24 h. Confocal microscopy (original magnification ×360). Nuclei counterstained with propidium iodide.

Caspase-9 is eventually cleaved and promotes activation by cleavage of other caspases, such as caspase-3. 3,4-DGE resulted in the appearance of cleavage fragments from caspases-3 and -9, as well as a cleavage fragment of the caspase-8 target Bid (Fig. 4A). Caspase-3 is the central executioner caspase. Functional evidence of caspase-3 activation was also observed (sixfold increase in caspase-3 activity) (not shown). A broad-spectrum caspase inhibitor (zVAD-fmk) prevented both caspase-3 activation (not shown) and the development of features of apoptosis (Fig. 4B, *inset*). However, zVAD-fmk did not prevent eventual cell death (Fig. 4B). Specific inhibitors of caspases -2, -3, -8, and -9 also decreased apoptosis induced by 3,4-DGE (Table 3), suggesting the involvement of multiple caspases in a positive feedback loop mode.
Bax is central to cell death induced by 3,4-DGE in tubular epithelium. We then explored the role of Bax in cell death induced by 3,4-DGE in tubular epithelium. We used two independent approaches to inhibit or antagonize Bax. First, the level of expression of Bax was decreased by

TABLE 2
3,4-DGE-induced apoptosis was dose dependent

	Control	3,4-DGE (72 h)			
		1 μmol/l	10 μmol/l	25 μmol/l	50 μmol/l
Apoptosis (%)	3.5 ± 0.8	3.6 ± 1.05	4.7 ± 1.1	7.1 ± 2.8*	22.8 ± 1.3†

Data are the means ± SD of four independent experiments. Quantification of flow cytometry results: dose response at 72 h. *$P < 0.05$, †$P < 0.01$ vs. control.
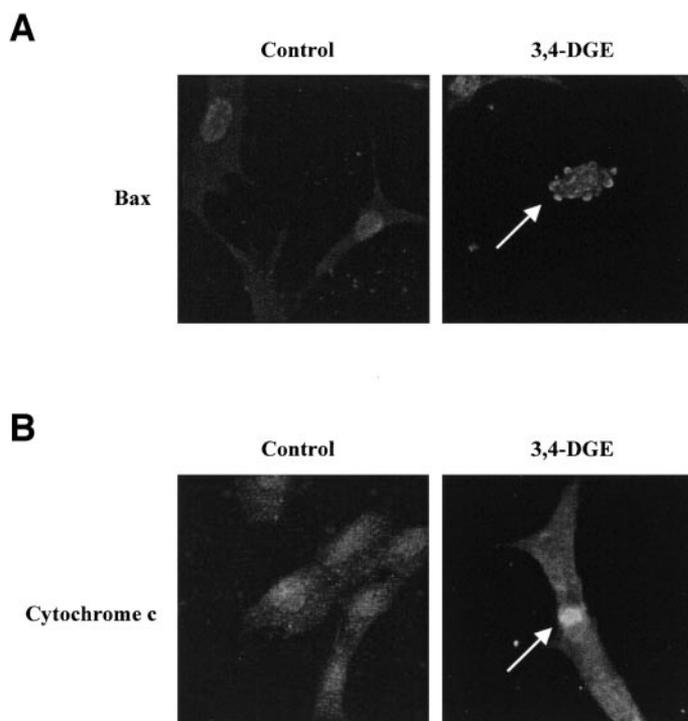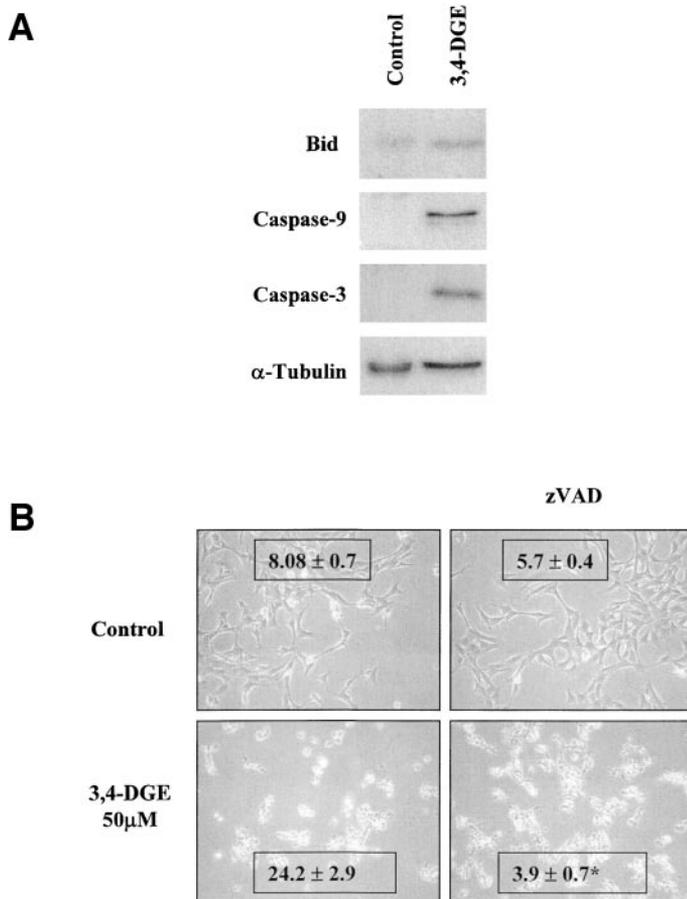
A

B

FIG. 4. 3,4-DGE–induced tubular cell apoptosis is caspase dependent, but caspases do not prevent eventual cell death. *A*: Bid and caspase-3 and -9 are processed in the course of 3,4-DGE–induced apoptosis (Western blot). *B*: However, zVAD-fmk failed to prevent eventual cell death. Contrast phase microscopy is shown (original magnification ×200). In all experiments cells were treated with 50 μmol/l 3,4-DGE for 72 h. Note the discrepancy between the low percentage of apoptosis and the fact that 3,4-DGE/zVAD cells are rounded and detached in the same manner as 3,4-DGE cells. Percentage of apoptosis (flow cytometry of DNA content) is presented as insets and expressed as the means ± SD of four independent experiments. *$P < 0.05$ vs. 3,4-DGE.

Bax antisense oligonucleotides (23). Western blot confirmed that antisense oligonucleotides decrease Bax protein levels (not shown) (23). Decreased Bax expression protected from both apoptosis and cell death (Fig. 5). Then, Bax function was antagonized by a Ku-70–derived Bax antagonistic peptide (25). This intervention also resulted in decreased apoptosis at 72 h (3,4-DGE: 23.7 ± 4.4%; 3,4-DGE/Ku70 peptide: 9.7 ± 2.5%, $P < 0.05$ vs. 3,4-DGE; 3,4-DGE/control peptide: 20.7 ± 2.9%) and cell death (not shown).

DISCUSSION

We have shown that the glucose degradation product 3,4-DGE promotes apoptosis in cultured tubular epithelial cells through the activation of a Bax-mediated pathway. 3,4-DGE had initially been isolated from a brown seaweed with immunosuppressive activity in vivo (28). 3,4-DGE had marked suppressive effects on T-cell proliferation, antibody production from B cells, and interleukin-1 production from macrophages (28). 3,4-DGE was also the main cytotoxic compound from glucose-containing peritoneal dialysis fluids (17). Induction of leukocyte apoptosis may underlie both the cytotoxicity observed in the peritoneal dialysis system and the immunosuppressive properties (24).

The current study expands the range of cellular targets of 3,4-DGE to renal tubular epithelial cells and unravels the molecular mechanisms underlying its lethal potential. Information was gathered on the molecular pathways of 3,4-DGE–induced apoptosis. Bax appears to be a central mediator of cell death in this system. A stimulus-specific role of Bax in tubular epithelial cell apoptosis had previously been described. Bax is required for cyclosporine A–induced apoptosis, but it is not implicated in death promoted by paracetamol (23,29). In addition, Bax had previously been implicated in apoptosis found in a diabetic milieu. Indeed high glucose–induced blastocyst apoptosis does not take place in the absence of Bax (10). Our results suggest that either high glucose and 3,4-DGE share similar intracellular death pathways, or that high ambient glucose increases intracellular 3,4-DGE levels, and this leads to apoptosis. After recruitment of Bax to the mitochondria, cytochrome c is released and the caspase cascade activated. Inhibition studies suggest that in apoptosis induced by 3,4-DGE, multiple caspases are activated and that individual inhibition of any of them is enough to prevent apoptosis. Previous experience with caspase inhibitors in this cell line argues against nonspecific inhibition of multiple caspases by a single caspase inhibitor because different patterns of protection by caspase inhibitors were observed in a stimulus-specific fashion (23,29). The pattern of protection offered by caspase inhibitors is similar to that observed in another form of Bax-mediated cell death induced by cyclosporine A (23). However, contrary to Bax inhibition, caspase inhibition did not prevent eventual cell death induced by 3,4-DGE. This suggests that Bax may be a more significant target for eventual therapeutic intervention and that once the mitochondria has been injured, caspase-independent lethal pathways are activated (30).

The current experimental data may be relevant to two clinical situations: peritoneal dialysis and diabetic nephropathy. Glucose degradation products are thought to

TABLE 3
Specific inhibitors of caspase-2, -3, -8, and -9 decreased apoptosis induced by 3,4-DGE

	Vehicle	z-VDVAD-fmk	DEVD-fmk	IETD-fmk	LEHD-fmk
Control	10.5 ± 1.1	11.2 ± 1.3	11.4 ± 1.5	8.2 ± 2.1	8.4 ± 1.3
3,4-DGE 72 h	25.4 ± 2.1	7.6 ± 1.01*	17.5 ± 3.0†	6.6 ± 1.1*	5.8 ± 1.08*

Data are the means ± SD of four independent experiments (% apoptosis). Hypodiploid cells were quantified by flow cytometry. In all experiments cells were treated with 50 μmol/l 3,4-DGE for 72 h. *$P < 0.05$ vs. 3,4-DGE alone; †$P < 0.08$ vs. 3,4-DGE alone. z-VDVAD-fmk, benzyloxycarbonyl-Val-Asp-Val-Ala-Asp-fluoromethylketone.

FIG. 5. Role of Bax in 3,4-DGE–induced tubular cell apoptosis. Bax depletion by antisense oligonucleotides prevents 3,4-DGE–induced cell death. Contrast phase microscopy is shown (original magnification ×200). In all experiments cells were treated with 50 μmol/l 3,4-DGE for 72 h. Percentage of apoptosis (flow cytometry of DNA content) is presented as insets and expressed as the means ± SD of four independent experiments. *$P < 0.05$ vs. 3,4-DGE. ODN, oligodeoxynucleotide.

be pivotal mediators of the poor biocompatibility of peritoneal dialysis solutions. Peritoneal dialysis solutions may have local (peritoneal) and systemic adverse effects as a consequence of poor biocompatibility. There is evidence that glucose degradation products from peritoneal dialysis fluid enter the systemic circulation, where they promote an increase in plasma advanced glycation end product compounds (13,14) and may influence appetite (12). More recently, a randomized clinical trial reported that glucose degradation product–poor peritoneal dialysis solutions better preserve residual renal function in peritoneal dialysis patients than traditional glucose degradation product–rich peritoneal dialysis solutions (14). 3,4-DGE is the most cytotoxic glucose degradation product in peritoneal dialysis fluids, so it is conceivable that it plays a role in different aspects of the bioincompatibility of these solutions. We hypothesize that the nephrotoxic potential of 3,4-DGE may have contributed to the results observed by Williams et al. (14). The concentration of 3,4-DGE in peritoneal dialysis bags may be as high as 125 μmol/l in 1.5% glucose peritoneal dialysis solutions and even higher in 4.25% glucose bags (17,21). Although the plasma concentration of 3,4-DGE after infusion of bioincompatible peritoneal dialysis solutions is not currently known, the toxic range in cultured cells is relevant to the concentrations found in peritoneal dialysis fluids and the expected passage of the molecule to the organism from the dyalisate.

A role for 3,4-DGE in diabetes complications should also be explored. The concentration of several glucose degradation products (e.g., 3-DG) is increased in diabetic individuals (31,32). 3-DG levels are well correlated with plasma glucose and HbA$_{1c}$ levels (18). In addition, fasting serum 3-DG levels were higher in diabetic patients suffering from more severe evidence of diabetic kidney microangiopathy (19). 3-DG concentration is severalfold higher inside the cell than in the extracellular milieu (20). Intracellular 3-DG is also correlated with glycemic control in diabetes (20). 3,4-DGE is generated nonenzymatically from dehydration of 3-DG (Fig. 6). The reaction is reversible, and equilibrium between these two compounds exists. Heat moves the equilibrium toward 3,4-DGE. For example, raising the temperature from 30 to 40°C increases 3,4-DGE concentration by 50% (21). It could be speculated that in vivo factors such as fever may tilt the balance toward generation of 3,4-DGE. Although the serum or tissue levels of 3,4-DGE in diabetic patients are not known, the hypothesis that high 3,4-DGE levels may be generated systemically or locally in tissues in diabetic patients with poor glycemic control and/or fever merits further testing. Indeed, there are reports that acute renal failure may be more frequent in diabetic patients with bacteriemia than in nondiabetic individuals (33)

In summary, 3,4-DGE, a glucose degradation product that may be absorbed from the peritoneum during peritoneal dialysis or produced locally inside the cell in diabetic individuals, promotes apoptosis in renal tubular epithelial cells. The intracellular molecular pathways require Bax in a similar manner as glucose-induced apoptosis in cells such as those in the blastocyst (10). The hypothesis that 3,4-DGE may participate in the pathogenesis of diabetic renal injury should be further explored.

FIG. 6. Chemical structure of 3-DG and 3,4-DGE (21).

REFERENCES

1. Barnes DJ, Pinto JR, Viberti GC: The patient with diabetes mellitus. In *Oxford Textbook of Clinical Nephrology*. 2nd ed. Davison AM, Cameron JS, Grunfeld JP, Kerr DNS, Ritz E, Eds. Oxford, U.K., Oxford University Press, 1998, p. 723–775

2. Ginevri F, Picotti E, Alinovi R, DeToni T, Biagini C, Chiggeri GM, Gusmano R: Reversible tubular proteinuria precedes microalbuminuria and correlates with the metabolic status in diabetic children. *Pediatr Nephrol* 7:23–26, 1993

3. Mangano CM, Diamondstone LS, Ramsay JG, Aggarwal A, Herskowitz A, Mangano DT: Renal dysfunction after myocardial revascularization: risk factors, adverse outcomes, and hospital resource utilization: the Multicenter Study of Perioperative Ischemia Research Group. *Ann Intern Med* 128:194–203, 1998

4. Solez K, Morel-Maroger L, Sraer JD: The morphology of "acute tubular necrosis" in man: analysis of 57 renal biopsies and a comparison with the glycerol model. *Medicine* (*Baltimore*) 58:362–376, 1979

5. Olsen TS, Olsen HS, Hansen HE: Tubular ultrastructure in acute renal failure in man: epithelial necrosis and regeneration. *Virchows Arch A Pathol Anat Histopathol* 406:75–89, 1985

6. Ortiz A: Apoptotic regulatory proteins in renal injury. *Kidney Int* 58:467–485, 2000

7. Thomas GL, Yang B, Wagner BE, Savill J, El Nahas AM: Cellular apoptosis and proliferation in experimental renal fibrosis. *Nephrol Dial Transplant* 13:2216–2226, 1998

8. Ortiz A, Ziyadeh FN, Neilson EG: Expression of apoptosis-regulatory genes in renal proximal tubular epithelial cells exposed to high ambient glucose and in diabetic kidneys. *J Invest Med* 45:50–56, 1997

9. Baumgartner-Parzer SM, Wagner L, Pettermann M, Grillari J, Gessl A, Waldhausl W: High-glucose–triggered apoptosis in cultured endothelial cells. *Diabetes* 44:1323–1327, 1995

10. Moley KH, Chi MM, Knudson CM, Korsmeyer SJ, Mueckler MM: Hyperglycemia induces apoptosis in pre-implantation embryos through cell death effector pathways. *Nat Med* 4:1421–1424, 1998

11. Catalán MP, Reyero A, Egido J, Ortiz A. Acceleration of neutrophil apoptosis by glucose-containing peritoneal dialysis solutions: role of caspases. *J Am Soc Nephrol*; 12:2442–2449, 2001

12. Zheng ZH, Anderstam B, Qureshi AR, Heimburger O, Wang T, Sodersten P, Bergstrom J, Lindholm B: Heat sterilization of peritoneal dialysis solutions influences ingestive behavior in non-uremic rats. *Kidney Int* 62:1447–1453, 2002

13. Zeier M, Schwenger V, Deppisch R, Haug U, Weigel K, Bahner U, Wanner C, Schneider H, Henle T, Ritz E: Glucose degradation products in PD fluids: do they disappear from the peritoneal cavity and enter the systemic circulation? *Kidney Int* 63:298–305, 2003

14. Williams JD, Topley N, Craig KJ, Mackenzie RK, Pischetsrieder M, Lage C, Passlick-Deetjen J, the Euro Balance Trial Group: The Euro-Balance Trial: the effect of a new biocompatible peritoneal dialysis fluid (balance) on the peritoneal membrane. *Kidney Int* 66:408–418, 2004

15. Wieslander AP, Andren AH, Nilsson-Thorell C, Muscalu N, Kjellstrand PT, Rippe B: Are aldehydes in heat-sterilized peritoneal dialysis fluids toxic in vitro? *Perit Dial Int* 15:348–352, 1995

16. Witowski J, Korybalska K, Wisniewska J, Breborowicz A, Gahl GM, Frei U, Passlick-Deetjen J, Jorres A: Effect of glucose degradation products on human mesothelial cell function. *J Am Soc Nephrol* 11:729–739, 2000

17. Linden T, Cohen A, Deppisch R, Kjellstrand P, Wieslander A: 3,4-dideoxyglucosone-3-ene (3,4-DGE): a cytotoxic glucose degradation product in fluids for peritoneal dialysis. *Kidney Int* 62:697–703, 2002

18. Hamada Y, Nakamura J, Fujisawa H, Yago H, Nakashima E, Koh N, Hotta N: Effects of glycemic control on plasma 3-deoxyglucosone levels in NIDDM patients. *Diabetes Care* 20:1466–1469, 1997

19. Kusunoki H, Miyata S, Ohara T, Liu BF, Uriuhara A, Kojima H, Suzuki K, Miyazaki H, Yamashita Y, Inaba K, Kasuga M: Relation between serum 3-deoxyglucosone and development of diabetic microangiopathy. *Diabetes Care* 26:1889–1894, 2003

20. Tsukushi S, Kajita M, Nakamura S, Niwa T: Gas chromatographic-mass spectrometric determination of erythrocyte 3-deoxyglucosone in diabetic patients. *J Chromatogr B Analyt Technol Biomed Life Sci* 776:133–137, 2002

21. Erixon M, Linden T, Kjellstrand P, Carlsson O, Ernebrant M, Forsback G, Wieslander A, Jonsson JA: PD fluids contain high concentrations of cytotoxic GDPs directly after sterilization. *Perit Dial Int* 24:392–398, 2004

22. Haverty TP, Kelly CJ, Hines WH, Amenta PS, Watanabe M, Harper RA, Kefalides NA, Neilson N: Characterization of a renal tubular epithelial cell line which secretes the autologous target of autoimmune experimental interstitial nephritis. *J Cell Biol* 107:1359–1368, 1988

23. Justo P, Lorz C, Sanz A, Egido J, Ortiz A: Intracellular mechanisms of cyclosporine A-induced tubular cell apoptosis. *J Am Soc Nephrol* 14:3072–3080, 2003

24. Catalan MP, Santamaria B, Reyero A, Egido E, Ortiz A: 3,4-di-deoxyglucosone-3-ene promotes leukocyte apoptosis. *Kidney Int*. In press

25. Sawada M, Hayes P, Matsuyama S: Cytoprotective membrane-permeable peptides designed from the Bax-binding domain of Ku70. *Nat Cell Biol* 5:352–357, 2003

26. Lorz C, Ortiz A, Justo P, González-Cuadrado S, Duque N, Gómez-Guerrero C, Egido J: Proapoptotic Fas ligand is expressed by normal kidney tubular epithelium and injured glomeruli. *J Am Soc Nephrol* 11:1266–1277, 2000

27. Shou Y, Li N, Li L, Borowitz JL, Isom GE: NF-κB-mediated up-regulation of Bcl-Xs and Bax contributes to cytochrome c release in cyanide-induced apoptosis. *J Neurochem* 81:842–852, 2002

28. Kato F, Mizukoshi S, Aoyama Y, Matsuoka H, Tanaka H, Nakamura K, Tsukamoto M: Immunosuppressive effects of 3,4-dideoxyglucosone-3-ene, an intermediate in the Maillard reaction. *J Agric Food Chem* 42:2068–2073, 1994

29. Lorz C, Justo P, Sanz A, Subirá D, Egido J, Ortiz A: Paracetamol nephrotoxicity: a role for ER stress. *J Am Soc Nephrol* 15:380–389, 2004

30. Saelens X, Festjens N, Vande Walle L, van Gurp M, van Loo G, Vandenabeele P: Toxic proteins released from mitochondria in cell death. *Oncogene* 23:2861–2874, 2004

31. Phillips SA, Mirrlees D, Thornalley PJ: Modification of the glyoxalase system in streptozotocin-induced diabetic rats: effect of the aldose reductase inhibitor Statil. *Biochem Pharmacol* 46:805–811, 1993

32. Eriksson UJ, Wentzel P, Minhas HS, Thornalley PJ: Teratogenicity of 3-deoxyglucosone and diabetic embryopathy. *Diabetes* 47:1960–1966, 1998

33. Carton JA, Maradona JA, Nuno FJ, Fernandez-Alvarez R, Perez-Gonzalez F, Asensi V: Diabetes mellitus and bacteraemia: a comparative study between diabetic and non-diabetic patients. *Eur J Med* 1:281–287, 1992