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Differential impact of glucose levels and advanced glycation end-products on tubular cell viability and pro-inflammatory/profibrotic functions

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ABSTRACT

High glucose (HG) or synthetic advanced glycation end-products (AGE) conditions are generally used to mimic diabetes in cellular models. Both models have shown an increase of apoptosis, oxidative stress and pro-inflammatory cytokine production in tubular cells. However, the impact of the two conditions combined has rarely been studied. In addition, the impact of glucose level variation due to cellular consumption is not clearly characterized in such experiments. Therefore, the aim of this study was to compare the effect of HG and AGE separately and of both on tubular cell phenotype changes in the HK2 cell line. Moreover, glucose consumption was monitored every hour to maintain the glucose level by supplementation throughout the experiments. We thus observed a significant decrease of apoptosis and H_2O_2 production in the HK2 cell. HG or AGE treatment induced an increase of total and mitochondrial apoptosis as well as TGF-β release compared to control conditions; however, AGE or HG led to apoptosis preferentially involving the mitochondria pathway. No cumulative effect of HG and AGE treatment was observed on apoptosis. However, a pretreatment with RAGE antibodies partially abolished the apoptotic effect of HG and completely abolished the apoptotic effect of AGE. In conclusion, tubular cells are sensitive to the lack of glucose as well as to the HG and AGE treatments, the AGE effect being more deleterious than the HG effect. Absence of a potential synergistic effect of HG and AGE could indicate that they act through a common pathway, possibly via the activation of the RAGE receptors.

1. Introduction

Diabetic nephropathy (DN) remains the main cause of end-stage renal disease and is still a challenge [1], affecting vascular, glomerular and tubular components. Several pathways are studied in glomerular cells involving polyol and hexosamine pathways [2], such as oxidative stress [3]. Nonenzymatic linkage of glucose with amino groups leads to advanced glycation end-products (AGE), whose levels increase in diabetes [4] and chronic kidney diseases [5]. They are responsible for oxidative stress by inducing reactive oxygen species (ROS) production. ROS production is triggered by PKC, NADPH oxydase (NOX) or mitochondrial activation [6]. Furthermore, AGE and HG induce *TGF-β1* activation and ROS, leading to increased connective tissue growth factor [7], followed by extracellular matrix expansion, epithelial–mesenchymal transition and tubular atrophy [8]. Tubular injuries in DN have only recently been investigated and seem to be preferentially associated with the loss of kidney function rather than glomerular lesions [9].

Two *in vitro* conditions are currently available to mimic diabetes: high glucose (HG) and AGE exposure. However, only few studies have analyzed the effect of HG and AGE together. The effects of

Abbreviations: AGE, advanced glycation end-products; AGE-BSA, glycated bovine serum albumin; BSA, bovine serum albumin; DHR, Dihydrorhodamine; DIOC6, 3,3′-dihexyloxacarbocyanine iodide; DMEM, Dulbecco's minimum essential medium; DN, diabetic nephropathy; GLUT, glucose transporter; HG, high glucose condition; KSFM, keratinocyte-serum free medium; LG, low-glucose condition; M, Mannitol condition; NOX, NADPH oxidase; RAGE, receptor for advanced glycation end-product; ROS, reactive oxygen species; SGLT2, sodium/glucose co-transporter 2; TGF β, transforming growth factor beta.

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AGE involve its extracellular accumulation [10] and intracellular pathway activation mainly through the activation of the receptor for advanced glycation end-products (RAGE) [11]. An AGE increase may be driven by an excess of its intake or an increase of its synthesis. In addition, a high glucose level in culture medium leads to extracellular synthesis of AGE through the Maillard reaction as well as the intracellular production of methylglyoxal. Thus the HG condition may lead to changes in the tubular cell phenotype by a proper effect or by a local AGE synthesis effect and RAGE axis stimulation. On the other hand, the glucose level decrease during cell culture may induce a "hypoglycemic side-effect" [12], hypoglycemia being clinically defined as a blood glucose level below 2.8 mmol/L [13]. Therefore, this study was conducted (1) to quantify the glucose decrease during HK-2 cell culture and to analyze its impact on cellular survival in order to control the glucose level by supplementation and (2) to compare the HG, AGE and HG + AGE impacts on apoptosis, oxidative stress and the inflammatory response of HK2 cells in culture.

2. Material and methods

2.1. Cell culture and reagents

HK-2 cells, a proximal tubular cell line immortalized by HPV-16, were obtained from American Type Cell Collection (Molsheim, France), expended in keratinocyte-SF medium supplemented with L-glutamine, 5 ng/mL epidermal growth factor and 50 μg/mL bovine pituitary extract (from Invitrogen; Saint Aubin, France), at 37 °C, 5% CO_2. At 80% confluence, cells were growth-arrested for 24 h in serum-free Dulbecco's MEM (DMEM) from Sigma–Aldrich (St-Quentin-Falavier, France). Growth-arrested cells were exposed to the following conditions up to 72 h: KSFM as a control experiment or DMEM that contains 5.6 mmol/L glucose or without further supplementation for low-glucose experiments (LG) or with 25 mmol/L D-mannitol (M) or 25 mmol/L D-glucose for high-glucose conditions (HG) or 100 mg/L bovine serum albumin (BSA) from Sigma–Aldrich, 100 mg/L AGE-BSA (AGE) or 25 mmol/L glucose and 100 mg/L AGE-BSA (AGE+).

2.2. Advanced glycation end-product (AGE) synthesis

As previously described [14], AGEs were produced by incubating 20 mg/mL of BSA with 0.1 M D-glyceraldehyde in 0.2 M phosphate buffer for 12 days. Validation of BSA transformation was assessed by fluorimetry following the appearance of AGE (λ_{ex} 340 nm, λ_{em} 440 nm) and the loss of BSA (λ_{ex} 280 nm, λ_{em} 340 nm) (Varian Cary Eclipse Agilent Technologies, les Ullis, France).

2.3. Glucose supplementation

Glucose concentration was monitored in 50 μL of medium samples. For all analyses, except in experiments without supplementation, 280 mmol/L glucose in DMEM were added every 12 h to obtain a final glucose concentration of 6 mmol/L.

2.4. Apoptosis assay by flow cytometry

Annexin assay (total apoptosis): After trypsin treatment, 10^5 cells were stained with FITC-labeled annexin V and propidium iodide (PI) according to the supplier's recommendations (FITC-annexin V apoptosis detection kit from BD Pharmingen, Rungis, France). Cells positive for annexin V and negative for PI were considered as apoptotic cells.

DIOC6 assay: After trypsin treatment, 10^5 cells were incubated in DMEM with 0.1 μM 3,3′-dihexyloxacarbocyanine iodide (DIOC6) (Sigma–Aldrich) at 37 °C for 30 min [15]. Cells with a loss of fluorescence were counted as apoptotic cells. For each assay, data were collected by flow cytometry (FACS Canto II flow cytometer (Becton Dickinson, San Jose, CA, USA) from 10,000 events and analyzed using Diva® software (BD Biosciences, San Jose, CA, USA).

2.5. Measurement of the pro-apoptotic factor TGF-β1

After exposure of HK-2 cells to the different conditions, culture medium was collected without cell detachment and centrifuged. The supernatant was collected and TGF-β1 concentrations in supernatants were assessed using a colorimetric ELISA kit according to the supplier's recommendations (Quantikine Human *TGF-β1* immunoassay Lille, France). RAGE inhibition was performed with 5 μg/mL RAGE antibody (R&D System, Lille, France), incubated for 2 h before exposing the cells to the above-described different conditions.

2.6. Measurement of H_2O_2 production

Total H_2O_2 production by HK2 cells was measured using the fluorescent probe dihydrorhodamine (DHR, Anaspec, Fremont, France) as described previously [16]. For each assay, data were collected using flow cytometry from 10,000 events and analyzed using Diva® software (same material as for apoptotic assay).

2.7. Statistical analysis

Experiments were evaluated using the Wilcoxon test according to the replicates analyzed and are presented as the median (interquartile). Analyses were performed with *r* software. For all statistical analyses, a p-value < 0.05 was considered significant.

3. Results

3.1. Glucose consumption monitoring during HK-2 cells cultured in LG or HG conditions

The glucose concentration was monitored hourly in the culture supernatant. The glucose concentration decreased linearly in LG (Fig. 1). The same curve slopes were observed between conditions 0.25 mmol/L h^{-1} for LG (r^2 0.995) versus 0.26 mmol/L h^{-1} for HG (R^2 = 0.882), 0.28 mmol/L h^{-1} (r^2 = 0.899) for AGE, and 0.29 mmol/L h^{-1} (r^2 = 0.828) for BSA. However, under the HG condition the decrease was no longer linear but oscillated. The hypoglycemic threshold for plasmatic measurement in humans is defined by a glucose concentration in plasma lower than 2.8 mmol/L. With an initial glucose concentration of 5.6 mmol/L in the culture medium, HK-2 cells reached this "hypoglycemic" threshold after 12 h of culture and no glucose could be detected after 24 h.

3.2. Effect of glucose supplementation on apoptosis and ROS production in HK-2 cells cultured in LG or HG conditions

We analyzed apoptosis and H_2O_2 production of HK-2 cells with or without 12-h supplementation with glucose in LG or HG conditions (Fig. 2). Significant early and sustained H_2O_2 production was observed in control nonsupplemented conditions (p = 0.1 at 24 h, 0.03 at 48 h and 0.009 at 72 h for LG with supplementation versus without) (Fig. 2A). Glucose supplementation significantly reduced the ROS production observed in LG conditions at 24, 48 and 72 h of exposure. Glucose supplementation also protected HK-2 cells

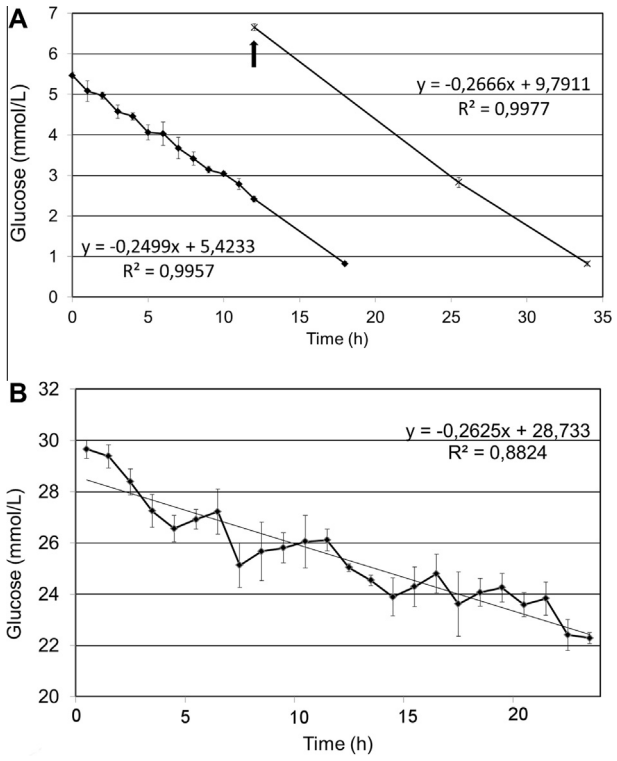

Fig. 1. Glucose consumption by HK-2 cells in LG or HG conditions. In the T25 plate, 0.5×10^6 cells were seeded, and after 80% confluence, cells were exposed to DMEM in LG conditions – 5.6 mmol/L glucose (A) – and HG conditions – 30 mmol/L glucose (B). 50-μL samples were assessed every hour for glucose concentration. The black arrow indicates glucose supplementation to obtain a final concentration of 6 mmol/L glucose in the medium. Error bars indicate the SEM from four independent experiments.

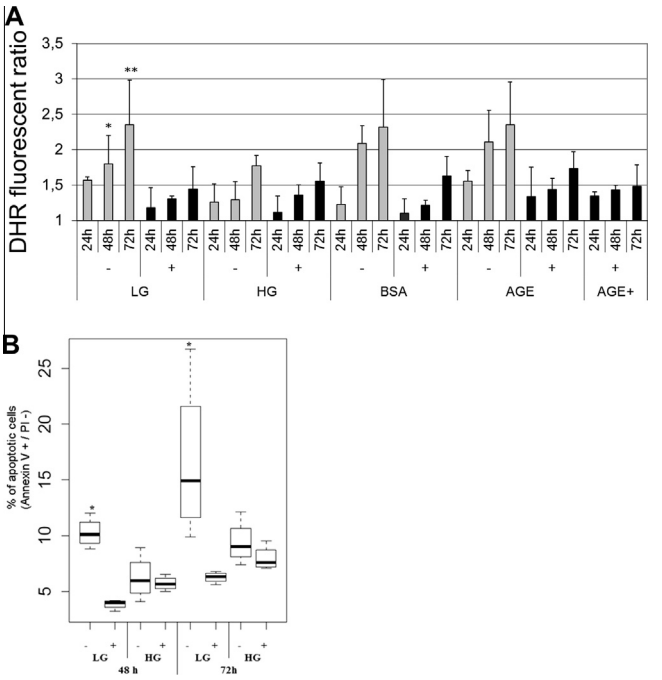

Fig. 2. Impact of glucose supplementation on apoptosis and ROS production by HK-2 cells in LG or HG conditions. HK-2 cells were seeded at 0.5×10^6 in T25 and after 80% confluence, cells were growth-arrested in FBS-free DMEM for 24 h and then exposed to LG or HG conditions. A supplementation of glucose, with 280 mmol/L glucose in DMEM solution, was made (+) every 12 h or not (−) for 48 or 72 h. H_2O_2 production was measured in presence of DHR (A) and total apoptosis was analyzed after annexin V and PI labeling (B). For each assay, data were collected by flow cytometry from 10,000 events. Error bars indicate the SEM from four independent experiments, $^*p < 0.05$, $^{**}p < 0.01$. For + versus − condition, results are shown as the median (interquartile).

against apoptosis in LG conditions only [4% versus 10% ($p = 0.03$) at 48 h and 6.3% versus 25% at 72 h ($p = 0.03$)] (Fig. 2B). However, there was no significant effect of supplementation in HK-2 cells under HG conditions (Fig. 2).

3.3. Global and mitochondrial apoptosis of HK-2 cells after 48 h exposure to different diabetic conditions

Global and mitochondrial apoptosis of HK-2 cells measured by flow cytometry with annexin V/PI and DIOC6 probes, respectively, were evaluated under different conditions mimicking diabetes. A greater apoptosis percentage was found at 48 h in HG conditions compared to mannitol conditions with both annexin (5.45% for HG versus 3.9% for mannitol) and DIOC6 probes (18.95% for HG versus 13.3% for mannitol) ($p < 0.005$). AGE and AGE+ treatments for 48 h induced greater global apoptosis (annexin V) (7.6% and 7.3%, respectively) compared to the BSA treatment alone (5.25%, $p < 0.01$). The same effect of AGE and AGE+ on the mitochondrial apoptosis level (DIOC6) was observed compared to the BSA condition (20.2% and 20.7% versus 14.95% ($p < 0.01$)) (Fig. 3). Addition of high glucose to AGE (AGE+ condition) did not modify annexin V and DIOC6 apoptosis. In addition, AGE and AGE+ conditions induced higher global apoptosis than HG alone ($p = 0.007$ for AGE+ and 0.0003 for AGE), but not on mitochondrial apoptosis. Finally, the increase of the apoptosis ratio (defined as the condition/specific control ratio) was around 30–35% in diabetic conditions (AGE/BSA or HG/M) and was the same for global or mitochondrial apoptosis. RAGE blockade by antibody pretreatment reduced AGE-induced apoptosis ($p < 0.01$ for both DIOC6 and annexin V/PI versus BSA) (Fig. 3), whereas it decreased only partially HG-induced apoptosis ($p = 0.002$ for DIOC6, $p = 0.1$ for annexin V/PI versus M).

3.4. TGF-β release and oxidative stress by HK-2 cells under diabetic conditions

After 48 h exposure, TGF-β1 release in the culture medium by HK2 cells was higher after HG than mannitol treatment (138 pg/ml versus 78 pg/ml, $p = 0.03$). In addition, in AGE+ or AGE treatment for 48 h, a significant increase of TGF-β1 release was observed compared to the BSA condition (210 pg/ml and 191 pg/ml versus 129 pg/ml, $p = 0.03$) (Fig. 4). This was also observed after 72 h of exposure with AGE and AGE+. We did not find any differences in TGF-β1 concentrations between M and LG conditions, whereas it increased with BSA exposure ($p = 0.03$). In addition, AGE and AGE+ induced the same but higher TGF-β1 release than HG. We did not find any significant differences in H_2O_2 production after 48 h of exposure in all the conditions tested.

4. Discussion

The impact of glucose uptake by tubular cells and pathways leading to interstitial fibrosis and tubular atrophy are poorly understood. In the present experiments, slopes of glucose levels in culture media are the same between HG and LG. However, the kinetics are not comparable: we observed a continuous decrease with an initial glucose concentration of 5.6 mmol/L, whereas an oscillating curve with a 30-mmol/L initial glucose concentration was obtained. Two transporters act in proximal tubular cells: GLUT2 is a facilitative and bidirectional insulin-independent

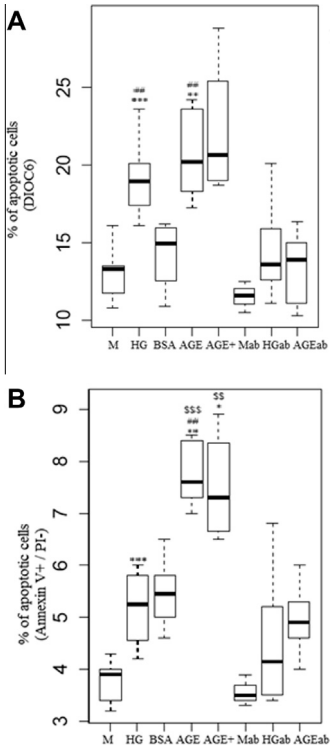

Fig. 3. Global and mitochondrial apoptosis of HK-2 cells after 48 h exposure to different diabetic conditions, HK-2 cells were seeded at 0.5×10^6 in T25 and after 80% confluence, cells were growth-arrested in FBS-free DMEM for 24 h. Then cells were exposed to different conditions for 48 h; KSFM medium as control (C), 25 mmol/L D-mannitol and 5.6 mmol/L glucose (M); 30 mmol/L D-glucose (HG); 100 mg/L BSA (BSA); 100 mg/L AGE-BSA (AGE); 30 mmol/L glucose and 100 mg/L AGE-BSA (AGE+), 30 mmol/L D-glucose and a preincubation with RAGE antibodies (HGab), 100 mg/L AGE-BSA and a preincubation with RAGE antibodies (AGEab). Mitochondrial apoptosis (A) was measured in presence of the DIOC6 probe, and global apoptosis (B) by annexin V/Pi labeling by flow cytometry. For each assay, data were collected by flow cytometry from 10,000 events. Error bars indicate the SEM from five independent experiments. Results are shown as median (interquartile). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$ versus M for HG, versus BSA for AGE and AGE+; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus RAGE. Antibody condition; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$ versus HG.

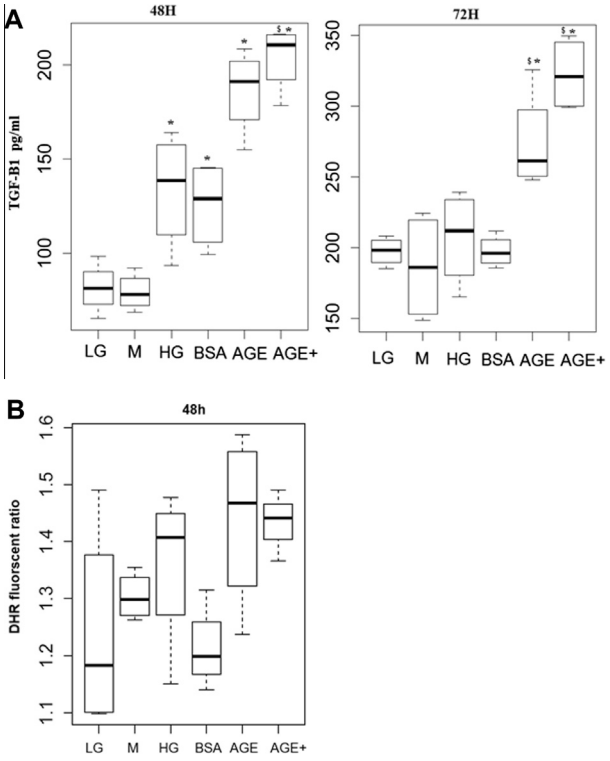

Fig. 4. TGF β1 and H_2O_2 production by HK-2 cells exposed to different diabetic conditions HK-2 cells were seeded at 0.5×10^6 in T25 and after 80% confluence, cells were growth-arrested in FBS-free DMEM for 24 h. Then cells were exposed to different conditions as for the apoptosis assay for 48 or 72 h. A: TGF-β1 level in supernatant of HK-2 cells was assessed as described in Section 2. (B) H_2O_2 production in supernatant of HK-2 cells was assessed by flow cytometry using DHR probe. For each assay, data were collected by flow cytometry from 10,000 events. Error bars indicate the SEM from five independent experiments. Results are shown as median (interquartile). $^{*}p < 0.05$ versus M for HG or versus BSA for AGE and AGE+, $^{\$}p < 0.05$ versus HG.

transporter and SGLT2 drives active uptake of glucose [17]. We assume that when HK-2 cells are in LG, glucose uptake could be mediated by GLUT2 and SGLT2 in the same manner. Indeed in HG an active entrance of glucose through SGLT2 could be maintained, whereas an alternative influx/efflux of glucose through GLUT2 could explain the steady-state phases. Studies of glucose consumption with the blockade of SGLT2 or GLUT2 or both will be useful to understand these molecular kinetic differences.

Then we wished to highlight the side effect of the "hypoglycemia-like condition" (levels of glucose < 2.8 mmol/L) on HK2 cell culture. We found severe cell injuries (increase of apoptosis and H_2O_2 production) that could bias functional studies in these cells. This sensitivity to the decrease in glucose may support the impact of frequent hypoglycemia episodes on progressive loss of renal function and patient prognosis as evidenced by recent clinical trials [18]. However, in our cell model, low glucose lasted for long periods (>12 h) and only animal models can reproduce frequent short-term hypoglycemia.

The glucose consumption values measured in our study are close to what is found in the literature [12,19]. Indeed two methods were proposed to thwart the low-glucose effect: by increasing the initial glucose concentration (>12 mmol/L) or by frequently refreshing the medium. We suggest adding a small volume of high-concentrated glucose solution to maintain the glucose concentration above 2.8 mmol/L without adding a significant amount of fresh medium (1% of total volume). The potential bias control conditions used in previous studies can thus be avoided. This method avoids increasing the initial glucose concentration (far from physiology) or frequently refreshing the medium (then clearance of secreted mediators) previously used.

HG and AGE models are often studied separately in the literature. AGE induces significantly higher global apoptosis than HG. The greater impact of AGE compared to HG may be explained by BSA and AGE cumulative toxicity on proximal tubular cells, instead of BSA toxicity alone [19]. This is supported by the fact that RAGE blockade in the AGE condition by specific antibodies decreases the level of apoptosis, as under the BSA condition but not the mannitol condition. TGF-β1 is known to be produced in either the HG or AGE condition [20]. Previous studies have evidenced a very early effect of AGE on TGF-β1 release from tubular cells in 4–24-h exposure but not for longer exposure times [21–22]. On the contrary, a significant increase in TGF-β1 under HG conditions was seen after 12–72 h [8,23–25], but not earlier. We confirmed that the increase of TGF-β1 was greater in AGE than HG. The earlier effect of AGE compared to HG could be explained by differential activation pathways. AGE could act mainly through RAGE, whereas the HG effect could use the polyol pathway or late RAGE activation caused by glucose-induced glycation of proteins. This underlines the need to develop other glycation products than BSA-AGE in proximal tubular cell models.

We observed for the first time the effect of HG and AGE used at the same time on a proximal tubular cell line and found no evidence of an additive effect. Therefore, we hypothesized that AGE and HG pathways use a common limiting step. Under HG

conditions, AGE could be synthesized *de novo*. However, direct glycation by glucose level is probably negligible because *in vitro* synthesis of BSA-AGE by glucose exposure requires more than 4 weeks [26]. Thus in HG conditions, part of glucose could serve for methylglyoxal synthesis, which is a highly reactive polysaccharide like glyceraldehyde, and requires less time to create AGE (7 days). Therefore, the amount of AGE produced after 48 h of HG exposure is probably low, explaining the lower effect of HG observed compared to AGE treatment. In addition, RAGE blockade completely decreases global and mitochondrial apoptosis obtained under the AGE condition, whereas only mitochondrial apoptosis was induced by HG. We assume that under the AGE condition, all the apoptosis pathways are dependent on RAGE activation, whereas under HG conditions only the mitochondrial part of apoptosis is triggered by RAGE.

Both global and mitochondrial apoptosis are rarely studied at the same time in diabetic models. However, levels of apoptosis measured in the present study are consistent with the literature. What is new is that a similar ratio of apoptosis (HG/M or AGE/BSA) was observed for both mitochondrial and global apoptosis. The DIOC6 probe, which assesses the opening of mitochondrial pore transition, makes it possible to observe an early state of mitochondrial apoptosis. This led us to assume that tubular apoptosis in diabetic conditions is mainly driven by the mitochondria. This is consistent with the observation of mitochondrial apoptosis under HG conditions showed by mitochondrial translocation of cytochrome *c*, increased caspase 9 expression, and a decrease in BCL2 [6,27,28].

This study has several limitations. We did not found significant H_2O_2 overproduction. In LG conditions, H_2O_2 production was highly variable, probably due to the culture conditions. This affects the power to show differences between controls and conditions. Another explanation may be the specificity of the DHR probe, which detects mainly H_2O_2 and not all the ROS components, whereas DCF-DA or DHE detects several ROS or superoxides, respectively. In addition, H_2O_2 overproduction is usually observed after a short exposure according to reports in the literature (6–24 h for HG and 4 h for AGE) [6,22,27,29–30].

In conclusion: (1) Tubular cells are sensitive to a lack of glucose; strict glucose monitoring and supplementation are required for *in vitro* models. (2) Diabetic lesions on tubular cells seem to be more severe through AGE than HG treatment. (3) There is no evidence for an additive effect between HG and AGE models. (4) RAGE seems to be a common pathway in AGE- and HG-induced tubular lesions.

Acknowledgments

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References

[1] E.T. Rosolowsky, J. Skupien, A.M. Smiles, M. Niewczas, B. Roshan, R. Stanton, et al., Risk for ESRD in type 1 diabetes remains high despite renoprotection, J. Am. Soc. Nephrol. 22 (2011) 545–553.
[2] M. Brownlee, Biochemistry and molecular cell biology of diabetic complications, Nature 414 (2001) 813–820.
[3] J.M. Forbes, M.T. Coughlan, M.E. Cooper, Oxidative stress as a major culprit in kidney disease in diabetes, Diabetes 57 (2008) 1446–1454.
[4] K. Nakamura, S.I. Yamagishi, T. Matsui, H. Adachi, M. Takeuchi, T. Imaizumi, Serum levels of soluble form of receptor for advanced glycation end products (sRAGE) are correlated with AGEs in both diabetic and non-diabetic subjects, Clin. Exp. Med. 7 (2007) 188–190.
[5] P. Papanastasiou, L. Grass, H. Rodela, A. Patrikarea, D. Oreopoulos, E.P. Diamandis, Immunological quantification of advanced glycosylation end-products in the serum of patients on hemodialysis or CAPD, Kidney Int. 46 (1994) 216–222.
[6] L. Sun, L. Xiao, J. Nie, F.-Y. Liu, G.-H. Ling, X.-J. Zhu, et al., P66Shc mediates high-glucose and angiotensin II-induced oxidative stress renal tubular injury via mitochondrial-dependent apoptotic pathway, Am. J. Physiol. Renal Physiol. 299 (2010) F1014–F1025.
[7] S.C.W. Tang, L.Y.Y. Chan, J.C.K. Leung, A.S. Cheng, M. Lin, H.Y. Lan, et al., Differential effects of advanced glycation end-products on renal tubular cell inflammation, Nephrology (Carlton Vic.) 16 (2011) 417–425.
[8] C.E. Hills, E. Siamantouras, S.W. Smith, P. Cockwell, K.-K. Liu, P.E. Squires, TGFβ modulates cell-to-cell communication in early epithelial-to-mesenchymal transition, Diabetologia 55 (2012) 812–824.
[9] K.A. Nath, The tubulointerstitium in progressive renal disease, Kidney Int. 54 (1998) 992–994.
[10] M.E. Cooper, V. Thallas, J. Forbes, E. Scalbert, S. Sastra, I. Darby, et al., The cross-link breaker, N-Phenacylthiazolium bromide prevents vascular advanced glycation end-product accumulation, Diabetologia 43 (2000) 660–664.
[11] A. Miyazaki, H. Nakayama, S. Horiuchi, Scavenger receptors that recognize advanced glycation end products, Trends Cardiovasc. Med. 12 (2002) 258–262.
[12] C. Morais, J. Westhuyzen, B. Pat, G. Gobe, H. Healy, High ambient glucose is effect neutral on cell death and proliferation in human proximal tubular epithelial cells, Am. J. Physiol. Renal Physiol. 289 (2005) F401–F409.
[13] A. Dufey, B. Ballan, J. Philippe, Non diabetic hypoglycemia diagnosis and management, Rev. Méd. Suisse 9 (2013) 1186–1188. 1190–1191.
[14] S. Yamagishi, S. Amano, Y. Inagaki, T. Okamoto, K. Koga, N. Sasaki, et al., Advanced glycation end products-induced apoptosis and overexpression of vascular endothelial growth factor in bovine retinal pericytes, Biochem. Biophys. Res. Commun. 290 (2002) 973–978.
[15] S. Moriceau, C. Kantari, J. Mocek, N. Davezac, J. Gabillet, I.C. Guerrera, et al., Coronin-1 is associated with neutrophil survival and is cleaved during apoptosis: potential implication in neutrophils from cystic fibrosis patients, J. Immunol. (Baltim. Md) 1950 (182) (2009) 7254–7263.
[16] L. Carrichon, A. Picciocchi, F. Debeurme, F. Defendi, S. Beaumel, A.J. Jesaitis, et al., Characterization of superoxide overproduction by the D-LoopNox4-Nox2 cytochrome b558 in phagocytes—Differential sensitivity to calcium and phosphorylation events, Biochim. Biophys. Acta BBA – Biomembr. 2011 (1808) 78–90.
[17] Y.J. Lee, Y.J. Lee, H.J. Han, Regulatory mechanisms of Na(+)/glucose cotransporters in renal proximal tubule cells, Kidney Int. Suppl. (2007) S27–S35.
[18] Action to Control Cardiovascular Risk in Diabetes Study Group H.C. Gerstein, M.E. Miller, R.P. Byington, D.C. Goff Jr, J.T. Bigger, et al., Effects of intensive glucose lowering in type 2 diabetes, N. Engl. J. Med. 358 (2008) 2545–2559.
[19] M. Sargazi, N.B. Roberts, A. Shenkin, In-vitro studies of aluminium-induced toxicity on kidney proximal tubular cells, J. Inorg. Biochem. 87 (2001) 37–43.
[20] D. Fraser, N. Brunskill, T. Ito, A. Phillips, Long-term exposure of proximal tubular epithelial cells to glucose induces transforming growth factor-beta 1 synthesis via an autocrine PDGF loop, Am. J. Pathol. 163 (2003) 2565–2574.
[21] S.-I. Yamagishi, Y. Inagaki, T. Okamoto, S. Amano, K. Koga, M. Takeuchi, Advanced glycation end products inhibit de novo protein synthesis and induce TGF-beta overexpression in proximal tubular cells, Kidney Int. 63 (2003) 464–473.
[22] T. Matsui, S.-I. Yamagishi, M. Takeuchi, S. Ueda, K. Fukami, S. Okuda, Irbesartan inhibits advanced glycation end product (AGE)-induced proximal tubular cell injury in vitro by suppressing receptor for AGEs (RAGE) expression, Pharmacol. Res. Off. J. Ital. Pharmacol. Soc. 61 (2010) 34–39.
[23] X.-J. Ren, G. Guan, G. Liu, T. Zhang, G.-H. Liu, Effect of activin A on tubulointerstitial fibrosis in diabetic nephropathy, Nephrology (Carlton Vic.) 14 (2009) 311–320.
[24] Z.-M. Lv, Q. Wang, Q. Wan, J.-G. Lin, M.-S. Hu, Y.-X. Liu, et al., The role of the p38 MAPK signaling pathway in high glucose-induced epithelial–mesenchymal transition of cultured human renal tubular epithelial cells, PLoS ONE 6 (2011) e22806.
[25] M.G. Wong, U. Panchapakesan, W. Qi, D.G. Silva, X.-M. Chen, C.A. Pollock, Cation-independent mannose 6-phosphate receptor inhibitor (PXS25) inhibits fibrosis in human proximal tubular cells by inhibiting conversion of latent to active TGF-beta1, Am. J. Physiol. Renal Physiol. 301 (2011) F84–F93.
[26] H. Vlassara, M. Brownlee, A. Cerami, High-affinity-receptor-mediated uptake and degradation of glucose-modified proteins: a potential mechanism for the removal of senescent macromolecules, Proc. Natl. Acad. Sci. USA 82 (1985) 5588–5592.
[27] E.-G. Yoo, W.J. Lee, J.H. Kim, H.-W. Chae, S.E. Hyun, D.H. Kim, et al., Insulin-like growth factor-binding protein-3 mediates high glucose-induced apoptosis by increasing oxidative stress in proximal tubular epithelial cells, Endocrinology 152 (2011) 3135–3142.
[28] L. Sun, P. Xie, J. Wada, N. Kashihara, F. Liu, Y. Zhao, et al., Rap1b GTPase ameliorates glucose-induced mitochondrial dysfunction, J. Am. Soc. Nephrol. JASN 19 (2008) 2293–2301.
[29] D. Verzola, M.B. Bertolotto, B. Villaggio, L. Ottonello, F. Dallegri, F. Salvatore, et al., Oxidative stress mediates apoptotic changes induced by hyperglycemia in human tubular kidney cells, J. Am. Soc. Nephrol. JASN 15 (Suppl. 1) (2004) S85–S87.
[30] S. Maeda, T. Matsui, M. Takeuchi, Y. Yoshida, R. Yamakawa, K. Fukami, et al., Pigment epithelium-derived factor (PEDF) inhibits proximal tubular cell injury in early diabetic nephropathy by suppressing advanced glycation end products (AGEs)-receptor (RAGE) axis, Pharmacol. Res. Off. J. Ital. Pharmacol. Soc. 63 (2011) 241–248.