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Diabetes and exposure to peritoneal dialysis solutions alter tight junction proteins and glucose transporters of rat peritoneal mesothelial cells

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ABSTRACT

Aim: To evaluate alterations in tight junction (TJ) proteins and glucose transporters in rat peritoneal mesothelial cells (RPMC) from diabetic rats and after treatment with peritoneal dialysis solutions (PDS) in vitro.
Methods: Diabetes was induced in female Wistar rats by streptozotocin (STZ)-injection. Twenty-one days after STZ-injection, peritoneal thickness and mesothelial cell morphology were studied by light microscopy and microvilli length and density by atomic force microscopy. RPMC were obtained from healthy and diabetic rats. Mesothelial phenotype, evaluated by cytokeratin and pan-cadherin, epithelial to mesenchymal transition (EMT), evaluated by alpha-smooth muscle action (α-SMA) and vimentin, TJ proteins, claudins-1 and -2, and occludin, and glucose transporters, sodium and glucose co-transporters (SGLT) -1 and -2 and facilitative glucose transporters (GLUT) -1 and -2 were analyzed. Also, transepithelial electrical resistance (TER) was measured. Oxidative stress was estimated by measuring reactive oxygen species production, and protein carbonylation, receptor for advanced glycation end products (RAGE), nuclear factor erythroid related factor-2 (Nrf-2), and expression of antioxidant enzymes.
Key findings: Peritoneal damage was present 21 days after STZ-injection. Diabetes induced changes in TJ and glucose transporters in RPMC together with decreased TER. RPMC from diabetic rats showed oxidative stress, which was enhanced by exposure to PDS. In addition, RPMC from diabetic rats showed early EMT.
Significance: To our knowledge, this is the first study that shows changes in TJ proteins and glucose transporters of RPMC from diabetic rats. All these alterations might explain the increased peritoneal permeability observed in diabetic patients undergoing peritoneal dialysis.

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1. Introduction

Diabetes is a worldwide public health problem and is associated to complications such as end stage renal disease (ESRD), neuropathy and retinopathy [1]. In Mexico peritoneal dialysis therapy is widely used in ESRD patients [2]. Peritoneal dialysis solutions (PDS) are hypertonic, acidic (pH 5) and with high glucose content (1500–2500 mg/dl). PDS are infused into peritoneal cavity, a residence time is allowed and then solutions are drained. During the residence time, a bidirectional flow is established between blood and the peritoneal cavity. However, the efficiency of this treatment is limited in diabetic patients, due to generalized vascular damage [3–6]. Diabetic patients show poor vascularization of peritoneal membrane which alters peritoneal permeability [7]. Long term exposure to PDS damages peritoneum, leading to decreased water and solutes exchange, known as ultrafiltration failure [8], which is associated to hypertonicity and acid pH of PDS. In addition, PDS contain glucose degradation products (GDP), which participates in this failure [9,10].

Mesothelial cells (MC) constitutes the external layer of the peritoneum; these cells have polygonal cobblestone morphology and participate in solute and water transport [11,12]. MC have profuse microvilli and one or two cilia at the apical surface. In these cells, microvilli participate in solutes transport and cilia protects mesothelium through the secretion of surfactants [13]. Also, both structures respond to microenvironment changes [14]. Finally, loss in the number of microvilli decreases peritoneal function.

Cellular transport occurs through trans- and para-cellular pathways. The trans-cellular transport of glucose occurs through sodium and glucose co-transporters (SGLT) -1 and -2, and facilitative glucose transporters (GLUT) -1 and -2. On the other hand, paracellular pathway is mediated by tight junction (TJ) proteins such as claudins and occludin. TJ proteins of MC are damaged by glucose in dialyzed ESRD patients [15]. However, peritoneal TJ features under early diabetic conditions, in the absence of renal insufficiency are unknown. It is known that high glucose levels induces oxidative stress that alter TJ proteins [16].

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In human MC glucose-induced damage is concentration-dependent and is associated with glucose levels and not with osmolarity, since mannitol, an hyperosmolar agent, does not alter TJ morphology [15].

Exposure to PDS induces MC detachment, and reduces β-catenin, E-cadherin, ZO-1 and claudin-1 expression, thus affecting adherens and TJ proteins [17]. PDS also accelerates cell cycle inducing early senescence and apoptosis [18,19]. In addition, PDS induce epithelial to mesenchymal transition (EMT) in human MC, leading to a fibroblastic mesenchymal appearance, with reduced intercellular adhesions and increased motility [20,21]. Also, It has been reported that EMT is induced by transforming growth factor β-1 (TGF-β1) and increased expression of receptor for advanced glycation end products (RAGE) [22,23]. During EMT, the transcriptional factor snail-1 is induced and down-regulates E-cadherin and cytokeratin expressions [24,25]. Based on the findings described above we aimed: 1) to characterize rat peritoneal mesothelial cells (RPMC) derived from healthy and diabetic rats; 2) to evaluate EMT in RPMC; and 3) to study glucose transporters (SLGT-1, SLGT-2, GLUT-1 and GLUT-2) and TJ proteins (claudin-1, claudin-2 and occludin). Additionally, all the above-described parameters were evaluated in MC from healthy and diabetic rats exposed to PDS in vitro. Based on the findings described above we hypothesize that diabetes modifies the expression of tight junction proteins and glucose transporters which worsen after PDS exposure.

2. Material and methods

2.1. Reagents and chemicals

Anti-vimentin antibody was from Zymed (CA, USA); α-SMA antibody was from Merck Millipore (CA,USA); streptozotocin (STZ), antibodies anti-cytokeratin 4.62, anti-RAGEs and anti-nuclear factor erythroid related factor-2 (Nrf-2), were purchased from Sigma-Aldrich (Mo, USA); antibodies anti-occludin, anti-claudin-1, anti-claudin-2; anti-pan-cadherin, rabbit anti-Alexa Fluor 488, goat anti-Alexa Fluor 488, mouse anti-Alexa Fluor 594, mouse anti-peroxidase conjugated and rabbit anti-peroxidase-conjugated were purchased from Thermo Scientific (CA, USA). The anti-SGLT-1, anti-GLUT-1, and anti-GLUT-2 antibodies were purchased from Millipore Corp. (MA, USA). Antibodies anti-catalase, anti-γ-glutamyl-cysteine-synthetase (γ-GCSc), anti-superoxide dismutase (SOD) -1 and -2, anti-SGLT-2 and anti-goat peroxidase-conjugated were purchased from Santa Cruz Biotechnology (CA, USA), α-actin antibody was donated by Prof. Jose Manuel Hernandez (Cell Biology Department from CINVESTAV-IPN, Mexico City) [26]; and anti-hemeoxygenase (HO-1) antibody was obtained from ENZO Life Sciences (NY, USA).

2.2. Experimental design

Female Wistar rats were obtained from UPEAL-Cinvestav and experimental procedures were performed under Institutional Animal Care Committee guidelines (protocol No. 491 approved for this study). Animal housing temperature and humidity were maintained at 22 ± 1 °C and 50 ± 5%, respectively. 12:12 h light:dark cycles were established and rodents were dispensed food and water ad libitum. Type 1 diabetes was induced in rats with a body weight of 200-250 g by a single tail vein injection of STZ (60 mg/Kg) diluted in citrate buffer (pH 6.0). Diabetes was confirmed 72 h after STZ-injection by measuring blood glucose with a strip glucose test (OneTouch Ultra blood glucose meter, CA, USA). Two groups of rats were studied (n = 12/group): (1) control (CTR), rats treated with a single injection of citrate buffer (STZ vehicle) and (2) diabetic (DBT) rats, STZ-treated group.

2.3. Physiological parameters

Blood glucose and body weight were monitored before STZ administration and at 3, 7, 14 and 21 days after STZ-injection. Rats were individually placed in cages with purified water and standard rat food (5008, Purina, TX, USA). Water and food consumption were measured along the study. Rats were individually housed in metabolic cages 24 h before sacrifice (from days 20–21 after STZ-injection). Urine samples were collected and volumes were measured.

2.4. Isolation and culture of RPMC

A small incision on rat abdominal wall was made and catheter was inserted and secured. Subsequently, 20 ml of trypsin and EDTA solution (0.11% and 0.1%, respectively) was introduced into the cavity and maintained for 10 min, and then collected. Once the perfusion was performed, omentum was removed and placed in phosphate buffer (PBS, pH 7.4 at room temperature). Samples were then incubated in trypsin (0.03%) and EDTA (0.1%) for 15 min, at 37 °C. After incubation, omentum was discarded. The recovered perfusion solution samples were centrifuged at 1000*g* for 5 min. RPMCs pellet was resuspended and cultured in DMEM F-12 medium (12% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 μg/ml L-glutamine, 5 μg/ml transferrin, and 0.4 μg/ml hydrocortisone). Finally, every 48 h medium was replaced until cultures reached confluence. DMEM 2× was prepared with 50% DMEM F-12 medium and 50% the peritoneal dialysis solution (PDS) 2.5% of glucose. Four groups of cultures were studied: (1) CTR; (2) DBT; (3) CTR + PDS; (4) DBT + PDS.

2.5. Atomic force microscopy (AFM)

RPMC were seeded on coverslips (3 mm) precoated with collagen. Once the cells reached confluence, they were washed with phosphate solution, fixed with methanol (4 °C) for 10 min and examined with a N8 NEOS Atomic Force Microscope (Bruker, Germany). Images were obtained by Amplitude Modulation (AM-AFM) and the cantilevers used were Point Probe Plus NonContact Long cantilever Reflex coated (PPP-NCLR) from Nanosensors. Randomly captured images from at least 10 cells of three independent cultures were obtained. The images were processed with the program Gwyddion 2.40.

2.6. Confocal microscopy and immunofluorescence

Cells were seeded on coverslips (3 mm) pre coated with collagen. Once the cells reached confluence, they were separated into four groups: a) control group (CTR), b) diabetic group (DBT): c) control group exposed to DMEM 2× for 1 h (CTR + PDS), and d) diabetic group exposed to DMEM 2× for 1 h (DBT + PDS). After incubation in those media, cells were washed with phosphate solution (PBS) containing 1 mM Ca^{2+}. Monolayer was fixed with absolute methanol at −20 °C for 10 min, and hydrated (5 min with PBS with 1 mM Ca^{2+} at room temperature). Cells were permeabilized with PBS-Triton X-100 0.2% for 30 min. Then, cells were blocked for 1 h at room temperature with 1% (wt/vol) IgG-free albumin (1331-A, Research Organics, Cleveland, OH, USA), and incubated overnight at 4 °C with one of the following primary polyclonal antibodies: anti α-SMA (dilution 1:500), vimentin (dilution 1:1000), Pan-cadherin, cytokeratin 4.62, occludin, claudin-1 and -2, SGLT-1 and -2 and GLUT-1 and -2 (dilution 1:100), and incubated with the secondary anti-rabbit Alexa Fluor 488, anti-goat Alexa Fluor 488, anti-mouse Alexa Fluor 488, anti-rabbit Alexa Fluor 594 anti-mouse Alexa Fluor 594 anti-goat Alexa Fluor 594 (dilution 1: 200) antibodies for 1 h at room temperature. 4′,6-diamidino-2-phenylindole (DAPI, 1:1000) was used to visualize nuclei. Immunofluorescence was analyzed by using a confocal inverted microscope (TCS-SP8, Leica,Heidelberg, Germany) and images were obtained in three different fields for each sample from four different animals per group.

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