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Original Article

Effect of aldosterone on epithelial-to-mesenchymal transition of human peritoneal mesothelial cells

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ABSTRACT

Background: Peritoneal fibrosis is one of the major causes of technical failure in patients on peritoneal dialysis. Epithelial-to-mesenchymal transition (EMT) of the peritoneum is an early and reversible mechanism of peritoneal fibrosis. Human peritoneal mesothelial cells (HPMCs) have their own renin–angiotensin–aldosterone system (RAAS), however, it has not been investigated whether aldosterone, an end-product of the RAAS, induces EMT in HPMCs, and which mechanisms are responsible for aldosterone-induced EMT.
Methods: EMT of HPMCs was evaluated by comparing the expression of epithelial cell marker, E-cadherin, and mesenchymal cell marker, α-smooth muscle actin after stimulation with aldosterone (1–100nM) or spironolactone. Activation of extracellular signal-regulated kinase (ERK)1/2 and p38 mitogen-activated protein kinase (MAPK) and generation of reactive oxygen species (ROS) were assessed by western blotting and 2′,7′-dichlorofluororescein diacetate staining, respectively. The effects of MAPK inhibitors or antioxidants (*N*-acetyl cysteine, apocynin, and rotenone) on aldosterone-induced EMT were evaluated.
Results: Aldosterone induced EMT in cultured HPMCs, and spironolactone blocked aldosterone-induced EMT. Aldosterone induced activation of both ERK1/2 and p38 MAPK from 1 hour. Either PD98059, an inhibitor of ERK1/2, or SB20358, an inhibitor of p38 MAPK, attenuated aldosterone-induced EMT. Aldosterone induced ROS in HPMCs from 5 minutes, and antioxidant treatment ameliorated aldosterone-induced EMT. *N*-acetyl cysteine and apocynin alleviated activation of ERK and p38 MAPK.
Conclusion: Aldosterone induced EMT in HPMCs by acting through the mineralocorticoid receptor. Aldosterone-induced generation of ROS followed by activation of ERK, and p38 MAPK served as one of the mechanisms of aldosterone-induced EMT of HPMCs.

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Introduction

Long-term peritoneal dialysis (PD) results in peritoneal damage that is characterized by a decreased ultrafiltration

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capacity associated with submesothelial fibrosis, accumulation of extracellular matrix, and neoangiogenesis [1–3].

Recent data have revealed that peritoneal mesothelial cells play an important role in peritoneal fibrosis via phenotype transition and production of extracellular matrix. Yáñez-Mó et al [4] found that mesothelial cells isolated from dialysate effluents showed phenotypic transition. This phenomenon of epithelial-to-mesenchymal transition (EMT) denotes loss of epithelial characteristics and acquisition of a fibroblast-like phenotype, and is suggested as a key process in the onset and progression of peritoneal fibrosis.

There are several lines of evidence that the renin–angiotensin–aldosterone system (RAAS) is involved in organ fibrosis [5–8]. Activation of the RAAS is related to the development of tubulointerstitial fibrosis in the kidney, and hepatic and lung fibrosis [6,9,10]. Aldosterone, a final end-product of RAAS, is reported to be an important mediator of cardiac fibrosis [11], and spironolactone ameliorates peritoneal fibrosis in animal models of peritoneal fibrosis and inflammation [12].

To determine the effect of aldosterone on peritoneal EMT, we investigated whether aldosterone induced EMT of peritoneal mesothelium and examined the mechanism of aldosterone-induced phenotypic transition of the peritoneum.

Methods

Reagents

All chemicals and tissue culture plates were purchased from Sigma–Aldrich (St. Louis, MO, USA) and Nunc Labware (Waltham, MA, USA), unless otherwise stated.

Isolation and culture of human peritoneal mesothelial cells

Human peritoneal mesothelial cells (HPMCs) were isolated from a piece of omentum, obtained from consenting patients receiving elective abdominal surgery according to the method described by Stylianou et al [13]. HPMCs were identified by phase-contrast microscopy according to morphological criteria and by immunofluorescence staining for the markers of mesothelial cells [14]. All experiments were performed using cells of the second to fifth cell passages. Tissue collection was approved by the Institutional Review Boards and informed consent was obtained from each patient.

Reverse transcriptase polymerase chain reaction of mineralocorticoid receptor

Total RNA was extracted from HPMCs that were prepared by using TRIzol reagent (Gibco Invitrogen, Carlsbad, CA, USA). The RNA pellet was suspended in DNase/RNase-Free distilled water and stored at −70°C until subsequent analysis. Mineralocorticoid receptor oligonucleotide primers used for the polymerase chain reaction (PCR) were as follows: forward primer: 5′-ACCAAGCATTCATGTTCAGGCACC-3′ and reverse primer: 5′-AGCTCCCGTCATCTGGTTCTTGTT-3′. PCR was performed in 10mM Tris–HCl (pH 9.0), 40mM KCl, 1.5mM $MgCl_2$, and 0.1 U Taq polymerase (Promega), in a final volume of 20 μL. Reactions were carried out in a DNA thermal cycler (Perkin–Elmer, Boston, MA, USA). Following initial denaturation at 94°C for 5 minutes, mixtures were subjected 35 cycles at 94°C for 30 seconds, annealing at 55°C for 30 seconds, primer extension at 72°C for 45 seconds, and a final extension at 72°C for 10 minutes. PCR products were fractionated on 1% agarose gel, followed by staining with 0.5 μg/mL ethidium bromide (Gibco BRL, Grand Island, NY, USA). The amount of PCR products was normalized with a housekeeping gene, GAPDH (forward primer: 5′-ACCACAGTCCATGCCATCAC-3′ and reverse primer: 5′-TCCACCACCCTGTTGCTGTA-3′).

MTS assay for assessing cell proliferation

To measure cell proliferation, HPMCs (10^4 cells/well) were incubated with aldosterone (1–100nM) in 96-well plates. After incubation for 2–7 days at 37°C, MTS reagent [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; Promega] was added, and the amount of metabolized formazine was measured at 490 nm with an automated plate enzyme-linked immunosorbent assay reader (Molecular Devices, Sunnyvale, CA, USA) according to the manufacturer's instructions.

Cell morphology and immunocytochemistry

The morphology of HPMC cells was observed by an inverted phase contrast microscope (Axiovert 200; Carl Zeiss, Oberkochen, Germany) and the images were obtained using a digital camera (AxioCam HRC; Carl Zeiss). For staining, cells were washed with phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde in PBS (25 minutes at 20°C) and permeabilized with 1% Triton X-100 in PBS (15 minutes at 4°C). After washing with PBS, the cells were treated with 5% bovine serum albumin in PBS for 1 hour before incubation with primary antibodies specific for cytokeratin (1:50; DAKO Cytomation, Carpinteria, CA, USA) or α-smooth muscle actin (SMA,

Figure 1. Expression of mineralocorticoid receptor mRNA in HPMCs. Mineralocorticoid receptor mRNA detected in cultured HPMCs. Representative reverse transcriptase polymerase chain reaction bands from three different patients are shown. NC (negative control) denotes the sample containing all reactants except cDNA. HPMC, human peritoneal mesothelial cell; MR, mineralocorticoid receptor.

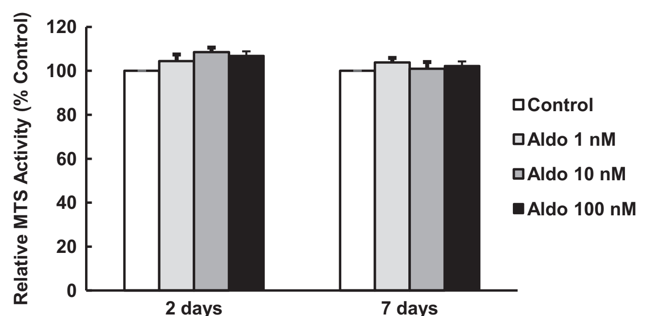

Figure 2. Effect of aldosterone on the proliferation of human peritoneal mesothelial cells. There was no effect of aldosterone (1–100nM) on MTS activity on Day 2 and 7. Data are presented as means ± standard deviation. Aldo, aldosterone; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt.

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