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High glucose induces dysfunction of airway epithelial barrier through down-regulation of connexin 43

Hongmei Yu [a,*], Juan Yang [b], Xiangdong Zhou [c], Qian Xiao [a], Yang Lü [a], Li Xia [a]

[a] Department of Geriatrics, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China
[b] Division of Respiratory Medicine, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China
[c] Division of Respiratory Medicine, The Affiliated Hospital of Hainan Medical College, Hainan, China

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ABSTRACT

The airway epithelium is a barrier to the inhaled antigens and pathogens. Connexin 43 (Cx43) has been found to play critical role in maintaining the function of airway epithelial barrier and be involved in the pathogenesis of the diabetic retinal vasculature, diabetes nephropathy and diabetes skin. Hyperglycemia has been shown to be an independent risk factor for respiratory infections. We hypothesize that the down-regulation of Cx43 induced by HG alters the expression of tight junctions (zonula occludens-1 (ZO-1) and occludin) and contributes to dysfunction of airway epithelial barrier, and Cx43 plays a critical role in the process in human airway epithelial cells (16HBE). We show that high glucose (HG) decreased the expression of ZO-1 and occludin, disassociated interaction between Cx43 and tight junctions, and then increased airway epithelial transepithelial electrical resistance (TER) and permeability by down-regulation of Cx43 in human airway epithelial cells. These observations demonstrate an important role for Cx43 in regulating HG-induced dysfunction of airway epithelial barrier. These findings may bring new insights into the molecular pathogenesis of pulmonary infection related to diabetes mellitus and lead to novel therapeutic intervention for the dysfunction of airway epithelial barrier in chronic inflammatory airway diseases.

1. Introduction

The airway epithelial barrier provides defenses against inhaled antigens and pathogens [1]. Disruption of the epithelium integrity is one of the most important pathological characteristics of chronic airway inflammatory diseases [2]. It starts with pathogen infection or other biophysical injurious agents [3]. Diabetes mellitus predisposes the host to bacterial infections. Hyperglycemia has been shown to be an independent risk factor for respiratory infections [4–7]. Hyperglycemia is associated with increased morbidity and mortality in patients in intensive care units and patients with respiratory disease. Therefore, the mechanisms of the respiratory infections promoted by hyperglycemia are critical for the patients suffering from hyperglycemia. Previous studies reported that high glucose (HG) induced the dysfunction of the endothelial barrier in diabetic retinopathy and brain [8–10]. It is reported that hyperglycemia-induced reduction in transepithelial electrical resistance (TER) across polarized airway epithelial cultures [11]. However, it is not well understood how HG induces the dysfunction of airway epithelial barrier (reduction in TER), and then maybe contributes to respiratory infections.

The integrity of the epithelium that protects multicellular organisms from the external environment is maintained by intercellular junctional complexes composed of tight junctions, adherens junctions and gap junctions [12]. Connexins (Cx) are considered to play a crucial role in the differentiation of airway epithelial cells and to be associated with tight junctions [13]. Connexin 43 (Cx43), a gap junction protein, associated with ZO-1(a tight junction protein) occurred through the extreme carboxyl terminus of Cx43 and the second PDZ domain of ZO-1 in lung epithelial cells [14]. The interaction of Cx43 and ZO-1 was decreased by mutated and phosphorylated Cx43 [15]. Cx43 played critical role in the regulation of the tight junctions and the integrity of the blood-testis barrier [16]. Studies have indicated that endothelial Cx43 regulates injury-based permeability of pulmonary microvascular [17] and plays a critical role in the maintenance of the lung endothelial barrier function [18]. It has shown that HG or diabetes reduces Cx43 expression, and promotes vascular damage associated with diabetic retinopathy [19–22]. Studies have

Abbreviations: HG, high glucose; NG, normal glucose; Cx43, connexin43; ZO-1, zonula occludens-1; TER, transepithelial electrical resistance

* Corresponding author.
E-mail address: yhm920@126.com (H. Yu).

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shown that Cx43 colocalizes and coprecipitates with tight-junction molecules occludin and ZO-1 [23]. HG-induced Cx43 down-regulation contributed to the breakdown of endothelial barrier tight junctions (such as ZO-1 and occludin) associated with diabetic retinopathy [24]. However, it is currently unknown whether or not HG influences the interactions between the tight junction and gap junction proteins in airway epithelial cells, and if Cx43 influences the airway epithelial barrier.

Based on the findings mentioned above, we hypothesized that the down-regulation of Cx43 induced by HG alters the expression of tight junctions and contributes to the dysfunction of airway epithelial barrier, and Cx43 plays a critical role in the process induced by HG. In this study, to examine this hypothesis, first we investigated whether HG down-regulated Cx43 expression in 16HBE cells (immortalized human bronchial epithelial cells). Next, we examined which tight junction protein was regulated by HG and whether Cx43 involved in the alteration of tight junction proteins induced by HG. Finally, we examined whether airway epithelial TER and permeability were regulated by Cx43 which was down-regulated by HG. We show that HG down-regulated tight junction proteins and induced disassociation between Cx43 and tight junction proteins (Cx43/ZO-1 and Cx43/occludin), and then induced dysfunction of airway epithelial barrier by down-regulation of Cx43 in airway epithelial cells.

2. Materials and methods

2.1. Materials

Roswell Park Memorial Institute (RPMI) 1640 medium, TRIzol, fetal bovine serum (FBS) and dimethyl sulfoxide (DMSO) were purchased from Sigma (St Louis, MO). FuGENE 6 reagent was purchased from Roche (Indianapolis, IN). Rabbit Cx43, ZO-1 and occludin were from Cell Signaling (Danvers, MA); Cx43 siRNA and scramble siRNA were from Santa Cruz Biotechnology (Santa Cruz, CA); pEGFP-N1 was purchased from Clontech (Mountain View, CA).

2.2. Small interfering RNA preparation and transfection

One day before transfection, cells were plated in growth medium without antibiotics per well so that they would be 90–95% confluent at the time of transfection. Then, cells were transfected with 0.5 μg/ml siRNA duplexes (either the scramble or Cx43) using FuGENE 6 according to the manufacturer's recommendations. Concentrations of siRNAs were chosen on the basis of dose–response studies (data not shown).

2.3. Plasmids transfection

Cells were transfected with plasmid pEGFP-N1 containing full length Cx43 cDNA (constructed by our team) or pEGFP-N1 using FuGENE 6 according to the manufacturer's recommendations. After transfection, protein was isolated to determine Cx43, ZO-1, and occludin protein levels in the transfected cells. Cells transfected with Cx43 plasmid were also subjected to TER and permeability analysis as described above.

2.4. Cell culture

16HBE, SV-40 virus-transformed, immortalized human bronchial epithelial cells, was seeded on Transwell inserts (Costar, Corning, NY, USA) in RPMI 1640 medium containing 10% FCS, penicillin (100 U/mL), streptomycin (100 μg/ml) and 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (25 mM) at 37 °C in a humidified 5% CO2 atmosphere. Cells were treated with glucose (5 mM as normal glucose (NG) and 30 mM as high glucose (HG)) or osmotic control (mannitol, 30 mM final concentration). The cells in control group were incubated in a physiological concentration (5 mM) [25]. 16HBE cells seeded on Transwell inserts were cultured with or without glucose for up to 72 h. Experiments presented here were repeated at least four times.

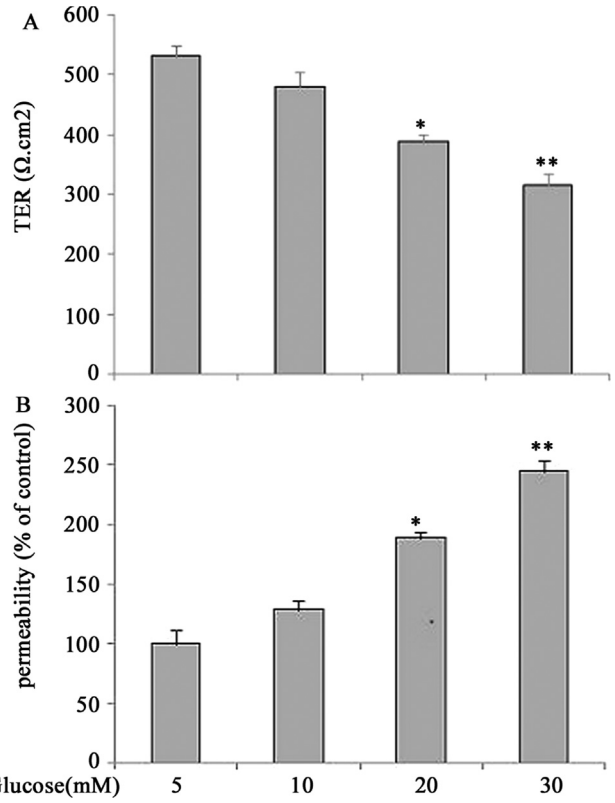

Fig. 1. Regulation of TER (A) and permeability (B) by HG. TER (A) and permeability (B) were analyzed as described in Section 2. 16HBE cells were treated with increasing concentrations of HG (5, 10, 20 and 30 mM). Results were expressed as fold change versus NG group (5 mM). $^{*}P<0.05$, $^{**}P<0.01$, compared with the NG group (5 mM). Figure is representative of $n=4$.

2.5. Cytotoxicity detection

All reagents used were tested for cytotoxicity using a Promega Cytotox 96 nonradioactive cytotoxicity assay kit according to the manufacturer's instructions. The data were expressed as the ratio of released lactate dehydrogenase to total lactate dehydrogenase.

2.6. Western blot analysis

Western blot analysis was performed with samples containing equal amounts of protein (20 μg) in a 6% or 10% SDS–PAGE. The separated proteins in the gel were then transferred onto a PVDF membrane. Nonspecific binding sites were blocked by incubating the polyvinylidene difluoride (PVDF) membrane in Tris-buffered saline containing 0.1% Tween-20 (TTBS) with 5% nonfat dry milk. Membranes were then incubated overnight at 4 °C with rabbit Cx43, rabbit ZO-1, and rabbit occludin antibodies, washed with TTBS three times each for 10 minutes, and then incubated with the anti-rabbit secondary antibody conjugated with alkaline phosphatase (1:3000) (Cell Signaling). Spots were developed with the enhanced chemiluminescence reagent kit (Keygen, Nanjing, China) according to the manufacturer's instructions.

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