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All-trans retinoic acid can regulate the expressions of gelatinases and apolipoprotein E in glomerulosclerosis rats

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ABSTRACT

Apolipoprotein E (apoE) is an important plasma protein in cholesterol homeostasis and plays a key role in the pathogenesis of glomerulosclerosis (GS). Gelatinases include matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9). The abnormal expressions of gelatinases are implicated in the pathogenesis of extracellular matrix accumulation. All-trans retinoic acid (ATRA) is an import biological agent which can play a protective role against GS. We performed this investigation to explore whether ATRA could regulate the expressions of gelatinases and apoE in the glomerulus of GS rats. 120 Wistar rats were randomly divided into three groups: sham operation group (SHO), glomerulosclerosis model group without treatment (GS) and GS model group treated with ATRA (GA). The GS disease was established by unine-phrectomy and adriamycin injection. At the end of 9 and 13 weeks, the relevant samples were collected and determined. Compared with GS group at 9/13 weeks, values of 24-hour urine total protein, 24-hour urine excretion for albumin, blood urea nitrogen, serum creatinine and glomerulosclerosis index, and protein expressions of apoE, transforming growth factor-βl (TGF-β1), α-smooth muscle actin, collagen-IV and fibronectin in glomerulus and mRNA expressions of apoE and TGF-β1 in renal tissue were significantly down-regulated by ATRA (each $P<0.01$). However, the expressions of MMP-2 and MMP-9 (mRNA, protein and activity) were enhanced in GA group than those in GS group. In conclusion, gelatinases are associated with apoE expression, and ATRA can increase the gelatinases expressions and reduce the accumulation of apoE in glomerulus of GS rats, but the detailed mechanism needs to be elucidated in the future.

1. Introduction

Apolipoprotein E (apoE) is a secreted protein that plays an important role in the systemic lipoprotein metabolism and vascular wall homeostasis (Yue and Mazzone, 2011). apoE is a main ligand for the clearance of lipids via low density lipoprotein receptor and other related receptors (Reilly and Rader, 2006). Some investigations found that the expressions of lipids and apoE in renal glomerulus were elevated in some renal diseases, especially in lipoprotein glomerulopathy (Calandra et al., 1981; Deighan et al., 2000; Russi et al., 2009). Lipid deposition in glomerulus, which can be mediated by apoE, is an important characteristic of nephrotic syndrome. apoE involves in the pathogenesis of atherosclerosis (Choi et al., 2010; Fenyo et al., 2011), and atherosclerosis lesion is similar to glomerulosclerosis (GS) pathological change (Sarnak et al., 2002; Joss et al., 2005). apoE might play a key role in the pathogenesis of GS.

Matrix metalloproteinases (MMPs), a family of proteolytic enzymes, plays a major role in the remodelling and homeostasis of the extracellular matrix (ECM) (Lamy et al., 2010; Mehan et al., 2011; Setz et al., 2011). More than twenty MMPs have been identified and are separated into six groups based on their structure and substrate specificity (collagenases, gelatinases, membrane type matrix metalloproteinase, stromelysins, matrilysins, and others) (Hu and Beeton, 2010). Gelatinases, a key subgroup of MMPs, include gelatinase A and gelatinase B which are also called matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) respectively (Mathivadhani et al., 2007; Duran-Vilaregut et al., 2011). MMP-2 and MMP-9 participate in extracellular protein remodeling (Madro et al., 2009) and gelatinases may cleave the collagen IV (Col-IV) (Ha et al., 2004; Cortes-Reynosa et al., 2008) and the fibronectin (FN) (Pal et al., 2010). Col-IV and FN are the major component of ECM (Qin et al., 2003; Abe et al., 2011). Accumulation of glomerular ECM may result in GS (Zhang et al., 2010). So, the abnormal expressions of gelatinases would play a most important role in the pathogenesis of GS.

As the evidences presented above, gelatinases and apoE might take part in the pathologicprocess of GS. Whether there was an association between gelatinases and apoE, it was not elucidated, and there was rare report to investigate this association. Hwang et al. (2004) found

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that apoE was a substrate of matrix metalloproteinase-14 (MMP-14) and apoE was cleaved by MMP-14 in vitro. Park et al. (2008) confirmed that apoE was a substrate of MMP-14 and also of matrix metalloproteinase-7 (MMP-7) and MMP-2 to a lesser extent. Those two studies might draw a conclusion that MMPs might regulate the apoE expression and apoE might be a downstream protein for MMPs. In this context, we hypothesize that gelatinases might be associated with the accumulation of apoE in glomerulus of GS rats.

Nuclear receptors, such as retinoic acid receptor (RAR) and vitamin D receptor (VDR) (Edwards et al., 2002), can play a regulation role on the transcriptional as well as epigenetic level of several genes. All-trans-retinoic acid (ATRA) is a ligand of retinoic acid receptor (RAR) (Saito et al., 2010) and exerts biological effects via binding to RAR. In the past years, various investigations were performed and those studies found that ATRA might increase the expressions of MMP-2 and MMP-9 (Joshi et al., 2007; Darmanin et al., 2007; Lackey et al., 2008; Lackey and Hoag, 2010). Therefore, there might be an association between ATRA and gelatinases expressions. In this study, we drew a hypothesis that ATRA might regulate the expressions of gelatinases and apoE in GS rats. 120 male Wistar rats were divided into sham operation group (SHO), GS model group without treatment (GS) and GS model group treated with ATRA (GA) at random, in order to investigate the relationship between ATRA and gelatinases/apoE in glomerulus of GS rats induced by adriamycin.

2. Materials and Methods

2.1. Animal Model

120 healthy male rats, 180 g to 200 g, of Wistar backgrounds were purchased from the Experimental Animal center of Guangxi Medical University, Nanning, China. All experiments were performed in accordance with the Ethics Review Committee for Animal Experimentation of Guangxi Medical University. The rats were divided into three groups at random: sham operation group (SHO, n = 40), GS model group without treatment (GS, n = 40) and GS model group treated with ATRA (GA, n = 40). The SHO group received a sham operation and tail vein injection of normal saline solution alone. GS disease in GS group and GA group were induced by uninephrectomy and, after 1 week, a single tail vein injection of adriamycin (Wanle Pharmaceutical Co., Shenzhen, China) at a dose of 5 mg/kg, which was dissolved in sterilized water. The rats in GA group were treated with ATRA (Sigma Co., USA; 15 mg/kg·d) in corn oil once daily by oral gavage, from the time of injection of adriamycin to the end of the experiment. At the end of 9 and 13 weeks, serum and urine from 20 rats in each group were collected and stored at −20 °C; and the renal tissues were collected for the determination of histological and molecular biology.

2.2. Laboratory Analysis

All the serum and urine specimens were removed from the refrigerator and balanced to the room temperature for detection. 24-hour urine total protein (24UTP) and 24-hour urine excretion for albumin (24Ualb) were measured by the sulfosalicylic acid method. Levels of blood urea nitrogen (BUN) and serum creatinine (Scr) were determined by standard enzymatic method.

2.3. Renal Morphology

Renal tissues were fixed in 10% neutral formaldehyde, and they were dehydrated through a graded ethanol series and embedded in paraffin. 4 μm sections were prepared on a microtome and stained with hematoxylin and eosin (H&E). Renal damage was viewed by light microscopy, and the severity of the renal lesion was defined by the glomerulosclerosis index (GSI). The GSI was counted according to the method of Raij et al. (1984). The severity of the lesions was examined in 100 glomeruli selected at random, graded from 0 to 4 points in accordance with the percentage of morphological changes on each glomerulus (0 = 0%, 1 + = 1%–25%, 2 + = 26%–50%, 3 + = 51%–75%, 4 + = 76%–100%). The number of glomeruli showing a lesion of 0 was n_0, of 1+ n_1, of 2+ n_2, of 3+ n_3, of 4+ n_4, respectively. 100 glomeruli were independently examined, and the GSI was obtained by the formula as follow: $GSI = (0\times n_0 + 1\times n_1 + 2\times n_2 + 3\times n_3 + 4\times n_4)/(n_0 + n_1 + n_2 + n_3 + n_4) = (0\times n_0 + 1\times n_1 + 2\times n_2 + 3\times n_3 + 4\times n_4)/100$. The scores obtained by two investigators were averaged.

Masson's trichrome staining for collagen detection in glomerulus was also performed. Semi-quantitative evaluation for collagen fibers was performed by Image-Pro P1uS Version 6.0 Imaging System (Media Cybernetics, USA) (Wang et al., 2010) at 400-folds original magnification in 100 glomeruli at random. Positive areas for collagen fibers (blue) were extracted by "hue saturation method" and expressed as percentage of discriminated regions (integral optical density/area) (Palese et al., 2003; Pan et al., 2009; Liu et al., 2010).

2.4. Immunohistochemical analysis of MMP-2, MMP-9, apoE, TGF-β1, α-SMA, Col-IV and FN

The operation was implemented using the streptavidin-peroxidase immunohistochemical method. Renal tissue samples were fixed in 10% neutral formaldehyde, dehydrated with ethanol, and embedded in paraffin. Serial 4 μm sections were collected sequentially on glass slides. The paraffin was removed from the sections with xylene and rehydrated in graded ethanol. In order to retrieve antigenicity from formalin fixation, we incubated the sections for 10 min in 10 mmol/l sodium citrate buffer using a microwave oven. Endogenous peroxidase activity was blocked by further pretreatment with 3% hydrogen peroxide and methanol. Finally, the sections were incubated with antibody against MMP-2 (1/200) (Neomarkers Co., Inc., USA), MMP-9 (1/500) (Chemicon Co., Inc., USA), apoE (1/150) (Bo Ao-Sen, Co., Inc., China), TGF-β1(1/100) (Wuhan Boshide, Co., Inc., China), α-SMA (ready-to-use kit) (Shanghai Changdao, Co., Inc., China), Col-IV (1/100) (Shanghai Changdao, Co., Inc., China) and FN (1/100) (Beijing Zhongshan Co., Inc., China) overnight at 4 °C. The sections were thoroughly washed in phosphate-buffered saline (PBS) solution and incubated with rabbit anti-mouse biotinylated second antibody immunoglobulin (Shanghai Changdao, Co., Inc., China) for 30 min. Finally, the sections were stained with diaminobenizidine (DAB, Maixin Bio, Co., Inc., China). We obtained negative controls by replacing specific antisera with PBS solution. Brownish yellow granular or linear deposits in the cells or matrix were interpreted as positive areas. For determination of the expression of MMP-2, MMP-9, apoE, TGF-β1, α-SMA, Col-IV or FN, semi-quantitative evaluation was performed by Image-Pro P1uS Version 6.0 Imaging System (Media Cybernetics, USA) at 400-folds original magnification in 100 glomeruli which were selected from coded sections for each rat at random. Positive areas for MMP-2, MMP-9, apoE, TGF-β1, α-SMA, Col-IV and FN were extracted by "hue saturation method" and expressed as percentage of discriminated regions (IOD/area) (Palese et al., 2003; Pan et al., 2009; Liu et al., 2010).

2.5. Gelatin zymography to detect MMP-2 and MMP-9 activity

Renal tissue was homogenized and the protein concentration was measured by the bicinchoninic acid method (Shanghai Jierui Biotechnology, Co. Ltd., China). Renal sample (50 μg protein) from each animal was mixed with the buffer at a ratio of 2:1 and then was subjected to electrophoresis (voltage 5–8 V/cm) on 10% sodium dodecylsulfate (SDS) polyacrylamide gel containing 0.1% gelatin. After electrophoresis, the gels were washed in 2.5% TritonX-100 (Shanghai Jierui Biotechnology, Co. Ltd., China) to displace the SDS. Then, the gels were incubated further for 18 h in 50 mM pH 7.4 Tris buffer containing 200 mmol/l sodium chloride and 10 mmol/l calcium chloride. We identified gelatin-degrading enzymes by staining for 1 h with Coomassie blue R250 and destaining in methanol/acetic acid/H_2O. After the above