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Research Paper

Glucocorticoids Regulate the Vascular Remodeling of Aortic Dissection Via the p38 MAPK-HSP27 Pathway Mediated by Soluble TNF-RII

Lei Zhang [a], Jian Zhou [a,*], Zaiping Jing [a,*], Yu Xiao [a], Yudong Sun [a], Yani Wu [b], Huiying Sun [a]

[a] Department of Vascular Surgery, Changhai Hospital, Second Military Medical University, Shanghai 200433, China
[b] Department of Breast and Thyroid Surgery, Changhai Hospital, Second Military Medical University, Shanghai 200433, China

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ABSTRACT

Increasing researches suggest that inflammatory response is involved in vascular remodeling, which plays an important role in the development of aortic dissection. Glucocorticoids have been widely used in the clinical practice due to its powerful and effective anti-inflammatory property. However, the potential relationship between glucocorticoids and aortic dissection was still obscure. This study sought to elucidate the effect of glucocorticoids on the development and progression of aortic dissection, and the potential mechanism involved. Serum cortisol in aortic dissection patients was significantly higher than that in non-ruptured aortic aneurysm patients and healthy volunteers by radioimmunoassay. In modified C57BL/6 mouse model of aortic dissection, glucocorticoids reduced the incidence of aortic dissection and protected the collagen from degradation. Furthermore, glucocorticoids inhibited the TNF-α secretion of THP-1 monocytes, decreased the migration, phenotype switch from contractile type to synthetic type, and the apoptosis of human aortic smooth muscle cells induced by TNF-α. Finally, TNF-sRII was identified as an important cytokine in cellular interaction that participated in vascular remodeling by targeting the p38 MAPK-HSP27 pathway. These results indicate that glucocorticoids inhibit the incidence of aortic dissection by decreasing the TNF-α secretion and increasing the uncombined TNF-sRII, positively participating in vascular remodeling.

1. Introduction

Aortic dissection is the most devastating disease of thoracic aorta (Goldfinger et al., 2014). The estimated annual incidence of aortic dissection is approximately 2–5 per 100,000 individuals (Clouse et al., 2004; Howard et al., 2013), which may underestimate the true incidence and case fatality by the incomplete inclusion of deaths before hospital admission (Nienaber and Clough, 2015). The most feared clinical consequence of aortic dissection progression is lethal rupture of aorta, which occurs in about 1–2% per hour within the initial 24 h and almost 50% by 1 week after clinical symptoms onset for acute type A dissection patients (Hagan et al., 2000). Risk factors of aortic dissection include hypertension, physical trauma, cigarette smoking, prior cardiac surgery, male sex, and genetic disorders, etc. (Goldfinger et al., 2014). In addition, increasing pathologic researches have shown that inflammatory mechanisms are involved in the process of vascular remodeling (Rodriguez et al., 2008; Rodriguez-Menocal et al., 2014), which plays an important role in the development and progression of aortic dissection (Luo et al., 2009; del Porto et al., 2010; Ait-Oufella et al., 2013).

Glucocorticoids have been widely used in clinical practice by reason of the powerful and effective anti-inflammatory properties (Rhen and Cidlowski, 2005; Owens et al., 2014). Previous studies demonstrated that glucocorticoids inhibited the immune-inflammatory responses mediated by macrophages (Usher et al., 2010), vascular smooth muscle cells (Pross et al., 2002) or mast cells (Zhou et al., 2011). To date, however, the effect of glucocorticoids on the incidence and development of aortic dissection was still unclear.

We hypothesized that the level of serum glucocorticoids in aortic dissection patients would be increased; the exogenous glucocorticoids intervention could influence the process of vascular remodeling and lower the incidence of aortic dissection, and thereby allowing us to potentially offer an attractive target for the intervention of aortic dissection.

2. Materials and Methods

The study protocol complied with the declaration of Helsinki, and was approved by the Institutional Review Board of our hospital. The human samples were collected in our hospital, and signed informed consent forms for using the samples in the experiments were obtained from all subjects. The animal experiments were performed conform the NIH guidelines on the protection of animals used for scientific purposes,

* Corresponding authors.
E-mail addresses: zhoujian1-2@163.com (J. Zhou), xueguanky@163.com (Z. Jing).

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and approved by the Institutional Animal Care and Use Committee of our university.

2.1. Human Blood and Aortic Samples Collection

Between October 2012 and December 2013, 82 patients diagnosed with Stanford type B aortic dissection, 68 patients with non-ruptured aortic aneurysm and 76 healthy volunteers were prospectively registered in the study. All the aortic dissection patients were free from connective tissue disorders such as Marfan syndrome, Ehlers-Danlos syndrome and aortitis. Diagnosis of aortic dissection and aneurysm were confirmed by computed tomography angiography. The venous blood was drawn in a fasting state at 8:00 AM on the second morning of hospital admission using minimally traumatic venepuncture. To maintain the biological activity, the blood was immediately stored at 4 °C and centrifuged within 10 min, and the supernatant was transferred to several freezing tubes and stored at −80 °C until being assayed for cortisol (Laboratory Medicine of Changhai Hospital), adrenocorticotropic hormone (ACTH, Phoenix Pharmaceuticals, RK-001-01, California, USA) using radioimmunoassay kit, and for soluble tumor necrosis factor receptor II (TNF-sRII, Raybiotech, ELH-TNFR2, Norcross, GA, USA) using a multiplex, bead based ELISA kit, according to the manufacturer's instructions.

At the same period of time, aortic specimens were obtained from eight aortic dissection patients and eight non-ruptured aortic aneurysm patients, who underwent open surgery for aortic graft. Additionally, healthy aortic specimens were obtained from eight body donation volunteers.

2.2. Entry Tear Number Counting

Entry tear was defined as the communication between true and false lumen that caused entry flow into the patent false lumen. Number of the entry tear was assessed by computed tomography angiography in transverse sections.

2.3. Definitions

Renal insufficiency was defined as the level of plasma creatinine >130 μmol/L. Smoking history was defined as those who had smoked no <100 cigarettes in their entire life. Coronary artery disease included stable angina, acute coronary syndrome, acute myocardial infarction, and major cardiac event.

2.4. Development of Aortic Dissection Model in Mice

Six-month old male wide-type C57BL/6 mice were purchased from the animal centre of our university and randomly allocated to one of the three treatment groups according to the computer-generated randomization sequence stratified by weight: the intervention group received Angiotensin II (AngII, Sigma-aldrich, A9525, St. Louis, MO) infusion followed by intraperionteal administration of corticosterone (Sigma-Aldrich, 27840), the aortic dissection model group received AngII infusion followed by intraperionteal administration of PBS, and the control group received saline infusion followed by intraperionteal administration of PBS. The animals were fed on a regular diet and drinking water with 12-hour dark/light circle for one-month. To eliminate the interference of endogenous glucocorticoids, bilateral adrenalectomy was performed in all mice.

After intraperitoneal anesthesia (ketamine, 5.6×10^{-3} g/mL), the mice received subcutaneous micro-osmotic pumps (Alzet, model 1004, Durect Corp., Cupertino, CA) delivering either saline (control group) or Ang II (intervention group and model group) at 2500 ng/kg/min for 28 days. The administration of either corticosterone (intervention group, 0.015 μg/g) or PBS (model group and control group) was started on the day of micro-pump implantation and administered at 9:00 AM daily until the end of the experiment.

2.5. Aortic Specimen Harvest

Ketamine-anesthetized mice were perfused with PBS via the left ventricle to remove blood from tissue; then the entire aorta from ascending aorta to iliac artery was excised in one piece and placed in sterile PBS. After the periadventitial fat was removed, the aortic specimen was observed and photographed. The location and scope of hematoma was carefully recorded. Then the whole aorta was averagely cut into nine segments in different processing.

2.6. External Aortic Diameter Measurement of Mice

External aortic diameter was measured in three transverse sections using image pro-plus software. Ascending aorta was defined as the proximal 2 mm section from the ostium of the innominate artery, aortic arch as the section between the ostia of left common carotid artery and left subclavian artery, descending aorta as the distal 2 mm section from the ostium of the left subclavian artery (Supplemental Fig. I).

2.7. Histology and Immunohistochemistry

Sections (4 μm) were cut from the paraffin-embedded aortic specimens obtained from the aortic dissection patients, non-ruptured aneurysm patients, healthy body donation volunteers or mice. Human aortic sections were immunostained with rabbit polyclonal antibody against human glucocorticoid receptor (GCR, 1:100, Abcam, ab3580, Manassas, VA, USA), and mouse monoclonal antibody against human macrophage (CD 64, 1:150, Abcam, ab140779). The negative control slides were stained with control IgG (Supplemental Fig. II). Blind evaluation of positive staining percentages was performed in 10 different fields at 400× magnification from two independent pathologists, with an intervariability <5%.

Mouse aortic tissues were stained with hematoxylin and eosin (HE) and Masson's trichrome staining. Photomicrographs were analyzed by investigators blinded to the experimental protocol using image pro-plus software to assess aortic media thickness and collagen volume fraction. Aortic dissection was confirmed by HE photograph, which was defined as the coexistence of true lumen and false lumen, even the thrombus in false lumen. They were also immunostained with rabbit polyclonal antibody against GCR (1:100, Abcam, ab3580), rat monoclonal antibody against macrophage (F4/80, 1:20, Abcam, ab6640) or control IgG.

2.8. Cell culture

Human aortic Smooth muscle cell line (HA-SMC, ScienCell, 6110, San Diego, CA) was cultured in dedicated SMC conditional medium (ScienCell, 1101) supplemented with 2% of heat-inactivated fetal bovine serum (FBS, ScienCell, 0010), growth supplement (ScienCell, 1152), penicillin (100 U/ml) and streptomycin (100 μg/ml) (ScienCell, 0503) at 37 °C in a humidified 5% CO_2 incubator. All cells used in this study were between passages 4 and 6. Human mononuclear macrophage cell line (THP-1, Chinese Academy of Science, TCHu 57, Shanghai, China) was cultured in 1640 medium (Gibco, RPMI 1640, 11875-093) supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100 μg/ml, Gibco, 10378-016) at the same condition of culture.

2.9. Supernatant Cytokine Analysis of THP-1 Cells

THP-1 cells were seeded at 5×10^4 cells per well in 24-well culture plates for 24 h. After incubation with starvation medium (0.5% FBS) for 24 h, cells were intervened with either human cortisol (H-CORT., 10^{-9} to 10^{-5} mol/L, Sigma-Aldrich, HA-8880) or PBS for 48 h. Positive

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