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EXPERIMENTAL STUDY

Influence of Sanao Tang on urine volume and expression of aquaporin 2 in rats with respiratory function impairment induced by passive smoking and lipopolysaccharide

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Abstract

OBJECTIVE: To investigate the effect of Sanao Tang (SAT) on urine volume and the expression of aquaporin-2 (AQP2) in rats with lung dysfunction induced by passive smoking and lipopolysaccharide.

METHODS: Totally 45 healthy Specific pathogen Free Wistar Rats were randomized into 3 groups: normal control group, model group and SAT group. A rat model of respiratory dysfunction induced by exposure to cigarette smoking and lipopolysaccharide (LPS). Lavage of decoction of the Chinese medicine was performed for rats in the SAT group. Anires 2005 System was used to analyze the pulmonary function. Urine of rats was collected through metabolism cage method. Enzyme-linked immunosorbent assay (ELISA) was used to determine content of antidiuretic hormone (ADH), angiotensin Ⅱ (Ang Ⅱ), atrial natriuretic factor (ANP), endothelin 1 (ET-1), nitric oxide (NO) and prostaglandin E2 (PGE2) in serum, lung and kidney. The expression of AQP2 in rat renal tissue was determined with real-time fluorescence quantitative PCR (RT-PCR).

RESULTS: (a) In comparison with the normal control, It was found that enforced vital capacity (FVC), 1-second forced expiratory volume/forced vital capacity% (FEV_1/FVC%), 24 h urine volume content of NO and PGE2 were decreased, while AQP2mRNA level and content of ADH, Agn Ⅱ, ANP and ET-1 were increased in the model group with statistical significance ($P < 0.05$). (b) In comparison with the model group, It was found that FVC, FEV_1, FEV_1/FVC%, 24 h urine volume, content of PGE2 and NO decreased, while AQP2mRNA level, content of ANP, ADH and Ang Ⅱ decreased in the SAT group with statistical significance ($P < 0.05$).

CONCLUSION: SAT might effectively regulate the urine volume in the modeled rats; ADH, Ang Ⅱ, ANP, ET-1, NO and PGE2 might play an important role in the regulation on urine volume by lungs. This might be the mechanisms underpinning the function of lung governing water passage in terms of the theory of Traditional Chinese Medicine.

Key words: Regulation of water passage; Lung *Qi*; Urine; Aquaporin 2

INTRODUCTION

Respiration is one of the biological processes necessary for maintaining metabolism, homeostasis and vital ac-

tivities.[1] Lung is the most important organ in the respiratory system, and the place that air-exchange takes place.[2] Besides breath function, lung also governs water passage and participates in regulation of water metabolism as argued by Traditional Chinese Medicine (TCM). Lung governing water passage means that the effects of dispersing and descending of lung have functions to dredge and regulate the water in human body, including liquid carried by urine, sweats and respiration. Some biological active substances may be elevated or activated when pulmonary hypoxia occurs, thereby resulting in pulmonary vasoconstriction. Many substances causes hypoxic pulmonary artery vasoconstriction and most of them are pulmonary vasoconstriction factors, such as antidiuretic hormone (ADH), angiotensin Ⅱ (Ang Ⅱ), atrial natriuretic factor (ANP), endothelin 1 (ET-1), nitric oxide (NO) and prostaglandin E2 (PGE2). Some of them may act upon kidneys directly or indirectly, inducing variation of renal blood flow, glomeruar filtration rate (GFR) and water re-absorption.[3-5]

It was reported that respiratory and renal physiology and pathophysiology has closely been related to various aquaporins (AQPs) systems. For example, the list of diseases known to involve AQPs now includes: early onset of cataracts, Sjogren's syndrome, cerebral and pulmonary edemas, cirrhotic liver development of ascites, and congestive heart failure (CHF),[6] Yu *et al* [7] found that AQP2 plays an important role in the occurrence of peripheral edema in paitets with acute exacerbations of chronic obstructive pulmonary disease, which are related to vasoconstriction. Sanao Tang (SAT), a representative of the prescriptions for ventilating the stagnated lung *Qi* in TCM, is usually used to treat respiratory system diseases and other illnesses caused by abnormality in Lung *Qi*. We established a model of lung defunction of rats treated with fumigation in combination with lipopolysaccharide (LPS), aiming to observe the function of SAT on their urine volume and AQP2, discuss biological active substances' variation correlating to regulation of urine volume, clarify the relationship between lung governing *Qi* and lung governing water passage, reveal the biological mechanism of lung governing water passage, and provide the scientific basis for the clinical application of this theory.

MATERIALS AND METHODS

Preparation of SAT

The prescription of SAT [containing Mahuang (*Herba Ephedra Sinica*), Kuxingren (*Semen Armeniacae Amarum*), Gancao (*Radix Glycyrrhizae*) and Jiegeng (*Radix Platycodi*)] was purchased from Beijing Tong Ren Tang Pharmacy. Dosage of the crude prescription was converted from that for human adults (9 g/day) based on body surface.[8] Conversion method based on the ratio of the surface area of the critter to its body weight, so the dose of crude drug is 0.162 g every day. In our experiment, the average weight of rat is 250 g, and our crude drug dosage is 0.2 g/day. Routine decoction was performed in preparing the TCM prescription.

Experimental animals

Sixty male Wistar rats were purchased from Beijing Wei tong Li hua experimental Animals Inc., [Qualification Certificate No. SCXK (JING) 2012-0001], and bred in Laboratory Animal Room in Beijing University of Chinese Medicine. The animals were accommodated for 1 week under (23 ± 1) ℃. All operations abided by the prescriptions by Animal Ethics Committee of Beijing University of Chinese Medicine.

Animal model and drug administration

The rats were randomized into a normal control, a model group and a SAT group with 15 animals in each group, using the random digital number method. Tracheal injection of lipopolysaccharide in combination with fumigation, as introduced by literature,[9] was used to establish rat model of respiration impairment as follows: on day 1 and day 14, rats were anesthetized with intraperitoneal injection of 1% pentobarbital sodium (40 mg/kg), and fixed on board in supine position. A No.18 vein detained needle was inserted swiftly into trachea through the exposed glottis, its core needle was pulled out, and then LPS (Sigma, Saint Louis, Missouri, USA) 200 μL (1 mg/mL) dissolved in normal saline was injected with one 1 mL syringe. The fixation board was erected and swirled, in order to make LPS distributed evenly in both lungs. 1 h-fumigation b. i. d. (ante meridiem and post meridiem respectively) was performed from day 2 to 28 (except for day 14) by making the rats exposed to cigarette smoke (Brand Da Qianmen, Beijing, China) in concentration of 5% in an animal fumigation box in a size of 60 cm × 50 cm × 40 cm. The SAT group was given 2 mL SAT soup intragastric during the 21st to 28th day, meanwhile, the model group and normal control group was given the same dose of distilled water.

Determination of urine volume

Urine volume was determined after administration on the 28th day according to methods introduced by literature.[8] Lower abdomen of each rat was tapped gently to let it void without residue urine in 30 min after 20 mL/kg water load was administered to it. Then the rat was put immediately into the metabolic cage for consecutive observation with records of 24 h urine volume.

Detection of respiratory function of rats

Seven rats were selected randomly from each of the two groups for detection of pulmonary function after the urine volume detection on the 29th. The rat was weighed and anesthetized deeply with peritoneal injection of 100 mg/kg pentobarbital sodium, followed by tracheotomy, intubation and ligation. The rat was put into the rat body scan box of the pulmonary function