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Hypotensive effects of renal denervation in spontaneously hypertensive rat based on ultrasonic contrast imaging

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ABSTRACT

Background: Sympathetic nerves-fire rate is generally enhanced in some types of hypertension models. Renal sympathetic denervation(RSD) by the radiofrequency ablation was used to treat the hypertension has achieved curative effect.HTN-1 and HTN-2 trial reported catheter-based renal denervation may cause substantial and sustained blood-pressure reduction in patients with resistant hypertension. However, recent controlled HTN-3 trial questioned the BP lowering effect of Renal denervation treatment. The controversial results maybe arised from the incompleted RSD which implemented inside the renal artery. Now renal denervation therapy for resistant hypertension is in attractive and controversial status. Our aim is to define the hyotensive value of complete renal denervation in adult spontaneous hypertensive rats.

Methods: Male spontaneous hypertensive rats(SHR) aged 12 weeks were randomly selected for either unilateral renal artery sympathetic nerves ablation (URSNA), or conventional technique of renal denervation (CRD), or bilateral renal artery sympathetic nerves ablation (BRSNA) and sham operation. Blood pressure, sodium and water balance,serum reninangiotensin II and Norepinephrine concentration were measured during 20 weeks after renal denervation operation. Internal diameters of renal arteries and renal blood flow rate was tested by ultrasonic contrast imaging.

Results: The continued increased blood pressure in SHR was delayed and significantly reduced by conventional renal denervation over a period of 8 weeks. Both the bilateral and unilateral renal sympathetic nerve ablation procedure did not prevent the development of hypertension in SHR. The attenuation of hypertension was accompanied with the increase of urinary sodium excretion and depression of rennin angiotensin system (RAS).

Conclusions: We concluded that renal denervation may not be an effective therapeutic method in the long-term control of hypertension in adult SHR.

1. Introduction

Although the pathogenesis of essential hypertension is effected by multifactors, sympathetic nervous system activation has been regarded as an important facor to the development of hypertension (Schlaich et al., 2009; Esler et al., 2006). Sympathetic nerves-fire rate is generally enhanced in some types of hypertension models. It is especially conceivable that activation of renal nerves contributes substantially to the physiological regulation of blood pressure(BP) (Bertog et al., 2012). The kidneys are endowed with abundant innervation of sympathetic nerves extending to the vasculature and tubules (Abdulla et al., 2008).

Experimental animal models and human investigations indicated that renal sympathetic nerves contribute to the pathogenesis of hypertension,depending on the setting. The physiologic effect may be mediated by activation of the efferent or afferent sympathetic renal nerves (Strazzullo et al., 2003; DiBona, 2002; Biaggioni, 2003). The technique using radiotracer-driven measurements of the appearance rates of norepinephrine in different organs has

Abbreviations: RSD, Renal sympathetic denervation; URSNA, unilateral renal artery sympathetic nerves ablation; CRD, conventional technique of renal denervation; BRSNA, bilateral renal artery sympathetic nerves ablation; SHR, spontaneous hypertensive rats; RAS, rennin angiotensin system; BP, blood pressure; ELISA, enzyme linked immunosorbent assay; HPLC, High Performance Liquid Chromatography; SBP, systolic blood pressure; DBP, distolic blood pressure.

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revealed a disproportionate increase in sympathetic activity to the heart and kidneys in hypertension, with half of the increase in noradrenaline being accounted for by the increased SNA to the heart and the kidneys (Malpas et al., 2006). Thus, disruption of sympathetic nerves and renal sympathetic nerves has long been considered an attractive therapeutic target for hypertension (Katholia et al., 2009).

Catheter-based renal sympathetic denervation (RSD) for resistant hypertension received great attention when the safety and proof-of-principle study was published in 2009 showing a marked BP lowering effect and only few complications (Krum et al., 2009). One year later, the randomized, but unblinded Symplicity HTN-2 study demonstrated very promising results (Esler et al., 2010). The Symplicity HTN-3 study, which was a prospective, randomized (2:1) sham vs. procedure, single-blinded study. Surprisingly, the study showed that both the RDN group and the sham procedure group had significant, but similar decrease in BP (Bhatt et al., 2014). It is therefore questioned whether RSD treatment is indeed effective.

Some researchers concluded that BP non-response to renal denervation in HTN-3 study may commonly be due to suboptimal denervation by operators inexperience and lack of training and skill (Esler, 2015). Preselection of patients remained problematic in many clinical researches. There are no exact methods to evaluate the specific effects of the RSD procedure which constitute a significant problem (Messerli and Bangalore, 2014). However,we can ablate the renal sympathetic nerves as direct viewing and verify the effect of RSD in the animal models.

Our aim in this study was to assess the hypotensive effects of renal denervation in adult growing SHR, and then we investigate the therapeutic value of renal denervation in established hypertension.

2. Material and methods

2.1. Animals

Animals were supplied by the Animal Experimental Center of Fuwai Hospital. All procedures were in compliance with the "Guidelines for Ethical Care of Laboratory Animals" and were approved by the Institutional Animal Care and Use Committee of Fuwai Hospital. Male spontaneous hypertensive rats, 12 weeks old, were randomly separated into three groups, which received three different procedures: conventional technique of renal denervation (CRD; n = 18), unilateral renal sympathetic nerves ablation (URSNA;n = 18), bilateral renal sympathetic nerves ablation (BRSNA;n = 18) and sham operation(Sham, n = 12). Six rats from each group were housed in metabolic cages (described below), the rest were in normal cages, with all cages having a controlled temperature($23 \pm 1\,^{\circ}C$) and lighting(under a 12:12-h light-dark cycle).

2.2. Surgical procedures

Overnight fasted rats were anaesthetized with pentobarbital sodium at a dose of 60 mg/kg (i.p.). A ventral midline abdominal incision was made to expose the renal arteries and veins. Then, with a dissecting microscope, the main renal artery was isolated from the renal vein. The proximal renal artery 3 mm parted from abdominal aorta's bifurcation was clamped by the clamping radiofrequency probe (Fig. 1).

Care was taken to avoid damage to the renal artery. Left renal sympathetic nerves were ablated in the URSNA. Both left and right renal sympathetic nerves were ablated in the BRSNA. The sham operation was performed by exposing the kidneys and gently manipulating the renal arteries and veins.

Radiofrequency ablations lasted up to 1 min each and 5 W to obtain the rotational ablation with each renal artery in URSNA and BRSNA.CRD was performed by stripping the renal artery out of its adventitia. All visible renal nerves passing from the celiac and aortico-renal ganglia to the kidney were isolated, dissected and cut. The remaining covering tissues were coated with a solution of 10% phenol in absolute alcohol as described previously (BelloReuss et al., 1975). During the 3 days after surgery, the adult SHRs were given an intramuscular antibiotic injection of 2.5 mg gentamycin and a subcutaneous injection of 0.075 mg butorphanol tartrate for analgesic purposes (Table 1).

2.3. Blood pressure and water-sodium balance

All the rats' basal body weight and blood pressure were determined before the morning surgical procedure and once per week post procedure. Blood pressure and heart rate were measured by the tail-cuff method with an instrument (softron/BP-98AL, Japan). A mean index of the measurements was created for each of the three groups. Six rats from each group were housed in metabolic cages individually. The rats' urine output, food, water intakes and urinary sodium were measured 1 day before operation and 2,4,6 and 8 weeks after operation. 24-h sodium intake was obtained by multiplying the food intake by the sodium content of the diet (0.07 mmol/g). Urinary sodium concentration was measured by a NOVA-5 sodium-potassium analyzer (Biomedical; Waltham, MA). 24-h urinary sodium excretion was calculated as the product of urine output and urine sodium concentration. Water and sodium balance was calculated as the difference between intake and urinary excretion of sodium and water.

Six rats from each group were killed by an overdose of pentobarbital at 4 and 8 weeks after operation. Blood samples(4 mL) and kidneys were obtained from inferior caval vein. The blood samples were subsequently centrifuged for 15 min at 1500 rev/min by a cooling ($4\,^{\circ}C$) centrifuge. The layer was decanted and subsequently stored at -80° C. Secretion of serum rennin activity, angiotensin and norepinephrine was later quantified with enzyme linked immunosorbent assay (ELISA) kits following the manufacturer's instructions (Table 2).

2.4. Verification of renal denervation

Internal diameters of renal arteries and renal blood flow rate were test by ultrasonic contrast imaging. Puncture the caudal vein with a needle. Imaging was conducted by using an Esaote Technos MPX DU8 color ultrasonographic device (Esaote, Italy). The probe was placed on the plank of the rat, and the position of the probe was adjusted after the clarity of the two-dimensional image, so that can obtain the largest long axis section of the kidney and make the renal artery display clearly. A total of 0.1 mL ordinary ultrasound microbubble Sulphur Hexafluoride Microbubbles was injected into the caudal vein of rat respectively, and then washed with saline.

Completeness of renal denervation was quantified by assaying for renal norepinephrine content. The kidneys were weighed, wrapped in aluminum foil, immediately frozen in liquid nitrogen, and then stored at $-70\,^{\circ}C$ until subsequent assay. Norepinephrine was extracted from the tissue and assayed for norepinephrine content by High Performance Liquid Chromatography(HPLC)with electrochemical detection, performed as previously described (Trostel and Osborn, 1992).

2.5. Statistical analysis

Continuous variables are given in mean and standard deviation. The change in blood pressure over time was analyzed by 2-way ANOVA for repeated measures (the first factor being the treatment

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