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Chemical denervation of the renal artery by vincristine in swine. A new catheter based technique

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ABSTRACT

Background: Renal sympathetic denervation is a promising technique for the treatment of resistant hypertension. We evaluated a novel method for chemical sympathetic denervation of the renal artery by local delivery of vincristine, an antineoplastic drug with potential for peripheral neurotoxicity, using a dedicated catheter in an animal model.
Methods: Local delivery of vincristine by a specially designed catheter, was performed unilaterally in the renal arteries of 14 juvenile Landrace swine. The procedure was then repeated in the contralateral renal artery using a placebo mixture. Animals were euthanized at 28 days and histological specimens of renal arteries and perirenal arterial stroma containing renal nerves were extracted and sectioned. The number of uninjured nerves in each histological section was then quantified, following identification by immunohistochemical staining.
Results: In all animals delivery of vincristine and placebo mixtures was successful and uncomplicated. Both vincristine- and placebo-treated renal arteries were angiographically patent at the end of the procedure. The mean number of intact nerves in all sections was significantly lower in the group of vincristine ($p<0.05$).
Conclusions: Catheter-based delivery of vincristine in the renal artery of an experimental model is feasible and results in significant reduction in the number of renal nerves. Our findings warrant further confirmation in animal and human studies.

1. Introduction

Effective treatment of arterial hypertension remains a worldwide concern, despite the availability of new pharmaceutical agents and lifestyle changes [1]. It is estimated that 30–40% of the adult population in the developed world suffers from this condition and that the prevalence of resistant hypertension-defined as blood pressure ≥140/90 mm Hg, despite the concurrent use of three antihypertensive agents, at full doses, one of them being a diuretic- is 12–15% of the treated hypertensive population [2–5].

There is accumulating evidence that renal efferent sympathetic nerves and afferent sensory nerves that lie at the wall of the renal artery play a crucial role in the initiation and maintenance of systemic hypertension [6,7], and that norepinephrine-containing renal sympathetic nerve terminals make contact with the renal tubular epithelial cell basement membrane, suggesting a closer relation between neural control of the kidney and arterial hypertension [8]. The denervation of the renal sympathetic nervous system for the treatment of resistant hypertension has been the point of interest for many years, and non-selective surgical sympathectomy, had been used with unsatisfactory results due to its side effects [9]. Recently-developed endovascular catheter technology enabled selective denervation of the human kidney, with radiofrequency energy delivered to the renal artery wall, targeting the renal nerves located in the adventitia of the renal arteries [10,11]. Early clinical results demonstrated the efficacy of this technique in producing renal denervation and significant reductions in blood pressure over a 12 month period [12]. However, several concerns have been raised over the possible side effects of radiofrequency denervation [13] and the regenerative potential of the afferent sensory fibers of the renal nerves [14].

In order to address these limitations, the local delivery of neurotoxic agents has emerged as an alternative method for renal denervation. Vincristine is an antineoplastic drug with a broad spectrum of activity against hematological malignancies and childhood sarcomas. Moreover, vincristine has potent neurotoxicity by causing giant axonal swellings and secondary demyelination of the paranodal type mainly in the proximal portions of the peripheral nerves outside the spinal canal [15–19].

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The aim of this study was to evaluate in an animal model, a novel method for chemical denervation of the renal artery by local delivery of vincristine using a dedicated catheter.

2. Methods

2.1. Animals

This protocol was approved by the Institutional Laboratory Care and Use Committee and was conducted in accordance with regulatory guidelines for the care of laboratory animals. We used 14 juvenile Landrace swine (3–5 months old, weight 28 ± 0.5 kg).

2.2. Catheter

In order to create a dedicated system for the delivery of vincristine into the vascular wall, we used a conventional non-compliant balloon angioplasty catheter. The catheter was modified in the laboratory of the 1st Department of Cardiology, University of Athens by creating circumferentially across the balloon 6 sideholes of 25 μm diameter, in fixed intervals of 60°.

2.3. Mixture preparation

For local delivery and in order to allow for intraprocedural visualization, vincristine was diluted to a final concentration of 25 mg/l in a mixture consisting of 20 ml of sterile 0.9% NaCl solution and 20 ml of contrast. The mixture used for the placebo procedure, contained 20 ml of sterile 0.9% NaCl solution and 20 ml of contrast, without the addition of vincristine. A schematic representation of the catheter is presented in Fig. 1.

2.4. Procedure

Animals underwent catheterization under general anesthesia with continuous monitoring of the vital signs. A 6Fr introducer sheath was inserted though the right femoral artery. An angiogram was performed in both renal arteries using a 6Fr JR4 guiding catheter in order to evaluate the preprocedural anatomy of the arteries and define the desired segment for chemical denervation. A guidewire was advanced to the distal segment of the renal artery. The modified balloon catheter (4–5 mm in diameter, 20–25 mm in length, dependent on the length of the main branch of the renal artery) was advanced in the main branch of the renal arteries. The diameter of the balloon for both treated and control segments was selected on a 1:1 ratio according to the diameter of the target vessel. The non-compliant balloon was then inflated at 20 atm to cover the target segment, using the mixture containing vincristine, allowing for delivery of vincristine into the vascular wall. The inflation was interrupted when a total of 4 ml of the mixture (containing 0.1 mg of vincristine) was delivered. The procedure was then repeated in the contralateral renal artery using the placebo mixture for inflation. The femoral catheter was used to obtain blood samples and to monitor the mean arterial pressure and heart rate, before, immediately after the procedure and at follow-up. The mean arterial pressure and heart rate, derived from pulsatile arterial pressure, were recorded continuously during the procedure. All angiograms before and after the procedure were recorded on video. Blood samples were drawn before, immediately after and 28 days after the procedure in order to determine serum creatinine levels.

2.5. Tissue processing

Animals were euthanized 28 days after the index procedure and both kidneys, including the renal arteries and the segment of the abdominal aorta containing the ostia of the renal arteries were explanted. Explanted renal arteries and kidneys were flushed using pressure perfusion with saline, followed by 10% buffered formalin. The renal arteries were carefully dissected from the kidney, and embedded in methyl acrylate after processing. Macroscopic examination for evidence of injury, thrombus or restenosis was then performed in the renal arteries. The renal arteries and perirenal arterial stroma containing renal nerves were sectioned transversely at 8 equal intervals of 2 mm.

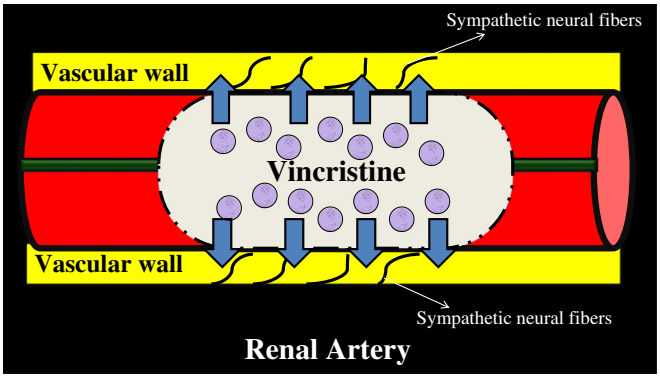

Fig. 1. Schematic representation of the release of the vincristine by the modified balloon catheter into the vascular wall.

2.6. Histopathology

Staining of the histological slides was performed with standard eosin and hematoxylin stain. Histological slides were reviewed by two independent, experienced pathologists (GA and EP), and a consensus report was generated. Pathologists were blinded to the treatment allocation of each renal artery. The analysis and measurements were performed separately in each section. Histopathologic assessment of the renal arteries included evaluation for significant stenosis, luminal and mural thrombosis or intramural hemorrhage. The intima and the media of the renal arteries were also assessed for disruption or injury.

2.7. Immunohistochemical analysis

Immunohistochemical staining was performed for the visualization of the nerves using a specific antibody for neuron-specific enolase (Cell Science, Canton, MA, USA). The total number of nerves in the vessel wall was quantified in each histological section. Results are expressed as the mean number of nerves identified within the sections.

2.8. Statistical analysis

Statistical analysis was performed with commercially available software (SPSS Statistics Version 19, SPSS Inc., Chicago, Illinois). Quantitative data are presented as mean ± SD. Probability values are two-sided from the paired t-test for continuous variables. For immunohistological measurements, inter- and intra-observer variability was assessed by reanalysis of a representative sample of histological sections. A value of $p<0.05$ indicated statistical significance.

3. Results

3.1. Procedural data

In all animals delivery of vincristine and placebo mixtures was successful and uncomplicated. There were no acute or short-term complications related to the intervention. Serum creatinine was 1.37 ± 0.12 mg/dl before the procedure, 1.41 ± 0.09 mg/dl after the procedure, and 1.36 ± 0.24 mg/dl 28 days after the procedure (p = NS for all comparisons). A representative case of the procedure is depicted in Fig. 2. Angiographically, diffusion of the contrast medium beyond the lumen borders was evident (Fig. 2C) implying delivery of the mixture in the deeper layers of the vascular wall. Mean duration of the inflation was 47 ± 15 s for the vincristine group versus 48 ± 12 s (p = NS) for the placebo group. All vincristine- and placebo-treated renal arteries were angiographically patent at the end of the procedure, without evidence of thrombus formation, abrupt occlusion of the arterial segments or aneurysmal dilatation of the target segments at the post-procedural angiography. The mean arterial pressure did not change before, after the procedure and at follow-up (114 ± 7, 112 ± 7 and 113 ± 6 mm Hg respectively, $p = 0.94$). Similarly the heart rate did not change before, after the procedure and at follow-up (184 ± 7, 184 ± 6 and 184 ± 4 bpm, $p = 0.75$).

3.2. Histological analysis

All of the animals survived until 28 days after the procedure, when they were euthanized. All kidneys and renal arteries were successfully harvested post-mortem and sent for histological analysis.

A total of 112 sections were assessed in each treatment group. Histological analysis did not show any evidence of significant stenosis, luminal or mural thrombosis, or intraplaque hemorrhage. In all arteries, i.e. placebo- and vincristine-treated, there was no evidence of intimal damage (occupying at least 10% of the intima) or intimal hyperplasia. Medial damage was not observed in any if the treated arteries, as well.