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Therapeutic resistance to angiotensin converting enzyme (ACE) inhibition is related to pharmacodynamic and -kinetic factors in 5/6 nephrectomized rats

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Abstract

Proteinuria plays a pathogenic role in the development of end stage renal disease. Angiotensin converting enzyme (ACE) inhibitors lower proteinuria and are renoprotective. However, large inter-individual variation in antiproteinuric response to ACE inhibitors exists. In this study, we explored the mechanism of therapeutic resistance to an ACE inhibitor in the rat 5/6 nephrectomy model. At week 6 after 5/6 nephrectomy, treatment with lisinopril was initiated for 6 weeks. Proteinuria and blood pressure were evaluated weekly. At the end of the experiment, rats were divided into tertiles according to their antiproteinuric response: (1) responders ($n=9$), (2) intermediate responders ($n=8$) and (3) non-responders to ACE inhibitor therapy ($n=9$). At the start of treatment, proteinuria had progressively increased to 154 (95% confidence interval [CI]: 123–185) mg/24 h in the entire cohort, with comparable proteinuria and blood pressure in all groups. Following treatment with ACE inhibitor, proteinuria was significantly lower in the responders (68, CI: 46–89 mg/24 h) compared to the non-responders (251, CI: 83–420) mg/24 h). Similarly, blood pressure was reduced in the responders, but unaffected in the non-responders. At autopsy, renal ACE activity and renal ACE expression were significantly lower in the responders compared to the non-responders. Although lisinopril intake was comparable in all animals, urinary drug excretion was increased in the non-responders, demonstrating increased drug clearance. Average urinary lisinopril excretion was correlated with antiproteinuric response ($R^2=0.32$, $P=0.003$). In conclusion, both pharmacodynamic and -kinetic factors account for the non-response to lisinopril. Whether these can be overcome simply by increasing drug dosage in non-responders should be investigated.

Keywords: Angiotensin converting enzyme inhibitor; Renal ACE activity; Therapy response; Proteinuria; 5/6 Nephrectomy

1. Introduction

Angiotensin converting enzyme (ACE) inhibitors improve renal outcome in renal disease, in humans as well as in the experimental setting (Adamczak et al., 2004; GISEN Group, 1997; Maschio et al., 1996; Remuzzi et al., 1995, 2005). Both the reduction in proteinuria and blood pressure appear to play a role in the prevention of structural renal damage. However, not every patient with chronic renal failure benefits from ACE inhibitors optimally (Haas et al., 1998), due to the large inter-individual variation in therapy response to these drugs, which seems only marginally modulated by changing dose within the recommended range or the class of drug (Bos et al., 2000, 2002). Because the reduction in proteinuria is correlated to renal prognosis (Apperloo et al., 1994; Wapstra et al., 1996), it is of the utmost importance to reduce proteinuria to the lowest possible level. Insight in the mechanisms of the resistance to the antiproteinuric efficacy of ACE inhibitors may provide clues for

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optimizing therapy and renal prognosis in patients with chronic renal failure.

Different mechanisms underlying therapy resistance to ACE inhibitors, have been identified both in clinical and experimental settings. Therapy response to ACE inhibitors has been shown dependent on sodium status (Kramer et al., 2006), ACE gene polymorphism (Scharplatz et al., 2005), activation of the renin angiotensin aldosterone system (RAAS) (Buter et al., 1998), and the extent of renal damage prior to ACE inhibition therapy (Kramer et al., 2003). It is however unclear to what extent these factors alter pharmacodynamic or -kinetic properties of ACE inhibitors. In previous experiments, we found a large variation in therapeutic response to lisinopril in 5/6 nephrectomized rats. As in untreated animals, renal ACE expression predicts the progression of renal disease (van Dokkum et al., 2003) and in humans renal ACE is up regulated in renal disease (Metzger et al., 1999), we hypothesized a critical role for renal ACE activity in therapy resistance to ACE inhibitors. To test this hypothesis, we compared renal ACE expression and ACE activity in responders and non-responders to lisinopril treatment in 5/6 nephrectomized rats. With regard to possible differences in the pharmacokinetic profile explaining therapeutic resistance to ACE inhibitors, we investigated drug concentrations and excretion. Renal ablation by 5/6 nephrectomy is a hypertensive model provoking both renal and cardiac damage (Amann et al., 2000; Dikow et al., 2004; Raine et al., 1993). While ACE inhibitors exert beneficial effects on both the heart and the kidney, its effects on cardiac parameters were measured as well to investigate variation in cardiac therapy response.

2. Materials and methods

2.1. Experimental protocol

Male Wistar rats (275–350 g; $n=33$) were housed under standard conditions with free access to food and drinking water. Rats received a standard chow diet. Animal experiments were approved by the institutional animal ethical committee. One rat died within 24 h after the surgical procedure.

At initiation of the experiment ($t=0$ weeks), animals underwent 5/6 nephrectomy. After 6 weeks, the animals were randomized based on their proteinuria prior to drug treatment into a group subsequently treated with lisinopril (Merck Sharp & Dohme, Haarlem, The Netherlands) 2.5 mg/kg/day in the drinking water (ACE inhibitor, $n=26$), and a vehicle treated group (vehicle, $n=7$). In both groups, the experiment was terminated 12 weeks after nephrectomy. To standardize drug intake in individual animals at the end of the experiment, the final dose of lisinopril (2.5 mg/kg in 500 μl water) or vehicle was administered by gavage 24 h before sacrification. At the end of the experiment, at a through level of lisinopril, functional cardiac parameters were measured under 2.5% isoflurane anesthesia, laparotomy was performed and renal blood flow was measured, followed by exsanguination by taking blood samples from the abdominal aorta for plasma measurements. The remaining kidney was flushed with saline and the heart and kidney were removed, weighed and processed further.

2.2. Surgical interventions

5/6th Nephrectomy was performed under anesthesia with 2.0% isoflurane in N_2O/O_2 (2:1) as described before (Johnston et al., 1983; Leenen et al., 1999; Schoemaker et al., 1991). Shortly, the right kidney was removed after ligation of the renal artery, vein and urethra. In addition, the proximal branch of the left renal artery (often responsible for 2/3 of the blood supply to the kidney) was ligated upon and interruption of 2/3 of the blood supply to this kidney was determined by visual inspection. If necessary, additional (smaller) branches of the renal artery were ligated.

2.3. Functional cardiac and renal characteristics

Cardiac performance was measured with a pressure transducer catheter under anesthesia, using 2.5% isoflurane in O_2, through the right carotid artery (Micro-Tip 3French, Millar Instruments Inc., Houston, TX, USA), connected to a personal computer equipped with an analog-to-digital converter and appropriate software (Millar Instruments, Germany). After a 3-min period of stabilization, left ventricular end-diastolic pressure, left ventricular end-systolic pressure and heart rate were recorded. Thereafter, the catheter was withdrawn into the aortic root to measure central systolic blood pressure. As a parameter of global myocardial contractility and relaxation, we determined the maximal rates of increase and decrease in left ventricular pressure (systolic $+dP/dt_{max}$ and diastolic $-dP/dt_{max}$), which were normalized to left ventricular pressure change (*i.e.*, left ventricular end-systolic pressure – left ventricular end-diastolic pressure) for individual rats (Windt et al., 2006).

Renal blood flow was measured using a 1 mm flow probe around the left renal artery (1RB; Transonic, Ithaca, NY, USA), connected to a flow meter (T106 Small Animal Research Flow meter, Transonic, Ithaca, NY, USA).

2.4. Histology

Kidneys were fixed by immersion for 48 h in a 4% buffered formaldehyde solution (Klinipath, Duiven, The Netherlands) after longitudinal bisection and subsequently embedded in paraffin according to standard procedures.

2.4.1. Focal glomerulosclerosis

Sections of 3 μm were stained with periodic acid Schiff (PAS). The degree of focal glomerulosclerosis was assessed in 50 glomeruli by scoring semi-quantitatively on a scale of 0 to 4. Focal glomerulosclerosis was scored positive when mesangial matrix expansion and adhesion to Bowman's capsule was present in the same quadrant. When 25% of the glomerulus was affected, a score of 1+ was adjudged, 50% was scored as 2+, 75% as 3+ and 100% as 4+. Overall focal glomerulosclerosis score is expressed as arbitrary units with a