Time-course characterization by DCE-MRI of kidney dysfunction in rats over-expressing the human renin and angiotensinogen genes

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Abstract

This *in vivo* study explored the time-course development of kidney failure in a high renin-high angiotensin rat model of hypertension using DCE-MRI. Obtained results allowed to identify changes in tubular reabsorption of water as early signs of kidney dysfunction as measured from an index of glomerular dysfunction.

Introduction

Double transgenic rats (dTGR) harbouring the human renin and angiotensinogen genes are considered as a relevant model of hypertension to investigate a new class of hypertensive agents called renin inhibitors, to which wild-type rodents do not respond. The lifespan of dTGR generally does not exceed 7 to 8 weeks of age, and while dying of heart failure, dTGR also quickly develop severe kidney dysfunction which itself participates in the maintenance of hypertension (1). Acting at the top of the renin angiotensin system, renin inhibitors may be able to induce renal vasodilation beyond that of other anti-hypertensive agents, conferring them potential renoprotection properties (2). To verify this, it is important that development of kidney failure be first well defined in terms of its relation to hypertension in dTGR. Therefore, the objective of this study was to monitor the development of kidney dysfunction in dTGR under preventive treatment with enalapril, an angiotensin converting enzyme inhibitor which curtails the conversion of Angiotensin I to the powerful vasoconstrictor Angiotensin II. Kidney clearance was here assessed by using our previously published method to measure Gd(DTPA) tracer uptake and release within specific areas of the kidney, e.g. cortex, inner and outer medulla (3) and by assuming that the paramagnetic agent was essentially cleared through the kidney.

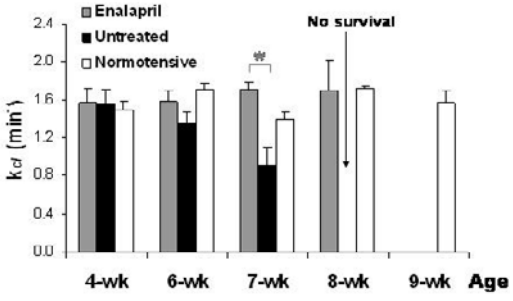
Methods

Experiments were carried out on untreated dTGR, enalapril-treated dTGR (40mg enalapril/liter of drinking water, onset of treatment at 4 weeks of age) and normotensive Sprague-Dawley rats at 4 different time-points (i.e. 4, 6, 7 and 8-week old). For each rat, blood pressure was accurately measured during the imaging session by means of an ultraminiature MRI compatible catheter inserted in the carotid artery (Millar Instruments). MR measurements of renal clearance were made under anesthesia with 2% isoflurane. Images were obtained on a Pharmascan 4.7T/16cm magnet equipped with a 90mm i.d. gradient system (max. strength 300mT/m) and using a 62mm ^{1}H volume resonator. After slice positioning using orthogonal scout scans, 256 consecutive snapshot images (TR=6.72ms, TE=2.6ms, SW 200kHz, FOV 5x5cm, matrix 128x64, slice thickness 1.2mm, flip angle 25°) were collected in the coronal plane of the animal. Motion artefact in images was minimized with sufficient signal averaging (16 averages) resulting in a 6s acquisition time per image. Immediately after acquiring the 10th image of the series, 84 µmoles/kg of Gd(DTPA) (or ~167 µl/kg of Magnevist) were injected in the tail vein and then flushed with 0.5ml of saline within 2 s. Time-course changes in signal intensities were translated into local Gd(DTPA) concentrations assuming a linear dependence with signal enhancement as described previously (3). Ultimately, transverse and longitudinal relaxation times as well as region-specific Gd(DTPA) relaxivities were used as fitting parameters to describe the time-course of changes in Gd(DTPA) concentration in selected areas of the kidney. The kidney clearance index was derived from the first-order rate constant k_{cl} according to $dc_m/dt=k_{cl}c_c(t)$ and assuming that the tracer transport from cortex to medulla was dominant during the initial phase (typically during the first 30 sec after injection of the contrast agent). We hypothesized that an in-depth kinetic analysis of Gd(DTPA) concentration curves would make possible to inquire into additional complex mechanisms of kidney function, particularly those occurring after glomerular filtration. In formulating the model, the assumption was made that any change in Gd(DTPA) concentration beyond glomerular filtration would primarily reflect modifications in the water reabsorption of the specific renal region investigated. In other words, the amount of water loss (i.e. water infiltrating the interstitial space) would be reflected by the transient Gd(DTPA) concentrations while it passes through the proximal tubules, Henle's loop, distal tubules and collecting duct. Therefore, a careful analysis of Gd(DTPA) concentration curves may help detect impairment of renal function caused by tubular failure. Such analysis was conducted by fitting concentration data obtained from the cortex and the outer medulla against a linear function, due to flow-dominated clearance, during both an early phase (i.e. EP*slope* determined during the first 30 sec after peak Gd concentrations are reached) and a later phase (i.e. . LP*slope* determined from 3 to 7 min post Gd(DTPA) injection). All data are presented as means±SE.

Results

The mean arterial blood pressure (MAP) increased by 60% (i.e. ~100 to 160 mmHg, $p<0.05$) between 4 weeks and 7 weeks of age. In untreated dTGR, whereas MAP was well controlled in enalapril treated rats (~80-90 mmHg). Consistent with this, there was a progressive decrease in glomerular filtration (Fig. 1) of non-treated dTGR already visible at 6 weeks of age (K_{cl} -10% vs. enalapril, ns), becoming highly significant at 7 weeks of age (K_{cl} -40% vs enalapril, $p<0.05$). A significant delay in time to peak (TTP), a marker of tracer uptake, was measured in every kidney region of untreated dTGR as young as 6-week old. Such a delay was successfully normalized by enalapril treatment. For every rat, initial clearance in both the cortex and the outer medulla was always faster (e.g. average slope 0.026±0.011 mM [Gd]/sec in the cortex of 6-week old rats) than the long-term clearance (e.g. average slope $7.16\text{x}10^{-4}\pm3.32\text{x}10^{-4}$ mM [Gd]/sec in the cortex of 6-week old rats) caused by decreasing amount of water due to reabsorption along the tubule, in line with our assumption of flow-dominated clearance. At 6 weeks of age, the LP*slope* (short-term clearance) was reduced in the cortex (-39%, p value = 0.019) and the outer medulla (-72%, p value = 0.012) of untreated dTGR vs. controls. On the contrary, at 4 weeks of age, the LP*slope* (long-term clearance) was higher in the cortex (~3 fold, p value = 0.0362) of untreated dTGR vs. controls. Enalapril treatment resulted in a normalization of these slopes.

Fig. 1 K*cl* as an index of kidney filtration



Discussion

This study allowed to characterize in details the time-course development of kidney dysfunction in non-treated dTGR. While a significant decrease in K_{cl} (i.e. GFR) was only apparent in 7-week old dTGR, early signs of an affected clearance were detected already at 4 weeks of age. The slower EP*slopes* measured in 6-week old dTGR may be explained by reduced transit time and water reabsorption in short Henle's loops (4). Due to reduced water reabsorption in the Henle's loop, the increased water flow into the cortex also supports faster LP*slopes* measured in 4-weekold dTGR. Such progressive deterioration of kidney function in dTGR could be prevented by enalapril treatment. These results will prove useful when defining the point of intervention at which renal dysfunction can be stopped or reversed.

References

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