

Signs of a tubular defect preceding the impairment of glomerular filtration in Alport mice as measured by DCE-MRI

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Introduction

Alport syndrome (AS) is an inherited disorder characterized by the progressive loss of kidney function. The glomeruli, along with other structures such as tubules, are gradually replaced by scar tissue, leading to kidney failure. This disease which is most prevalent in young men (X-linked) is caused by gene mutations that affect type IV collagen proteins in basement membranes [1]. While the primary symptom of the disease is the presence of blood in urine, deafness is also frequently observed since type IV collagen proteins are important to the structure of the inner ear. COL4A3-knockout (KO) mice can be used as a non-hypertensive, non-inflammatory animal model of AS [2]. With a lifespan of 10 to 11 weeks, these mice develop a progressive glomerulonephritis with microhematuria and proteinuria, consistent with the human disease. Of note, these previous studies did not fully address the progression of renal dysfunction in these mice, in terms of both glomerular function and tubular water reabsorption. Therefore, kidney function of COL4A3-KO mice was assessed repeatedly by measuring Gd(DTPA) tracer uptake and release within specific areas of the kidney, e.g. cortex, inner and outer medulla [3] and with the assumption that the paramagnetic contrast agent used in this method was cleared mainly through the kidneys.

Methods

Experiments were carried out on male, COL4A3-KO (n=3, 30±2 days, 14.8±0.5 g) and wild type (WT) (n=4, 33±1 days, 17.0±1 g) mice. Each animal was scanned for 7 consecutive weeks. MR measurements of renal clearance were made under anesthesia with 2% isoflurane. Images were obtained on a Pharmascan 4.7 T/16 cm magnet equipped with a 90 mm i.d. gradient system (max. strength 300 mT/m) using a 38 mm ¹H volume resonator. After slice positioning using orthogonal scout scans, 1024 consecutive FISP(TrueFISP) images (TR=3.0 ms, TE=1.5 ms, SW 200 kHz, FOV 3×3 cm, matrix 128×128, slice thickness 1.0 mm, pulse angle 60°, 1 average) were collected in the coronal plane of the animal resulting in a 0.834 s acquisition time per image. Immediately after acquiring the 30th image of the series, 84 umoles/kg of Gd(DTPA) (or ~167 µl/kg of Magnevist) were injected in the tail vein and then flushed with 50 µl of saline within 1 s. Time-course changes in signal intensities were translated into local Gd(DTPA) concentrations assuming a linear dependence with signal enhancement as previously described [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) concentrations in selected areas of the kidney. The kidney clearance index was derived from the first-order rate constant k_{el} according to $dc_m/dt = k_{el}c_c(t)$ and assuming that the tracer transport from cortex to medulla was dominant during the initial phase (typically during the first 10 seconds after injection of the contrast agent). The amount of time required for Gd(DTPA) to reach its peak concentration was denoted as time-to-peak (TTP) which can be used as a marker for tracer uptake. We hypothesized that an in-depth kinetic analysis of Gd(DTPA) concentration curves allows insight 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 primarily reflects modifications in the water reabsorption of the specific renal region being investigated. In other words, the amount of water loss (i.e. water infiltrating the interstitial space) is reflected by the transient concentration of Gd(DTPA) while it passes through the proximal tubules, Henle's loop, distal tubules and collecting duct. 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 after reaching the peak concentration (i.e. $EPslope$ determined during the first 5-8 s after peak Gd concentrations are reached). All data are presented as mean±SEM.

Results

Gd(DTPA) concentration-time curves obtained from the cortex of two representative mice (KO and WT) between 4-wk and 10-wk of age are shown in figure 1. For the WT mouse, tracer uptake and clearance profiles (representative of GFR and tubular function, respectively) remained unchanged with TTP values consistently <17 s throughout the study. On the contrary, the KO mouse showed signs of gradual renal dysfunction starting at 7 weeks of age characterized by a significant delay in the TTP value (>22 s), followed by a plateau of the tracer concentration during washout period, indicative of a defect in water reabsorption. On average, while no changes were detected over time in WT mice (k_{el} 1.27±0.1 min⁻¹), a 73% drop in k_{el} values was observed in KO mice only over the last 3 weeks of their life (p<0.05 one-way ANOVA). Interestingly, there was no detectable change in $EPslope/c$ values (-0.015±0.001 s⁻¹) in WT mice, while tracer elimination in KO mice was impaired already at 5-wk of age and became gradually non-existent by 10-weeks of age (p<0.05) (Fig. 2).

Discussion

This study allowed for a localized assessment of kidney disease progression in COL4A3-KO mice. Unlike WT mice, clear signs of gradual impairment in GFR and tubular water reabsorption were observed in KO animals over their last 6 weeks of life. The progressive deterioration of GFR in these animals may be due to an increased proteolytic degradation of the defective basement membrane, as reported previously [4]. The results on tubular dysfunction support the hypothesis that, fibrotic tissue formed in the tubulointerstitial compartment [5] at an early age (ie before adulthood), resulting in terminal kidney failure. These data may prove particularly useful when defining the point of intervention at which renal dysfunction in this mouse model of AS can be stabilized or reversed.

References

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