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Hyaluronidase 1 and hyaluronidase 2 are required for renal hyaluronan turnover

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ABSTRACT

Hyaluronidase 1 (HYAL1) and hyaluronidase 2 (HYAL2) are the major hyaluronidases acting synergistically to degrade hyaluronan (HA). In the kidney, HA is distributed heterogeneously. Our goal was to determine the consequences of a lack of either HYAL1 or HYAL2 (using specific knockout mice) on renal function and on renal HA accumulation. Experiments were performed in *Hyal1*$^{-/-}$ and *Hyal2*$^{-/-}$ mice and in their wild-type controls. HA concentration was measured in the plasma and kidney tissue and its distribution through the different kidney zones was examined by immunohistochemistry. Relative mRNA expressions of HYAL1, HYAL2 and the 3 main HA synthases were evaluated by quantitative RT-PCR.
Results: Kidney function was not impaired in the knockout mice but they displayed elevated HA concentrations in the plasma and in the kidney. *Hyal1*$^{-/-}$ mice presented an accumulation of HA inside the proximal tubular cells whereas *Hyal2*$^{-/-}$ mice showed HA accumulation in the interstitial space. In the cortex and in the outer medulla, *HYAL1* mRNA expression was up-regulated in *Hyal2*$^{-/-}$ mice. From our study we conclude that somatic hyaluronidases are not required for renal function. However, HYAL1 is necessary for the breakdown of intracellular HA in the cortex, whereas HYAL2 is essential for the degradation of extracellular HA in all kidney regions.

Introduction

Mammalian hyaluronidases (HYALs) are broadly distributed enzymes that display different catalytic mechanisms allowing them to degrade hyaluronan (HA). HA is a glycosaminoglycan (GAG) polymer, a major component of the extracellular matrix which can also be found within cells, mainly in the cytoplasm, nucleus and nucleolus (Hascall and Laurent, 1997). HA is composed of repeating units of the disaccharide [D-glucuronic acid-β1, *N*-acetyl-D-glucosamine]. The number of repeated units can reach 10,000, which corresponds to a molecular weight of 4.10^6 Da (Stern, 2003). It is distributed in most parts of the body including skin, lungs, kidneys and brain. HA is involved in many biological processes, such as cell proliferation, differentiation and migration, depending on its molecular weight. In contrast to most GAGs, HA is synthesized at the plasma membrane, more specifically at the inner face of the cell membrane, by three isoforms of HA synthases (HASs): HAS1, HAS2 and HAS3. HAS1 and HAS2 synthesize high molecular weight HA whereas HAS3 produces lower molecular weight HA (Weigel et al., 1997).

HYAL1 and HYAL2 are considered the major hyaluronidases in somatic tissues, acting synergistically to degrade HA. A catabolic scheme for HA degradation involving these two enzymes has been proposed (Stern, 2003). In this model, HYAL2, anchored to the plasma membrane by a glycosylphosphatidylinositol link (Andre et al., 2011), cleaves high molecular weight HA polymers into intermediate size fragments of approximately 20 kDa. The HA substrate may be presented to HYAL2 by cell membrane receptors such as CD44 (Harada and Takahashi, 2007). Subsequent fragments may be internalized through receptor-mediated endocytosis (Bourguignon et al., 2004). The model postulates that catabolic products of HA are delivered by endosomes to lysosomes where HYAL1 degrades the 20 kDa fragments to smaller units (Stern, 2003). Puissant et al. (2014) have recently shown in macrophages that HYAL1 is a partially secreted enzyme processed through the endosomal compartment to end up in lysosomes where its activity requires non-covalent associations with unknown partners.

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The functional importance of HYAL1 in HA turnover is underlined by the phenotype of patients exhibiting a genetic deficiency of HYAL1 in an autosomal recessive disease named mucopolysaccharidosis IX (MPS IX). These patients suffer from various forms of joint degeneration and synovitis (Natowicz et al., 1996; Imundo et al., 2011). Their circulating HA level is 40 times higher than normal (Triggs-Raine et al., 1999). A mouse model of MPS IX displays progressive alterations in cartilage (Martin et al., 2008). No human case of HYAL2 deficiency has been described to date but *Hyal2*$^{-/-}$ mice (Jadin et al., 2008) display extremely high plasma levels of HA, chronic hemolytic anemia, skeletal anomalies, and sometimes severe cardiopulmonary dysfunction (Chowdhury et al., 2013).

HA may play a role in kidney functions (Stridh et al., 2012). It is highly concentrated in the inner medulla of healthy kidney, but almost absent in the cortex, with intermediate levels in the outer medulla (Hällgren et al., 1990; Hansell et al., 2000). This differential distribution of HA may be key for urine concentration and water reabsorption (Knepper et al., 2003; Stridh et al., 2012). It has been shown that HA can affect physicochemical characteristics of the papillary interstitial matrix and therefore influence the interstitial hydrostatic pressure (Hansell et al., 2000; Rügheimer et al., 2009). During pathophysiological conditions such as diabetic nephropathy (Campo et al., 2010), allograft rejection (Wells et al., 1993), and ischemia-reperfusion injury (Johnsson et al., 1996; Declèves et al., 2006), HA rapidly accumulates in the cortex and the outer medulla and may increase renal damage (Colombaro et al., 2013). HA accumulation in renal post-ischemic settings is accompanied by temporal changes in its level of fragmentation, its molecular weight, and the level of expression of its metabolic enzymes: *HYAL1* and *HYAL2* expression are repressed in the post-ischemic kidney during the first 24 h whereas *HAS1* is transiently up-regulated; later on, *HAS2* takes on an important role in synthesizing increased amounts of HA (Declèves et al., 2012).

From these combined examples it can be concluded that regulation of HA levels is required to maintain tissue homeostasis and is achieved by coordinative expression of HASs and HYALs. Our study was set up to characterize the potential effects of a lack of HYAL1 or HYAL2 in the healthy kidney. To address these points, we characterized renal function and assessed kidney morphology of *Hyal1*$^{-/-}$ and *Hyal2*$^{-/-}$ mice. HA distribution and concentration in the kidneys were also analyzed in both knock-out mice.

Methods

Animals

The study conformed to APS's guiding principles in the Care and Use of Animals and was approved by the Animal Ethics Committee of the University of Namur. Experiments were performed on male age-matched, 8- to 10-wk old adult mice. *Hyal1*$^{+/-}$ mice raised in C57Bl/6 genetic background were purchased from MMRRC (Davis, CA, USA) and mated to obtain *Hyal1*$^{-/-}$ mice. *Hyal2*$^{-/-}$ mice (Jadin et al., 2008) were raised on a mixed genetic background (C129; CD1; C57Bl/6). Mice were genotyped using PCR-based experiment on DNA isolated from ear pieces. As *Hyal1*$^{-/-}$ and *Hyal2*$^{-/-}$ mice differ in their genetic background, we used wild type (WT) *Hyal1*$^{+/+}$ and *Hyal2*$^{+/+}$ littermates as controls, respectively. Mice were kept in a temperature and humidity controlled room, with a light/dark cycle of 12 h. They had free access to tap water and were fed with a standard chow diet (Safe, France). Four groups of male mice were used in the present experiments with a total of 10 animals per group (*Hyal1*$^{-/-}$ vs *Hyal1*$^{+/+}$ and *Hyal2*$^{-/-}$ vs *Hyal2*$^{+/+}$).

Urine collection

Mice housed in individual metabolic cages were allowed a 2-days acclimatization period to ensure they were adapted to their new environment. Subsequently, 24-h urine collection was performed for 2 consecutive days in order to measure diuresis (V). Urine samples were collected under water-saturated oil and centrifuged at 2500 rpm for 10 min at 4 °C to remove sediments. Supernatants were stored at −80 °C until use.

Plasma and tissue collections

At the end of the protocol, mice were anesthetized with an i.p. injection of a mixture of ketamine (80 mg/kg, CEVA, Belgium) and medetomidine (0.5 mg/kg, CEVA, Belgium). Blood was collected by intracardiac puncture and plasma samples were stored at −80 °C. Kidneys were harvested and immediately processed for further analytic purposes.

Osmolarity and electrolyte measurements

Urine osmolarity was measured from freezing point depression using a micro-osmometer (Fiske Model 210 Micro-Osmometer, Norwood, MA, USA). Plasma (P_{Na+}) and urine sodium (U_{Na+}) concentrations were measured using flame photometry (IL943, Instrumentation Laboratories, Lexington, KY, USA).

Renal function

Plasma creatinine concentration was determined using a sensitive and accurate HPLC method (Spherisorb 5 μm SCX column, 4.0 mm × 250 mm; Waters, Milford, MA). Urine creatinine (U_{creat}) was measured based on the Jaffé method, using a creatinine diagnostic kit (Sigma–Aldrich, St. Louis, MO). Urine albumin was measured with a mouse ELISA kit (Albuwell, Exocell, PA, USA). Urine glucose concentrations were determined using a glucometer and test strips (LifeScan Europe, Zug, Switzerland).

Renal histology

Tissue samples were fixed in Duboscq-Brazil fluid and embedded in paraffin wax. Five-micrometer sections were prepared and exposed to Schiff's reagent to perform PAS (Periodic Acid Schiff) staining in order to study the integrity of the brush border and the presence of casts in tubular lumen. The degree of tissue injury was assessed on a semi-quantitative basis as previously described (Declèves et al., 2006).

Histochemistry and immunostaining

Kidney sections were prepared for joint HA histochemistry and NEP (neutral endopeptidase, a brush border marker) immunostaining. Detection of HA was performed using a biotinylated HA-binding protein (b-HABP, 1:200, Sigma–Aldrich, St. Louis, MO, USA). After dewaxing and rehydration, tissue sections were incubated in 0.06% H_2O_2 for 5 min to quench any endogenous peroxidase activity. After rinsing in distilled water and PBS, sections were incubated with solutions of avidin and biotin (Vector Laboratories, Burlingame, CA, USA), respectively, then rinsed again and incubated in casein for 15 min. Without rinsing, the slides were incubated for 1 h with b-HABP, which binds specifically and strongly to HA ≥ 2000 Da. The presence of HABP was revealed by Tyramin-Signal Amplification (TSA, Perkin Elmer, Boston, MA, USA). Peroxidase activity was visualized by incubating tissue sections using a DAB (3,3′-diaminobenzidine) kit (Vector Laboratories,

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