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Proximal tubules and podocytes are toxicity targets of bucillamine in a mouse model of drug-induced kidney injury

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ABSTRACT

Effective detection of potential nephrotoxicity is crucial for pre-clinical drug development. We evaluated a sensitive animal model for drug-induced kidney injury, which includes hemi-nephrectomy of mice. Although bucillamine and D-penicillamine are used for the treatment of rheumatoid arthritis in Japan, drug-related adverse effects on the kidney can limit their therapeutic utilities. When bucillamine (1000 or 2000 mg/kg/day) or D-penicillamine (2000 mg/kg/day) were orally administered to hemi-nephrectomised BALB/c mice, the urinary protein levels of bucillamine-treated mice, but not of those treated with D-penicillamine, the vehicle, or in bucillamine treated unnephrectomized mice, were significantly increased and remained high during the 4-week drug-loading period. Membranous glomerulonephropathy occasionally seen in bucillamine/D-penicillamine-treated arthritis patients was not reproduced in mice. Instead, our mouse model showed proximal tubular injury and podocyte foot process effacement in the bucillamine-treated kidneys. These two cell types are also the primary targets of the experimental Heymann membranous glomerulonephropathy. Gene expression profiling of the bucillamine-treated mice identified lipocalin 2 as a significantly up-regulated transcript together with cytochrome P450 CYP4a14, a group-specific component, and proprotein convertase subtilisin/kexin type 9. Moreover, large amounts of lipocalin 2 were detected in the urine of the bucillamine-treated mice, but not in the hemi-nephrectomised control mice. These results indicate that hemi-nephrectomy effectively promotes acute kidney injury by bucillamine, which is accompanied by up-regulation of the urinary biomarker lipocalin 2. Our mouse model with initial stage of kidney injury should be useful to analyse the pathogenesis of drug-induced glomerular and tubular injuries.

1. Introduction

Investigation of the molecular pathogenesis of drug-induced kidney dysfunction can be quite challenging due to the lack of an appropriate animal model. Rodent models of proteinuria and glomerulopathy developed thus far are useful for evaluating therapeutic efficacy but are not intended for the study of kidney injury caused by drugs with known clinical efficacy (Barabas and Lannigan, 1974; Chen et al., 2004; Heymann et al., 1959; Lannigan et al., 1962; Thakur et al., 1988). Both bucillamine and D-penicillamine belong to a family of disease-modifying antirheumatic drugs used in Japan (Matsuno et al., 1993; Nakajima et al., 2009; Sekiguchi et al., 2006). The clinical benefits of bucillamine and D-penicillamine for the treatment of rheumatic arthritis may be reduced in cases of drug-induced renal injury by limiting the prescribed doses or by discontinuation of the treatment (Habib et al., 2006; Inokuma et al., 1996; Jaffe, 1986; Nagahama et al., 2002; Obayashi et al., 2003; Taylor and Samanta, 1992). In animal models of kidney injury induced by D-penicillamine, induction of proteinuria and glomerulonephropathy is highly dependent on the genetic background of the mouse or rat used (Robinson et al., 1986; Seelig et al., 1977; Tournade et al., 1990; Uetrecht, 2005). Brown Norway rats and A.SW mice, but not C57BL/10Sn or BALB/c mice, are susceptible to kidney injury caused by D-penicillamine treatment (Robinson et al., 1986; Tournade et al., 1990). However, it is not known whether these experimental conditions are applicable for the development of an animal model of bucillamine-induced kidney injury. Bucillamine has two thiol donor moieties compared to only one in D-penicillamine, and two different metabolites of bucillamine also have a pharmacological effect on the lymphocytes in suppressing interleukin production (Matsuno et al., 1998).

The histopathological features of bucillamine- and D-penicillamine-induced renal damage in humans include granular depositions of IgG and complement C3 in the sub-epithelial space of the glomerular basement membrane. These features are the hallmark of membranous

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glomerulonephritis (Bacon et al., 1976; Nagahama et al., 2002). Although the tubular injury or interstitial injury is common in drug-induced acute kidney injury, in the case of the bucillamine- and D-penicillamine-induced kidney injury, the proteinuria is caused by the drug-induced glomerular damage. Therefore, regular urinalysis is performed to monitor occurrence of proteinuria during the treatment (Inokuma et al., 1996; Jaffe, 1986; Nagahama et al., 2002; Obayashi et al., 2003; Taylor and Samanta, 1992). Despite long-term research, the mechanism of drug efficacy in arthritis treatment and the pathogenesis of drug-induced membranous glomerulonephritis remain elusive (Wood et al., 2008). Furthermore, an effective molecular marker for bucillamine- or D-penicillamine-induced kidney dysfunction is needed.

Here, we report the development and initial characterisation of a mouse model of bucillamine-induced kidney injury. Hemi-nephrectomy is found to be effective for establishing a mouse model with proteinuria. This model should be useful for understanding the causes and consequences of bucillamine-induced nephrotoxicity in mice. We also used DNA microarrays to assess bucillamine-dependent gene expression profiles, and found a set of renal genes that are up-regulated with bucillamine-induced kidney damage.

2. Materials and methods

2.1. Materials

Bucillamine was kindly provided by Santen Pharmaceutical Co., Ltd. (Osaka, Japan). D-Penicillamine was purchased from Sigma-Aldrich (St. Louis, MO, USA). Protein Assay CBB Solution was obtained from Nacalai Tesque (Kyoto, Japan). CRE-V solution and Can Get Signal Immunoreaction Enhancer Solution were obtained from TOYOBO (Osaka, Japan). Anti-mouse IgG and C3 antibodies were purchased from Cappel (Aurora, OH, USA). Anti-mouse Lcn2 antibody was purchased from Abnova (Taipei, Taiwan). TRIzol and superscript III reverse transcriptase were obtained from Invitrogen (Carlsbad, CA, USA). The 3′ IVT Express Kit and the Mouse Genome 430 2.0 DNA microarray were purchased from Affymetrix (Santa Clara, CA, USA). ECL Western Blotting Detection Reagent and horseradish peroxidase-labelled goat anti-rabbit IgG (1:5000) were obtained from GE Healthcare (Little Chalfont, UK). Methylcellulose, the Urea NB test kit, and all the other chemicals were purchased from WAKO (Osaka, Japan).

2.2. Development of a mouse model for bucillamine-induced kidney injury

We used males belonging to the BALB/c mouse strain, which were previously reported to be susceptible to cationic bovine serum albumin, and induced membranous glomerulonephritis (Chen et al., 2004). Seven-week-old mice weighing 20–22 g were anaesthetised with 50 mg/kg of intraperitoneal pentobarbital sodium injection, and the left kidney was nephrectomised as described previously with slight modifications (Johns et al., 1996). Briefly, a flank incision was made to expose the left kidney, which was ligated and removed. The incision was sutured. The mice were allowed to recover in a warm cage. Subsequently, they were given regular rodent chow and a drinking water. For the control experiment, unnephrectomized mice were treated with vehicle and urinalysis of these mice was within the normal range. For the control experiments of two interventions, nephrectomy and drug treatment, unnephrectomized mice with bucillamine treatment and uninephrectomised mice with vehicle treatment were used, respectively. Bucillamine and D-penicillamine were ground into powder with an agate mortar for 10 min and suspended in 0.5% methylcellulose. One week after the hemi-nephrectomy, the mice were orally administered a dose of 1000 or 2000 mg/kg/day (4.48 or 8.96 mmol/kg/day) of bucillamine, 2000 mg/kg/day (13.4 mmol/kg/day) of D-penicillamine, or 0.5% methylcellulose (10 ml/kg) via a feeding needle for 4 weeks. The numbers of survivors at the end of the drug administration period were as follows: 17 out of 19 mice survived in the vehicle group, 15 out of 22 mice survived in the group treated with 1000 mg/kg/day of bucillamine, 14 out of 28 mice survived in the group treated with 2000 mg/kg/day of bucillamine, and 7 out of 15 mice survived in the D-penicillamine-treated group. Using metabolic cages, 24-h urine samples were collected once each week (Chen et al., 2004; Salant et al., 1980). At the end of the drug administration period, the mice were sacrificed under pentobarbital sodium anaesthesia, and blood and tissue samples were collected and stored at −80 °C. All the mice were housed in a pathogen-free barrier facility, with access to regular chow and water ad libitum. All experiments were conducted in accordance with institutional guidelines.

2.3. Urinary protein measurement

Urine samples were centrifuged at 800 *g* for 5 min at 4 °C to remove any precipitates. Urinary protein concentrations were measured with Protein Assay CBB Solution (Nakalai Tesque, Japan). Bovine serum albumin was used to generate a standard curve.

2.4. Biochemical analysis of blood and urine

Serum total protein was measured by the biuret method using biuret reagent (0.3% $CuSO_4 \cdot 5H_2O$, 1.2% $KNaC_4H_4O_6 \cdot 4H_2O$, 3% NaOH, 0.5% KI, and 0.5% deoxycholic acid sodium salt). Blood urea nitrogen was determined using the Urea NB test kit based on the urease–indophenol method. Serum and urine albumin were determined using bromocresol green, as described by Doumas et al. (1971). Serum and urine creatinine were enzymatically measured using CRE-V solution.

2.5. Histological analysis of renal tissues

Mouse kidneys were fixed in 10% formalin/phosphate-buffered saline (PBS) overnight at 4 °C, dehydrated by passing through an ascending ethanol series, and embedded in paraffin wax. Sections (2-μm thick) were stained by periodic acid-Schiff (PAS) and periodic acid methenamine silver (PAM). For immunohistochemistry, frozen sections (4-μm thick) were fixed with acetone for 10 min at room temperature and incubated with fluorescein-5-isothiocyanate-conjugated goat anti-mouse IgG, or complement C3 antibody for 1 h at room temperature. After

Table 1
The gene-specific primers designed for quantitative RT-PCR.

Gene name	Left primer	Right primer
Actin, beta	5′-TTGCTGACAGGATGCAGAAG-3′	5′-ACATCTGCTGGAAGGTGGAC-3′
Cytochrome P450, family4, subfamily a, polypeptide 14	5′-TGCCATCTGGTCCCTACTGT-3′	5′-ACGCCAACCTGCATTTCTAC-3′
Group specific component	5′-TTGACTGAGGAGTGCTGTGC-3′	5′-TCAGGAGTTCCAGGGTGAAC-3′
Lipocalin 2	5′-AGGCAGCTTTACGATG-3′	5′-GGTTGTAGTCCGTGGT-3′
Proprotein convertase subtilisin/kexin type 9	5′-AGGTCCTTCAGAGCAGGTCA-3′	5′-CATGGACTCTTGCCACACAC-3′
Farnesyl diphosphate synthetase	5′-CTGAGAAGGAGCTGGGACAC-3′	5′-TCCTGGAAGGCTTGTACCAC-3′
Dual specificity phosphatase 14	5′-CTCCCTGGAAATCCTTAGCA-3′	5′-TCATGAAGATGCCAGTGGTC-3′