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Renal biomarker changes associated with hyaline droplet nephropathy in rats are time and potentially compound dependent

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ABSTRACT

Alpha 2u-globulin mediated hyaline droplet nephropathy (HDN) is a male rat specific lesion induced when a compound or metabolite binds to alpha 2u-globulin. The objective of this study was to investigate if the newer and more sensitive renal biomarkers would be altered with HDN as well as be able to distinguish between HDN and oxidative stress-induced kidney injury. Rats were dosed orally for 7 days to determine (1) if HDN (induced by 2-propanol or D-limonene) altered the newer renal biomarkers and not BUN or creatinine, (2) if renal biomarkers could distinguish between HDN and oxidative stress-induced kidney injury (induced by potassium bromate), (3) sensitivity of HDN-induced renal biomarker changes relative to D-limonene dose, and (4) reversibility of HDN and renal biomarkers, using vehicle or 300 mg/kg/day D-limonene with 7 days of dosing and necropsies scheduled over the period of Days 8–85. HDN-induced renal biomarker changes in male rats were potentially compound specific: (1) 2-propanol induced mild HDN without increased renal biomarkers, (2) potassium bromate induced moderate HDN with increased clusterin, and (3) D-limonene induced marked HDN with increased αGST, μGST and albumin. Administration of potassium bromate did not result in oxidative stress-induced kidney injury, based on histopathology and renal biomarkers creatinine and BUN. The compound D-limonene induced a dose dependent increase in HDN severity and renal biomarker changes without altering BUN, creatinine or NAG: (1) minimal induction of HDN and no altered biomarkers at 10 mg/kg/day, (2) mild induction of HDN with increased αGST and μGST at 50 mg/kg/day and (3) marked induction of HDN with increased αGST, μGST and albumin at 300 mg/kg/day. HDN induced by D-limonene was reversible, but with a variable renal biomarker pattern over time: Day 8 there was increased αGST, μGST and albumin; on Day 15 increased clusterin, albumin and Kim-1. In summary, HDN altered the newer and more sensitive renal biomarkers in a time and possibly compound dependent manner.

1. Introduction

Drug-induced kidney injury occurring in the glomerular, proximal tubule, Loop of Henle, distal tubule and/or collecting duct results in attrition throughout the drug discovery and development process as well as product withdrawal (Budnitz et al., 2006; Guengerich, 2011; Olson et al., 2000; Redfern et al., 2010). Historically these lesions were characterized in standard rat toxicology studies using traditional hematoxylin and eosin stained longitudinal and transverse sections of the kidney as well as the following biomarkers: blood urea nitrogen (BUN) and serum creatinine. These biomarkers are insensitive as greater than two-thirds loss of kidney mass or greater than 50% functional loss occurs before any biologically relevant biomarker changes occur (Bonventre et al., 2010; Pfaller and Gstraunthaler, 1998).

Within the past decade, health authorities approved new renal biomarkers for use in rat studies, including alpha glutathione-s-transferase (αGST), albumin, β2-microglobulin, clusterin, cystatin C, kidney injury molecule 1 (Kim-1), mu-glutathione-s-transferase (μGST), renal papillary antigen-1 (RPA-1), total protein, and trefoil factor 3 (TFF3) (Dieterle et al., 2010; Harpur et al., 2011).

Abbreviations: Alpha2u-g, alpha 2u-globulin; BUN, blood urea nitrogen; CBC, complete blood count; αGST, glutathione-s-transferase; μGST, mu-glutathione-s-transferase; H&E, hematoxylin and eosin; HDN, alpha2u-g-induced hyaline droplet nephropathy; Kim-1, kidney injury molecule 1; RPA-1, renal papillary antigen-1; TFF3, trefoil factor 3.

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Validation studies in rats, using compounds known to induce kidney injury in preclinical studies and clinical setting, were done by consortia comparing the newer renal biomarkers to BUN, serum creatinine and the 'gold standard' histopathology. In these rat validation studies, the newer renal biomarkers were determined to complement as well as be more sensitive than BUN and creatinine. However, to date no investigation has been made of the response of these more sensitive renal biomarkers to distinguish between rat specific lesions and lesions with translational potential. For example, potassium bromate induces both oxidative stress-induced kidney injury with translational potential and rat specific alpha 2u globulin (alpha2u-g) induced hyaline droplet nephropathy (HDN).

HDN is a kidney lesion induced as a result of a compound or metabolite binding to the protein alpha2u-g (Lehman-McKeeman and Caudill, 1992), which is produced in the liver of rats under the regulation of testosterone and other hormones (Kurtz and Feigelson, 1977); the protein is repressed by estrogen (Lehman-McKeeman et al., 1990a; Roy et al., 1975). Negative regulation of alpha2u-g by estrogen explains why HDN is considered male rat specific (Olson et al., 1990; Roy and Neuhaus, 1966). Alpha2u-g is a low molecular weight compound filtered through the glomerulus with approximately 40% excreted into the urine and 60% reabsorbed into the proximal tubule (Neuhaus et al., 1981) where it is normally degraded within the lysosome (Neuhaus et al., 1981). However, unlike most low molecular weight proteins that are easily degraded in the lysosome, with half-lives in minutes, alpha2u-g is relatively resistant to lysosomal degradation with a 5–8 h half-life (Geertzen et al., 1973; Lehman-McKeeman et al., 1990b). Decreased degradation rate of alpha2u-g when bound to compound or metabolite is the generally accepted mechanism for drug-induced HDN (Lehman-McKeeman et al., 1990b).

More than 30 chemicals of heterogeneous chemistry are known to induce HDN (Borghoff et al., 2009; Flamm and Lehman-McKeeman, 1991; Hildebrand et al., 1997; Saito et al., 1996), with accumulation of alpha2u-g and compound/metabolite conjugate typically in the P2 and occasionally in the P1 and P3 sections of the proximal tubule (Hildebrand et al., 1997), which can result in tubular cytotoxicity. Of these chemicals, D-limonene is one of the most studied HDN-inducers and considered a positive control in our studies since HDN is the kidney lesion reported with short-term dosing and increased HDN-associated kidney tumors in male rats with long-term exposure (Borghoff et al., 2009; Flamm and Lehman-McKeeman, 1991; Hard and Whysner, 1994; National Toxicology Program, 1990; Whysner and Williams, 1996). The compound 2-propanol and potassium bromate are known to induce HDN with short-term treatment, determined by immunohistochemistry staining of kidney sections with an alpha2u-g antibody (Hildebrand et al., 1997; Umemura et al., 1993). However, potassium bromate induces other toxicities as a result of oxidative stress, which would be detected in both male and female rats. For example, potassium bromate induces kidney tumors in male and female rats with long-term exposure, due to oxidative stress (Umemura et al., 1998). Therefore, potassium bromate was selected both to evaluate the impact of HDN on renal biomarkers but also to determine if renal biomarkers can help distinguish between different mechanisms of renal injury.

The objectives of our studies were to determine if (1) HDN altered any of the newer and more sensitive renal biomarkers even though BUN and creatinine are known to not be altered in HDN, (2) there was a single HDN renal biomarker signature that would enable the distinction between rat specific kidney injury and kidney injury with potential clinical relevance, and (3) HDN along with correlating renal biomarkers were reversible.

2. Materials and methods

2.1. Compounds

All compounds were purchased from Sigma (St. Louis, MO) unless otherwise stated. The compound D-limonene was diluted in corn oil (vehicle). Potassium bromate and 2-propanol were dissolved with 10% (w/v) PEG400 in water. All compounds were dosed orally.

2.2. Studies

Han Wistar rats (8 weeks of age; Harlan, Indianapolis, IN) were single housed at 23 ± 1 °C with food and water provided *ad libitum*. A standard light–dark cycle was maintained with a timer-regulated light period from 0600 to 1800 h. All procedures were approved by AstraZeneca's Institutional Animal Care and Use Committee in accordance with *The Guide for the Care and Use of Laboratory Animals*.

To determine if HDN altered renal biomarkers, male and female rats (n = 12/sex) were randomized into four groups (n = 3/sex/group) and orally dosed with corn oil (vehicle control), 1000 mg/kg/day 2-propanol, 100 mg/kg/day potassium bromate or 300 mg/kg/day D-limonene for 7 days. These doses were based on previous publications showing the three compounds induced moderate to marked HDN in male rats after short-term exposure (Borghoff et al., 2009; Hildebrand et al., 1997). Urine (approximately 18 h) was collected overnight prior to first dose and after the seventh dose. After the last urine collection, blood was collected for hematology and chemistry analysis, necropsies were performed (Day 8) and kidneys were fixed (10% neutral buffered formalin) and processed by routine histological methods for microscopic examination.

Sensitivity of renal biomarkers in detecting HDN was assessed using male rats (n = 6/group) dosed with 0, 10, 50 or 300 mg/kg/day D-limonene for 7 days. Urine was collected overnight before the first dose and following the seventh dose. After the last urine collection, necropsies were performed (Day 8) and kidneys fixed and processed by routine histological methods for microscopic examination.

To determine the reversibility of HDN and renal biomarkers, male rats were dosed with vehicle (n = 30) or 300 mg/kg/day D-limonene (n = 30) for 7 days. Urine was collected overnight prior to first dose (all rats), following the seventh dose (all rats), as well as with collection periods ending on Days 15, 29, 57, and 85 (all rats still on-study). After urine collection, necropsies were performed (n = 6/group) on Days 8, 15, 29, 57, and 85 with the kidneys fixed and processed by routine histological methods for microscopic examination.

2.3. Necropsy and histopathology

At necropsy, rats were euthanized with isofluorane and blood was collected from the vena cava into lithium heparin tubes for chemistry analysis and into EDTA tubes for hematology analysis. Kidneys were removed, fixed in 10% neutral buffered formalin, paraffin embedded, sectioned (5 μm) and stained with hematoxylin and eosin (H&E). H&E slides were graded on a scale of 0–4 for hyaline droplet nephropathy, with minimal, mild, moderate and marked being descriptors for scores 1–4, respectively. Slides were not stained with an alpha2u-g antibody to confirm presence of the alpha2u-g/compound complex since all three compounds were known to induce alpha2u-g-induced HDN (Borghoff et al., 2009; Hildebrand et al., 1997).

2.4. Hematology

Complete blood count (CBC) was determined using EDTA anti-coagulated blood on an Advia 120 Hematology System (Siemens Healthcare Diagnostics Inc., Tarrytown, NY).

2.5. Blood chemistry

Lithium heparin anti-coagulated blood samples were centrifuged at 2500 rpm for 15 min with plasma frozen until evaluated on the Cobas c501 (Roche Diagnostics, Indianapolis, IN) for the following parameters: creatinine, urea, total protein, albumin, cholesterol, triglycerides, total bilirubin, glucose, electrolytes (sodium, potassium and calcium), alanine aminotransferase, alkaline phosphatase, glutamate dehydrogenase, aspartate aminotransferase, globulin, albumin/globulin ratio, and inorganic phosphorus.

2.6. Urinalysis

Urine was collected overnight on ice, centrifuged and supernatant evaluated on the CliniTek 500 Urine Chemistry Analyzer (Siemens Healthcare Diagonstics Inc., Tarrytown, NY) and Cobas c501 for the following parameters: bilirubin-total, ketones, blood, creatinine, protein, urea, glucose, bilirubin, electrolytes (sodium, potassium, calcium, chloride, and phosphate), urobilinogen, lactate dehydrogenase, alkaline phosphate, N-acetyl-[beta]-D-glucosaminidase (NAG) and gamma glutamate transferase. Urine was also evaluated for specific gravity, color, pH, and clarity.