J. Comp. Path. 2014, Vol. 151, 264–270

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EXPERIMENTALLY INDUCED DISEASE

Comparison of Renal Biomarkers with Glomerular Filtration Rate in Susceptibility to the Detection of Gentamicin-Induced Acute Kidney Injury in Dogs

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Summary

Fourteen renal biomarkers were compared with measurement of glomerular filtration rate (GFR) in detecting acute kidney injury (AKI) in beagle dogs given gentamicin (40 mg/kg/day by subcutaneous injection) for 7 consecutive days. Serum and urinary biomarkers were measured before administration of gentamicin and then on days 4 and 8 after starting administration. GFR was derived by use of a simplified equation. Increased urinary cystatin C and decreased GFR occurred from day 4 and were detected before increases in blood urea nitrogen (BUN) and serum creatinine concentrations and changes in other urinary parameters. The closest correlation was between urinary cystatin C and GFR. At termination, microscopical examination revealed extensive necrosis of proximal tubular epithelium with hyaline casts in the kidney of treated dogs. These data indicate that urinary cystatin C is the most sensitive index of kidney injury and GFR reflects the kidney functional mass.
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Keywords: dog; gentamicin; glomerular filtration rate; kidney injury

Introduction

Acute kidney injury (AKI) involves sudden onset of renal parenchymal damage with loss of function, so its early detection is crucial for successful treatment in human (Nally, 2002) and animal (Ross, 2011) patients. There is growing interest in identifying an early-onset, sensitive and specific biomarker for predicting AKI (Vaidya *et al.*, 2008; Urbschat *et al.*, 2011; Mårtensson *et al.*, 2012). Seven biomarkers, including albumin (ALB), β_2-microglobulin, clusterin, cystatin C, kidney injury molecule (KIM)-1, trefoil factor-3 and total protein, have been used as an index to identify AKI (Dieterle *et al.*, 2010). More recently, urinary liver-type fatty acid binding protein (L-FABP) (Kamijo-Ikemori *et al.*, 2011) and neutrophil gelatinase-associated lipocalin (NGAL) (Kai *et al.*, 2013; Segev *et al.*, 2013) have been proposed as markers of milder forms of AKI that may be detected potentially earlier in the course of kidney damage.

Estimation of glomerular filtration rate (GFR) is generally accepted to be the best overall index of the level of kidney function in man (National Kidney Foundation, 2002) and animals (Heiene and Moe, 1998; Von Hendy-Willson and Pressler, 2011). However, there are few reports that deal with the relationship between changes in biomarkers and GFR, because estimation of GFR has proven to be methodologically difficult in animals. For example, the traditional method of urinary clearance measurement using inulin requires accurately timed, repeated blood and urine collection and inulin has an inherent disadvantage due to its extremely low solubility. Although endogenous creatinine clearance has been reported to have some advantages over measuring inulin, it is affected by other factors including active proximal tubular secretion (Darling and Morris, 1991). GFR estimated from a single blood sample using the new

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0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2014.06.001
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tracer iodixanol and based on Jacobsson's equation (1983), is correlated closely with the inulin detection method in clinically healthy dogs and dogs with spontaneously occurring kidney diseases (Sasaki *et al.*, 2014). The Jacobsson equation is derived from a simple one-compartment model combined with the volume of distribution (Vd) and optimum time for taking blood (Jacobsson, 1983).

The aims of the present study were to determine the relationship between 14 renal biomarkers and GFR in male beagle dogs given gentamicin sulphate (hereafter referred to as gentamicin) by subcutaneous injection for 7 consecutive days and to assess the relative sensitivity of these biomarkers. Gentamicin, a potent broad-spectrum aminoglycoside antibiotic, is a known nephrotoxicant that induces AKI in dogs (Cronin *et al.*, 1980; Kai *et al.*, 2013). Renal biomarkers utilized in this investigation included blood urea nitrogen (BUN) and serum creatinine and urinary ALB, cystatin C, KIM-1, L-FABP, *N*-acetyl-*β*-D-glucosaminidase (NAG), NGAL, glucose and minerals/electrolytes including calcium (Ca), inorganic phosphate (IP), sodium (Na^+), potassium (K^+) and chloride (Cl^-).

Materials and Methods

Experimental Protocol

Gentamicin was administered subcutaneously at 40 mg/kg/day into the dorsal neck region of four dogs once daily for 7 consecutive days. The dose and treatment period were chosen based on a previous report (Kai *et al.*, 2013). Four other dogs receiving 0.9% physiological saline solution (saline; 1 ml/kg/day) by the same route served as controls. Serum and urinary biomarkers and GFR were measured before administration of gentamicin (day − 1) and pre-dose on days 4 and 8 (24 h after the final administration). The first dosing day of gentamicin was regarded as day 1 of the study. After the estimation of GFR on day 8, all animals were killed by intravenous overdose (60 mg/kg) of sodium pentobarbital (Kyoritsu Seiyaku, Tokyo).

Drugs

Iodixanol (320 mg iodine/ml, 290 mOsm/kg H_2O) as the GFR tracer was provided by Daiichi Sankyo (Tokyo, Japan) and gentamicin (40 mg/ml) was purchased from FujiPharma (Shizuoka, Japan). All other chemicals and reagents were of the highest grade available from commercial sources, unless otherwise stated.

Animals

Eight clinically healthy male beagle dogs weighing 12.5 ± 1.2 kg and aged 6.6 ± 1.4 years were used. Dogs were regarded as healthy from the results of clinical examination and hematological, serum biochemical and urinalysis testing. The dogs were housed individually in stainless steel cages (93.5 × 80 × 78.5 cm) in an air-conditioned room (temperature, 18−28°C; relative humidity, 30−70%). The canine facility was kept at a light/dark cycle of 12 h and a ventilation rate of 10−20 air changes/h. Basal diet (220 g/day, Certified Canine Diet 5007, PMI Nutrition International Inc., St Louis, Missouri, USA) was given to each dog and tap water was available *ad libitum*. All experimental manipulations were performed in accordance with the Guidelines for Animal Experimentation issued by the Japanese Association for Laboratory Animal Science (1987) and approved by the Animal Experimental Ethics Committee of Daiichi Sankyo.

Laboratory Tests and Kidney Pathology

BUN and serum creatinine concentrations were measured with an autoanalyzer (Toshiba Medical Systems, Tochigi, Japan).

Urine was collected in a tray for 17 h. Volume was measured and approximately 5 ml of the supernatant of urine following centrifugation (4°C, 500g for 5 min) was filtered through a membrane filter (0.8 μm, ADVANTEC, Tokyo, Japan). Urinary creatinine, ALB, NAG, glucose, Ca, IP, Na^+, K^+ and Cl^- were measured using an autoanalyzer (TBA-200FR, Toshiba Medical Systems). Urinary cystatin C (canine cystatin C enzyme-linked immunosorbent assay [ELISA]; BioVender, Reckovice, Czech Republic), KIM-1 (Dog KIM-1 ELISA; Immunology Consultants Laboratory, Portland, Oregon, USA), L-FABP (human L-FABP Assay Kit; CMIC, Tokyo, Japan) and NGAL (BIOPORTO Diagnostics, Gentofte, Denmark) were measured using commercially available kits according to the manufacturer's instructions. The kits for L-FABP and NGAL were confirmed to cross-react with the canine equivalent molecules. The values of all urinary parameters were normalized based on the corresponding urinary creatinine (unit/g creatinine) as described by Waikar *et al.* (2010).

At termination on day 8, the left kidneys were removed, fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections were stained with hematoxylin and eosin (HE) and evaluated in a blinded fashion.

Estimation of Glomerular Filtration Rate

Iodixanol was administered as a bolus injection at 40 mg iodine per kg body weight (mg I/kg) into the

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