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Short communication

Urinary 3-hydroxypropyl mercapturic acid (3-HPMA) concentrations in dogs with acute spinal cord injury due to intervertebral disc herniation

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ABSTRACT

The aim of this study was to investigate urinary 3-hydroxypropyl mercapturic acid (3-HPMA), a metabolite of acrolein, as a novel biomarker in acute spinal cord injury (ASCI) due to intervertebral disc herniation in dogs. Urine from 10 client-owned dogs with ASCI collected at presentation and 10 control dogs was analyzed for 3-HPMA. The median urinary 3-HPMA concentration in ASCI dogs was significantly higher than in control dogs, but was not correlated with the severity of ASCI. The median urinary 3-HPMA concentration in intact dogs was higher than in neutered dogs. Higher urinary 3-HPMA concentrations in dogs after ASCI support a role for acrolein, a cytotoxic by-product of lipid peroxidation, in canine ASCI. Urinary 3-HPMA could be used as a biomarker in future clinical trials to measure the effect of therapeutic intervention of reducing acrolein after ASCI.

Acute spinal cord injury (ASCI) commonly occurs secondary to intervertebral disc herniation (IVDH) in dogs (Olby et al., 2003). The pathophysiology of ASCI consists of primary injury from mechanical insult and secondary injury, a biochemical cascade propagating tissue damage (Olby, 2010). Secondary injury includes ischemia, inflammation, ion dysregulation, excitotoxicity, production of reactive oxygen species and lipid peroxidation (LPO) (Olby, 2010).

Acrolein is a cytotoxic reactive aldehyde by-product of LPO, a known secondary injury mechanism following spinal cord injury (SCI) (Shi et al., 2011). A metabolite of acrolein-glutathione adduct found in urine, 3-hydroxypropylmercapturic acid (3-HPMA), reliably estimated acrolein in humans and in a rat model of ASCI (Carmella et al., 2007; Zheng et al., 2013). In this study, we evaluated acrolein as a potential biomarker and therapeutic target by measuring urinary 3-HPMA concentrations in dogs after naturally-occurring ASCI.

In a prospective, blinded, controlled study, urine was collected at presentation from 10 client-owned dogs with ASCI at Purdue University Veterinary Teaching Hospital and analyzed for 3-HPMA. Urine from 10 normal dogs was used as controls. This study was approved by the Purdue University Animal Care and Use Committee (approval number 1309000935; date of approval 3 January 2012). Informed consent was obtained from all pet owners.

Pre-operative neurological status was graded as follows: grade 1, spinal pain only; grade 2, ambulatory paraparesis; grade 3, non-ambulatory paraparesis; grade 4, paraplegia; grade 5, paraplegia without nociception. Dogs were eligible for inclusion if they suffered an acute, thoracolumbar SCI with grades 3–5 neurological deficits. Dogs with a history of SCI >72 h, concurrent illness or any other recent (<7 days) traumatic injury were excluded.

Magnetic resonance or computed tomography imaging to confirm IVDH and surgical decompression were performed in 19/20 dogs. Regaining ambulation was considered a successful outcome. Urine samples were immediately frozen at –80 °C, and stored for up to 2 weeks prior to analysis. The urinary concentration of 3-HPMA was quantified as described by Zheng et al. (2013). Creatinine was measured using a urinary creatinine assay kit (Cayman Chemical Company).

Due to the small sample size and some non-parametric distributions, Mann-Whitney *U* tests were used to compare urinary 3-HPMA concentrations between two independent groups (controls versus affected, neutered status, sex and outcome) and the Spearman rank correlation coefficient was used to assess the correlation between 3-HPMA concentration and continuous (weight and age) or ordinal (neurological grade) variables. Statistical analyses were performed using STATA SE version 14.1. $P < 0.05$ was considered to be significant.

The sample population included four Dachshunds and one of each of a Pug-Beagle cross, Cocker spaniel, Pekingese, Doberman pinscher, Shih tzu and Beagle. The median age was 5.5 years (range 1–10

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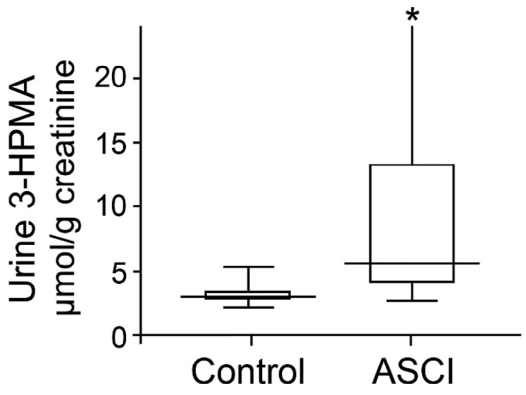

Fig. 1. Urinary 3-hydroxypropyl mercapturic acid (3-HPMA) in dogs with acute spinal cord injury (ASCI; $n = 10$) is statistically higher than in control dogs ($n = 10$; $P < 0.03$. The top, middle and bottom lines of the box correspond to the 75th, 50th (median) and 25th percentile, respectively. *Outlier (157.36 µmol 3-HPMA/g creatinine).

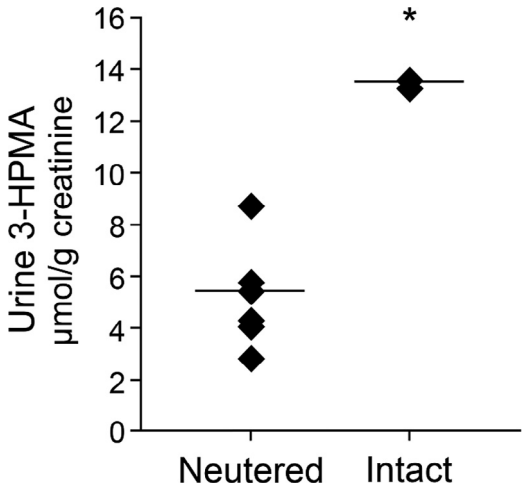

Fig. 2. Urinary 3-hydroxypropyl mercapturic acid (3-HPMA) concentrations in intact dogs were statistically higher than neutered dogs ($P < 0.02$). The horizontal line denotes the median. *Outlier (157.36 µmol 3-HPMA/g creatinine).

years). There were three neutered males, one intact male, four spayed females and two intact females. The neurological grades at admission were grade 3 ($n = 3$), grade 4 ($n = 3$) and grade 5 ($n = 4$).

One dog (grade 5) was euthanized at the owner's request (13.6 µmol 3-HPMA/g creatinine). Seven dogs recovered ambulation following surgery; the two dogs that failed to recover ambulation had grade 5 neurological status at presentation. One dog developed myelomalacia and was euthanized post-operatively (2.8 µmol 3-HPMA/g creatinine). The other dog (4.2 µmol 3-HPMA/g creatinine) remained paraplegic without nociception 1 year after surgery.

The median urinary 3-HPMA concentration in dogs with ASCI (5.8 µmol 3-HPMA/g creatinine; range 2.8–157.4 µmol 3-HPMA/g creatinine) was significantly higher than in control dogs (3.1 µmol 3-HPMA/g creatinine; range 2.4–5.4 µmol 3-HPMA/g creatinine; $P = 0.004$) (Fig. 1). There was no correlation between 3-HPMA concentration and neurological grade (Spearman's $\rho = -0.507$; $P = 0.135$). The 3-HPMA concentration in dogs with a successful outcome (median 5.8 µmol 3-HPMA/g creatinine, range 4.0–157.3 µmol 3-HPMA/g creatinine) was not significantly different from dogs that failed to recover (median 3.5 µmol 3-HPMA/g creatinine, range 2.8–4.2 µmol 3-HPMA/g creatinine; $P = 0.079$). The 3-HPMA concentration of intact dogs with ASCI (median 13.6 µmol 3-HPMA/g creatinine, range 13.2–157.4 µmol 3-HPMA/g creatinine) was significantly higher than neutered dogs with ASCI (median 5.4 µmol 3-HPMA/g creatinine, range 2.8–8.6 µmol 3-HPMA/g creatinine; $P = 0.017$) (Fig. 2). No weight, sex or age correlation was found ($P > 0.05$).

3-HPMA is the first assay to indirectly assess acrolein concentration by measuring a metabolite of acrolein-glutathione adduct in dogs. Acrolein is cytotoxic and may play a clinically relevant pathological role in canine ASCI, which may warrant therapeutic intervention. Plasma acrolein is a sensitive biomarker for human stroke and scavenging acrolein in animal models following SCI is neuroprotective (Saiki et al., 2011; Park et al., 2014). Therefore, detecting acrolein in canine ASCI may aid in the development of neuroprotective agents.

Increased urinary 3-HPMA concentrations were not correlated with neurological grade. Instead, the highest 3-HPMA concentrations were associated with dogs with the least severe neurological grade. This may be related to glutathione depletion rather than lower acrolein production, similar to an inverse correlation with severity observed in human stroke (Yoshida et al., 2012). Furthermore, extracellular glutamate accumulation prevents the uptake of cysteine, the rate limiting amino acid in glutathione production (Pereira and Oliveira, 2000). Glutamate cerebrospinal concentrations increase in dogs after ASCI, in a direct relationship to neurological grade (Olby et al., 1999). Taken together, the lack of a higher concentration of 3-HPMA in dogs with more severe ASCI is likely to be due to depletion of glutathione by acrolein. Concurrent measurement of plasma protein-acrolein, which is not dependent on glutathione levels, or measurement of cerebrospinal glutamate, might have assisted in elucidating the relationship between severity of ASCI and urinary 3-HPMA concentration.

Urinary 3-HPMA concentration was significantly higher in intact dogs compared to neutered males and females. Had our sample size been larger, multivariate analysis could have been used to investigate whether urinary 3-HPMA concentration was most strongly associated with neutering status or factors such as neurological grade.

In summary, the concentration of urinary 3-HPMA, a metabolite of acrolein, was significantly higher in dogs with ASCI due to IVDH than in unaffected dogs, but was not correlated with the severity of SCI. Urinary 3-HPMA may be used in future studies to evaluate pharmaceutical interventions that target LPO or sequester acrolein.

Conflict of interest statement

Riyi Shi is the co-founder of Neuro Vigor, a company interested in developing therapies for central nervous system disorders.

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