Journal of Veterinary Cardiology (2017) ■, ■–■

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Journal of Veterinary Cardiology
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Plasma L-citrulline concentrations in L-arginine—supplemented healthy dogs

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Received 22 November 2016; received in revised form 11 April 2017; accepted 18 April 2017

KEYWORDS
Nitric oxide;
Pulmonary hypertension;
Canine;
eNOS;
Sildenafil

Abstract *Introduction:* To determine whether oral L-arginine increases plasma [L-citrulline] in dogs.
Animals: Eleven healthy staff-owned dogs were used in this study.
Materials and methods: Dogs (n = 3) were given L-arginine (50mg/kg PO q8h) for 7 days, and plasma [L-arginine] and [L-citrulline] were analyzed by high performance liquid chromatography at baseline (BL), steady state trough, and 0.5, 1, 1.5, 2, 4, 6, and 8 h after final dosing on day 7. Eleven dogs were then treated with 100mg/kg L-arginine PO q8h for 7 days, and [L-arginine] and [L-citrulline] were measured at BL, steady state trough, and at peak 4 hrs after dosing (T4 hrs).
Results: — Plasma [L-arginine] and [L-citrulline] peaked at T4 hrs on the 50mg/kg dosage. Target outcome, modeled after human study results, of a doubling of [L-arginine] and a 25—30% increase in [L-citrulline] from BL were not reached. After the 100mg/kg dosage, plasma [L-arginine] increased from a BL median of 160.1 μM (range, 100.2—231.4 μM) to a peak of 417.4 μM (206.5—807.3 μM) at T4 hrs, and plasma [L-citrulline] increased from a BL median of 87.8 μM (59.1—117.1 μM) to peak of 102.2 μM (47.4—192.6 μM) at T4 hrs. Ten of eleven dogs showed a doubling of plasma [L-arginine] and 4/11 dogs achieved 25—30% or greater increases in plasma [L-citrulline]. No adverse effects on heart rate or blood pressure were noted.
Conclusions: — Oral L-arginine dosage of 100mg/kg q8h doubles plasma [L-arginine] in healthy dogs, but conversion to L-citrulline is quite variable. Further evaluation of this dosage regimen in dogs with pulmonary hypertension is warranted.

These data were presented in oral abstract form at the 2015 ACVIM Forum, Indiana Convention Center, Indianapolis, IN.

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http://dx.doi.org/10.1016/j.jvc.2017.04.005
1760-2734/

Please cite this article in press as: Flynn KM, et al., Plasma L-citrulline concentrations in L-arginine—supplemented healthy dogs, Journal of Veterinary Cardiology (2017), http://dx.doi.org/10.1016/j.jvc.2017.04.005

Abbreviations

ADMA	asymmetric dimethylarginine
BL	baseline
eNOS	endothelial nitric oxide synthase
HPLC	high performance liquid chromatography
HR	heart rate
NO	nitric oxide
PA	pulmonary arterial
PDE	phosphodiesterase enzyme
PH	pulmonary hypertension
T4 hrs	4 h after dosing, day 7
Tmax	time to peak measurements
TR	steady state trough, day 7

Introduction

Pulmonary hypertension (PH) is a complex syndrome that can cause relevant morbidity in both dogs and humans. While some dogs with PH may appear clinically normal, others present with clinical signs that decrease quality of life, including exercise intolerance, cough, dyspnea, syncope, and right-sided heart failure [1].

In veterinary medicine, PH is defined as an echocardiographically estimated pulmonary arterial (PA) systolic pressure ($>\sim$30 mmHg) [2—4]. While PH has various etiologies, all result in vasoconstriction and pathologic remodeling of the PA vasculature. Accordingly, therapies that promote PA vasodilation and inhibit PA vascular remodeling decrease pulmonary vascular resistance and are effective in managing PH [1].

One approach to treating PH is through manipulation of the nitric oxide (NO) pathway [5,6]. Nitric oxide is a potent vasodilator that is endogenously synthesized from L-arginine and oxygen by endothelial NO-synthase (eNOS). Nitric oxide diffusion into nearby smooth muscle cells leads to the generation of cyclic guanosine monophosphate (cGMP), promoting smooth muscle relaxation and subsequent vasodilation [7]. Phosphodiesterase enzymes (PDEs), including PDE-5 in pulmonary vessels, rapidly inactivate cGMP. Thus, PDE-5 inhibitors, such as sildenafil citrate, enhance pulmonary vasodilation by increasing cGMP, and have been successful in treating PH in dogs [3].

In addition to its vasodilatory actions, NO regulates various other processes within the vasculature. Pertinent to PH pathogenesis, NO inhibits inappropriate vascular remodeling via antimitogenic effects on pulmonary endothelial and smooth muscle cells, inhibition of smooth muscle cell migration, and also by reducing pressure and shear stress that result from increased vasoconstriction [8—12].

Phosphodiesterase enzyme inhibitors may be potentiated by concurrent treatment with agents that increase NO availability. In human patients with PH, administration of L-arginine led to reduced PA pressures and increased exercise tolerance [13—15]. L-citrulline is an amino acid by-product of NO synthesis from L-arginine, and plasma concentrations of L-citrulline have been previously used as a surrogate marker of NO synthesis [13,15—18]. In humans with precapillary PH, a single dose of L-arginine at 50 mg/kg PO led to a doubling of plasma L-arginine concentrations and a 25—30% increase in plasma L-citrulline concentrations, and was associated with relevant reductions in PA pressures, pulmonary vascular resistance, and mean systemic arterial pressure [13]. With chronic dosing (50 mg/kg PO q8h for 7 days), L-arginine improved several measures of exercise capacity in these same patients [13]. In canine PH patients oral L-arginine supplementation has not been evaluated nor has an appropriate dosage been established.

The primary aim of this study was to identify an oral dosage of L-arginine in healthy dogs that would lead to increases in plasma L-arginine concentrations and plasma L-citrulline concentrations that have been associated with hemodynamic improvement in human patients with PH. Because the addition of any exogenous substance has the ability to perturb physiologic function and because increased L-arginine concentrations can promote NO-induced vasodilation, a secondary aim was to evaluate for possible side-effects of this dosage in healthy dogs.

Materials and methods

Animals

Clinically healthy staff-owned dogs were recruited for the study. Dogs were determined to be healthy based on clinical history, physical exam, serum biochemical profiles, and non-invasive systemic blood pressure measurements (systolic blood pressure $<$160 mmHg) [19]. A systolic blood pressure measurement of $<$175 mmHg and a lack of evidence of target organ damage was considered acceptable for inclusion as slight elevations were considered attributable to anxiety [20]. With the exception of flea, tick, and heartworm preventatives, the dogs included in the study were taking no additional medications or supplements. The study was approved by the Animal Care and

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