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

ELSEVIER

Journal of Veterinary Cardiology
ESVC

www.elsevier.com/locate/jvc

Validation of a method for quantitation of the clopidogrel active metabolite, clopidogrel, clopidogrel carboxylic acid, and 2-oxo-clopidogrel in feline plasma

Janne G. Lyngby, DVM, MS , Michael H. Court, BVSc, PhD , Pamela M. Lee, DVM, MS*

Program in Individualized Medicine (PrIMe), Department of Veterinary Clinical Sciences, College of Veterinary Medicine, PO Box 647060, Washington State University, WA 99164-7060, USA

Received 3 September 2016; received in revised form 6 February 2017; accepted 9 March 2017

KEYWORDS
Plavix;
Cat;
Thromboprophylaxis;
Antithrombotic;
Antiplatelet

Abstract *Introduction:* The clopidogrel active metabolite (CAM) is unstable and challenging to quantitate. The objective was to validate a new method for stabilization and quantitation of CAM, clopidogrel, and the inactive metabolites clopidogrel carboxylic acid and 2-oxo-clopiodgrel in feline plasma.
Animals: Two healthy cats administered clopidogrel to demonstrate assay in vivo utility.
Materials and methods: Stabilization of CAM was achieved by adding 2-bromo-3'methoxyacetophenone to blood tubes to form a derivatized CAM (CAM-D). Method validation included evaluation of calibration curve linearity, accuracy, and precision; within and between assay precision and accuracy; and compound stability using spiked blank feline plasma. Analytes were measured by high performance liquid chromatography with tandem mass spectrometry. In vivo utility was demonstrated by a pharmacokinetic study of cats given a single oral dose of 18.75mg clopidogrel.
Results: The 2-oxo-clopidogrel metabolite was unstable. Clopidogrel, CAM-D, and clopidogrel carboxylic acid appear stable for 1 week at room temperature and 9 months at −80°C. Standard curves showed linearity for CAM-D, clopidogrel, and clopidogrel carboxylic acid ($r > 0.99$). Between assay accuracy and precision was ≤2.6%

Data presented on June 9, 2016 at the ACVIM forum in Denver, CO, USA.

* Corresponding author.
E-mail address: pamelamlee@vetmed.wsu.edu (P.M. Lee).

http://dx.doi.org/10.1016/j.jvc.2017.03.004

Please cite this article in press as: Lyngby JG, et al., Validation of a method for quantitation of the clopidogrel active metabolite, clopidogrel, clopidogrel carboxylic acid, and 2-oxo-clopidogrel in feline plasma, Journal of Veterinary Cardiology (2017), http://dx.doi.org/10.1016/j.jvc.2017.03.004

and ≤7.1% for CAM-D and ≤17.9% and ≤11.3% for clopidogrel and clopidogrel carboxylic acid. Within assay precision for all three compounds was ≤7%. All three compounds were detected in plasma from healthy cats receiving clopidogrel.
Discussion: This methodology is accurate and precise for simultaneous quantitation of CAM-D, clopidogrel, and clopidogrel carboxylic acid in feline plasma but not 2-oxo-clopidogrel.
Conclusions: Validation of this assay is the first step to more fully understanding the use of clopidogrel in cats.

Abbreviations

AUC	area under the curve
BMAP	2-bromo-3′methoxyacetophenone
CAM	clopidogrel active metabolite
CAM-D	derivatized clopidogrel active metabolite
EDTA	ethylenediaminetetraacetic acid
HPLC-MS/MS	high-performance liquid chromatography with tandem mass spectrometry

Introduction

Clopidogrel is an antiplatelet agent commonly used in cats and is superior to aspirin in the prevention of feline cardiogenic thromboembolism [1]. Prevention of thromboembolic disease is paramount since survival rates after embolism are low (less than 40%) with an approximate 17—75% recurrence rate [2,3]. In humans, individual response to clopidogrel has been demonstrated with some individuals being resistant to clopidogrel [4—6]. Variable response to clopidogrel has also been observed in cats [7—9]. Clopidogrel resistance in humans has been documented to be associated with increased risk for the development of major adverse cardiac events [4—6].

Clopidogrel is a prodrug and requires hepatic metabolic transformation for antiplatelet activity. In the human liver, metabolism of clopidogrel occurs through two main pathways. The clinically important pathway is the conversion of inactive parent drug clopidogrel by cytochrome P450 enzyme to an intermediate product 2-oxo-clopidogrel, which is then subsequently hydrolyzed to the clopidogrel active metabolite (CAM). The conversion of 2-oxo-clopidogrel can result in four diastereoisomers of CAM named H1, H2, H3, and H4. Only H4 and H2 have antiplatelet effects, with H4 exhibiting about twice the activity of H2 [10]. In the human body, only H3 and H4 isomers can be detected [11,12]. As a result, the H4 isomer is the only active circulating form of CAM in humans [10]. The H4 isomer selectively and irreversibly blocks adenosine diphosphate binding to the P2Y12 receptor thereby inhibiting adenosine diphosphate—induced platelet aggregation. In humans, about 15% of the parent clopidogrel undergoes bioactivation to both the H3 and H4 CAM isomers [11]. The remaining 85% is metabolized in the liver to an inactive clopidogrel carboxylic acid (Fig. 1).

Clopidogrel active metabolite is very unstable and has been challenging to quantitate thereby making complete pharmacokinetic studies in any species difficult. No pharmacokinetic studies of clopidogrel and/or its metabolites in cats have been reported. In humans and dogs, pharmacokinetic studies have focused instead on measuring plasma concentrations of clopidogrel or the clopidogrel carboxylic acid metabolite [13—15]. Only recently has a method to stabilize CAM in human plasma been developed by adding 2-bromo-3′methoxyacetophenone (BMAP) to blood tubes [12,16]. Once stabilized, the derivatized CAM (CAM-D, Fig. 1) can be readily quantitated using high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS). Validation of this assay has now allowed complete pharmacokinetic studies, pharmacodynamic—-pharmacokinetic correlations, medication interactions, and mechanisms of clopidogrel resistance to be more fully evaluated in humans [12,17,18]. In order to accomplish the same in cats, validation of this assay for use in cats is a critical first step.

The aim of this study was to validate this new method of BMAP addition and HPLC-MS/MS for CAM stabilization and quantitation in feline plasma. A secondary aim was to also validate this assay for quantitation of clopidogrel, clopidogrel carboxylic acid, and 2-oxo-clopidogrel. *In vivo* utility of this assay was evaluated by conducting a preliminary pharmacokinetic study in two healthy cats that received a single dose of 18.75 mg clopidogrel by mouth.

Please cite this article in press as: Lyngby JG, et al., Validation of a method for quantitation of the clopidogrel active metabolite, clopidogrel, clopidogrel carboxylic acid, and 2-oxo-clopidogrel in feline plasma, Journal of Veterinary Cardiology (2017), http://dx.doi.org/10.1016/j.jvc.2017.03.004