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Simultaneous determination of carboprost methylate and its active metabolite carboprost in dog plasma by liquid chromatography–tandem mass spectrometry with positive/negative ion-switching electrospray ionization and its application to a pharmacokinetic study

Lei Yin[a,b,c], Xiangjun Meng[b,c], Xiaotong Zhou[b,c], Tinglan Zhang[e], Heping Sun[b,c], Zhichao Yang[b,c], Bo Yang[b,c], Ning Xiao[b], J. Paul Fawcett[d], Yan Yang[b,c,**], Jingkai Gu[a,b,c,*]

[a] Clinical Pharmacology Center, Research Institute of Translational Medicine, The First Hospital of Jilin University, Dongminzhu Street, Changchun 130061, PR China
[b] College of Life Science, Jilin University, Qianjin Street, Changchun 130012, PR China
[c] Research Center for Drug Metabolism, Jilin University, Qianjin Street, Changchun 130012, PR China
[d] School of Pharmacy, University of Otago, PO Box 56, Dunedin, New Zealand
[e] Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, PR China

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ABSTRACT

A liquid chromatography–tandem mass spectrometric (LC–MS/MS) method using positive/negative electrospray ionization (ESI) switching for the simultaneous quantitation of carboprost methylate and carboprost in dog plasma has been developed and validated. After screening, the esterase inhibitor, dichlorvos was added to the whole blood at a ratio of 1:99 (v/v) to stabilize carboprost methylate during blood collection, sample storage and LLE. Indomethacin was added to plasma to inhibit prostaglandins synthesis after sampling. After liquid–liquid extraction of 500 μL plasma with ethyl ether-dichloromethane (75:25, v/v), analytes and internal standard (IS), alprostadil-d4, were chromatographed on a CAPCELL PAK Phenyl column (150 × 2.0 mm, 5 μm) using acetonitrile-5 mM ammonium acetate as mobile phase. Carboprost methylate was detected by positive ion electrospray ionization followed by multiple reaction monitoring (MRM) of the transition at m/z 400.5 → 329.3; the carboprost and IS were detected by negative ion electrospray ionization followed by MRM of the transitions at m/z 367.2 → 323.2, and 357.1 → 321.2, respectively. The method was linear for both analytes in the concentration range 0.05–30 ng/mL with intra- and inter-day precisions (as relative standard deviation) of ≤6.75% and accuracy (as relative error) of ≤7.21% and limit of detection (LOD) values were 10 and 20 pg/mL, respectively. The method was successfully applied to a pharmacokinetic study of the analytes in beagle dogs after intravaginal administration of a suppository containing 0.5 mg carboprost methylate.

1. Introduction

Carboprost [1] is a synthetic analog of PGF2α with oxytocic properties. It induces contractions and can trigger abortion in early pregnancy [2–11]. However, it can also reduce postpartum bleeding [12–15]. It is registered for termination of 2nd-trimester pregnancy, intrauterine fetal death, missed abortion, and hydatidiform mole in the 2nd trimester and for treatment of severe postpartum hemorrhage. Carboprost methylate (also known as 15-methyl PGF2α or methyl carboprost) is an ester prodrug of carboprosts, due to its better liposolubility, methyl carboprost is more easily absorbed across the biomembrane and its transdermal bioavailability was greatly improved compared with carboprost. As a prescribed drug, carboprost methylate has been used for many years for termination of pregnancy especially for high-risk pregnancies in women with scarred uterus, uterine malformations, and lactation pregnancy. It also can be used for prevention and treatment of postpartum hemorrhage caused by uterine atony [16,17]. Carboprost methylate is intended to undergo in vivo enzymatic

* Corresponding author. Fax: +86 431 85155380.
** Corresponding author.
E-mail addresses: yyan@jlu.edu.cn (Y. Yang), gujk@mail.jlu.edu.cn (J. Gu).

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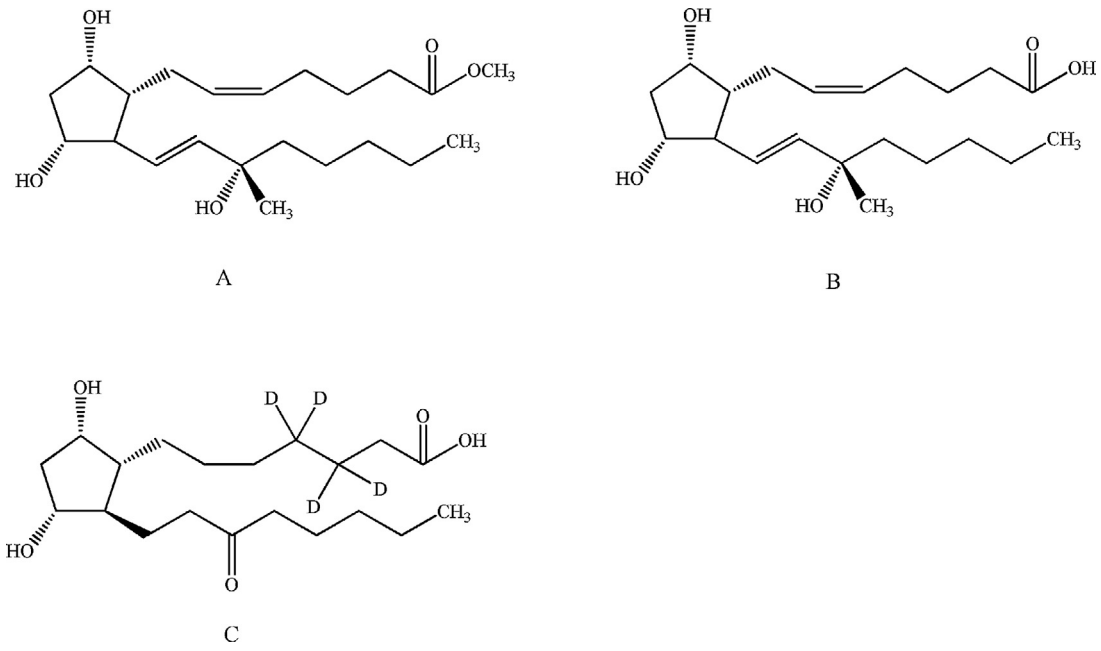

Fig. 1. Chemical structures of (A) carboprost methylate, (B) carboprost and the IS, alprostadil-d4.

transformation to release the active drug carboprost which can exert the desired pharmacological effect. It is important to quantify carboprost methylate in plasma at different stages in preclinical studies because exposure assessment is a necessary component of safety and efficacy evaluation.

Several studies have been reported concerned with the determination of prostaglandin drugs in biological matrix. However, the quantitative analysis of prostaglandin drugs especially in vivo analysis is still a very difficulty task which was not only challenged by its low levels (pg/mL) following a dose in microgram order, but also challenged by the endogenous prostaglandins interferents. To date, methyl carboprost and its acid-catalyzed degradation products have been determined in a polymeric controlled-release device by high performance liquid chromatography (HPLC) with UV detection [18]. In addition, carboprost has been determined in a sterile solution and infusion preparation using HPLC with UV and MS detection [19,20]. Green and Bygdeman [21] developed a GC–MS method for the quantification of carboprost and carboprost methylate in plasma, which required multiple step derivatization and was time-consuming. Only several samples could be analyzed per day. The sensitive and high throughput method for the simultaneous quantitation of carboprost and carboprost methylate in biological matrix has not been developed and validated owing to the interference from endogenous prostaglandins and the requirement for high sensitivity (pg/mL). Moreover, the esterases existing in bio-samples might hydrolyse carboprost methylate to carboprost. Therefore, effective esterase inhibator is crucial to prevent the cleavage occurred during blood collection, sample storage, and sample processing.

The ionization of the analytes is another challenge. Based on preliminary studies in our laboratory, it was found that liquid chromatography tandem mass spectrometry (LC–MS/MS) would allow simultaneous quantitation of the two analytes with the required sensitivity but only if positive ionization was used to determine carboprost methylate and negative ionization was used to determine carboprost. Furthermore, due to the different polarities and solubilities of the analytes, simultaneous analysis in biological samples posed a significant challenge. To meet these challenges, the present investigation aimed to develop an LC–MS/MS method to determine carboprost and carboprost methylate in dog plasma. This paper reports the first such assay and its application to a pharmacokinetic study of the analytes after an intravaginal administration dose of a carboprost methylate suppository containing 0.5 mg carboprost methylate.

2. Experimental

2.1. Chemicals and reagents

Carboprost methylate (purity > 98.9%), carboprost tromethamine (purity > 99.5%), and carboprost methylate suppositories (containing 0.5 mg carboprost methylate) were provided by the Northeast Pharmaceutical Co. (Shenyang, China). Alprostadil-d4 (purity > 99.5%) for use as internal standard (IS) was provided by Medical Isotopes, Inc., USA. The structures of the analytes and IS are shown in Fig. 1. Dichlorvos, diisopropyl fluorophosphate (DFP) phenylmethanesulfonyl fluoride (PMSF), paraoxon was obtained from Sigma (St Louis, MO, USA). Acetonitrile and isopropanol (HPLC-grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Distilled water, prepared from demineralized water, was used throughout the study. All other chemicals were of HPLC grade.

2.2. LC–MS conditions

The LC–MS system consisted of an Agilent 1100 Series HPLC (Agilent Technologies, Palo Alto, CA, USA) coupled to a Qtrap 5500 mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) equipped with a TurboIonSpray source. Data acquisition and integration were controlled by Analyst Software.

Chromatography was performed on a Capcell Pak Phenyl column (150 × 2.0 mm, 5 μm) maintained at 40 °C. Gradient elution utilized 5 mM ammonium acetate as solvent A and acetonitrile as solvent B delivered at a flow rate of 0.4 mL/min. The gradient elution program was as follows: 0–2 min 10% B; 2–4 min 10% → 40% B; 4–6 min 40% → 70% B; 6–6.5 min 70% B; 6.5–6.6 min 70% → 95% B; 6.6–8.1 min 95% B; 8.1–9.9 min 10% B. MS parameters optimized by infusing a standard solution of analytes and IS using a syringe pump and they were shown in Table 1.