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Determination of PMEA-Na in dog plasma by liquid chromatography and tandem mass spectrometry and its pharmacokinetic study

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ABSTRACT **AIM:** To established an HPLC/MS/MS method for the study of pharmcokinetics of PMEA-Na (the mono-sodium salts of 9-[2-(phosphonomethoxy) ethyl] adenine) in beagle dogs. **METHODS:** PMEA-Na and internal standard 9-(3-phosphony-methoxypropyl) adenine were isolated from plasma by protein precipitation with methanol, and then analyzed adopting multiple reaction monitoring (MRM) mode. Using Xterra MS column, the mobile phases consisted of methanol ∶water ∶formic acid (25 ∶75 ∶0.5) at a flow rate of 0.25 ml·min^{-1}. Beagle dogs received the intravenous dosage of PMEA-Na at 1.0, 3.0 and 6.0 mg·kg^{-1}. Pharmacokinetic parameters were obtained from concentration-time curves by non-linear least-squares regression using the program DAS. **RESULTS:** The linear calibration curve was obtained in the concentration range of 0.02 to 20 mg·L^{-1} (r＝0.999), and the limit of quantitition was 20 μg·L^{-1}. The within-day and internal-day precisions (RSD) were less than 6.5% and 10.8%, respectively. The accuracy was 97.1%～107.3%. After a single dose studies in dogs the *AUC* were 2.3±0.5, 8.2±1.3 and 18.5±1.3 mg·L^{-1}·h; the $t_{1/2}$ were 3.9±1.8, 8.4±1.5 and 8.9±0.6 h; the *CL* were 0.44±0.09, 0.35±0.05 and 0.31±0.03 ml·h^{-1}·kg^{-1} at the dose level of 1.0, 3.0 and 6.0 mg·kg^{-1} respectively. **CONCLUSION:** The analytical method is sensitive and specific for investigation the pharmacokintics of PMEA-Na in beagle dogs.

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PMEA-Na is mono-sodium salts of PMEA (9-[2-(phosphonomethoxy) ethyl] adenine), which is a nucleotide analogue of adenosine with potent activity against retrovirus replication that hasn't 3'-hydroxylic root so as to compete DNA polymerases and reverse transcriptases and inhibit DNA synthesis[1]. PMEA has been approved for the treatment of chronic hepatitis B in both Europe and USA[2]. Numerous studies demonstrated that PMEA has low oral bioavailability in a number of species including rats, cynomolgus monkeys and human[3-5]. So intravenous injection was considered an administrated route. In the paper, we established a specific and simple liquid chromatography and tandem mass spectrometry (HPLC/MS/MS) analysis method of PMEA-Na in dog plasma in order to carry out its pharmacokinetic studies in beagle dogs by intravenous administration.

1 MATERIALS AND METHODS

1.1 Chemicals and instrument PMEA-Na(99.4%, Fig1A) was obtained from the Jiangsu Wuzhong Suyao drug exploiture Ltd. 9-(3-phosphony-methoxypropyl) adenine (99.1%, Fig 1B) as internal standard (IS) was presented by Pro. ZHONG Da-fang of Shenyang Pharmaceutical University. Methanol was from Fisher, water was Millipore purity, and other chemicals and solvents were of analytical grade. Alliance 2695-Quattro Micromass API

(HPLC/MS/MS, Waters) including solvent delivery system, auto-injector, column oven, triple quadrupole tandem mass spectrometry detector equipped with an ESI source, Masslynx 4.0 workstation and quanlynx software.

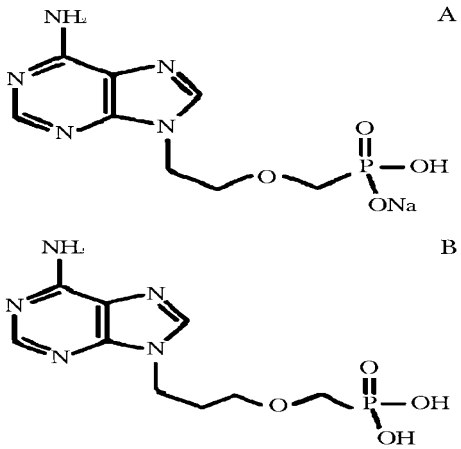

Fig 1 Chemical structure of PMEA-Na (A) and 9-[2-(phosphonomethoxy) propyl] adenine (B)

1.2 Animals 9 male and 9 female beagle dogs (4.7—7.1 kg) were purchased from Nanjing Xinxueren technology development Inc and used for the study. The certificate number was 2002-0028(SCXK). The dogs were housed in stainless-steel cages and were conscious throughout the entire study. The dogs were fasted 12 h prior to dosing and until 6 h post-dosing during the experiment, but were allowed to drink water freely.

1.3 Chromatogram conditions PMEA-Na was analyzed using Xterra MS C_{18} column (3.5 μm, 2.1 mm × 150 mm, Waters Corporation). The mobile phases consisted of methanol ∶water ∶formic acid (25 ∶75 ∶0.5). The column temperature was 25 ℃. The analysis time was 5 min at a flow rate of 0.25 ml·min^{-1}.

1.4 Mass spectrometry conditions Capillary voltage 3.5 kV, cone voltage 38 V, source temperature 100 ℃, desolvation temperature 350 ℃, desolvation gas flow rate 450 L·h^{-1}, collision induced voltage 28 V. Elecetrospary ionization (ESI) source was applied and operated in the positive ion mode. Multiple reaction monitoring (MRM) mode with parent/daughter mass transitions of 274.0/162.1 and 288.0/176.1 was used to quantity PMEA and the internal standard, respectively. Scan time was 0.1 s.

1.5 Disposition of plasma sample Plasma (100 μl), 25 μl water, and IS (25 μl of 1 μg·ml^{-1}) were mixed with 400 μl methanol and vortexed for 3 min to precipitate protein, then centrifuged at 14,000 r·min^{-1} for 10 min. The supernatant was dried with N_2 flow and reconstituted in 100 μl mobile phase, then vortexed and centrifuged at 14,000 r·min^{-1} for 5 min. The supernatant was transferred to auto-injector vials for HPLC/MS/MS analysis.

1.6 Pharmacokinetics in beagle dogs Beagle dogs were randomly divided into 3 groups, which received the intravenous dosage of PMEA-Na at 1.0, 3.0 and 6.0 mg·kg^{-1}, respectively. PMEA-Na was formulated for intravenous injection as solution in 0.9% NaCl by behind limb vein. Blood samples (0.4 ml) were collected for analysis of PMEA-Na from forelimb vein, and placed into heparinized tubes at 0 (predose), 1, 5, 15, 30, 45 min and 1, 2, 4, 6, 8, 12, and 24 h post dosing.

1.7 Data analysis All data are presented mean ± standard deviation ($\bar{x} \pm s$). Statistical analysis was performed by Student t-test, ANOVA analysis and Pearson-test. Pharmacokinetic parameters were obtained using the program DAS.

2 RESULTS

2.1 Validation of analytical method The analytical procedure was specific and sensitive for determination concentration of PMEA-Na in plasma. The peak shapes of PMEA-Na and IS were good, and matrix of plasma did not interfere the detection. The retention time of PMEA-Na and IS was 2.4 and 2.6 min, respectively, and performed determination only need 5 min (Fig 2). A linear relationship was good over the range of 0.02 to 20 mg·L^{-1} by calculation of a regression line by the method of least squares. The calibration curve equation was "f= 0.532× C+ 0.0332" (r= 0.999, f: the ratio of peak area of PMEA-Na to that of IS, C: the concentration of PMEA-Na), and the lower limit of quantitation (LOQ) was 20 μg·L^{-1} (S/N> 10 ∶1). The accuracy (recovery) of PMEA-Na was 97.1% ~107.3%. The within-day and between-day precisions were less than 6.5% and 10.8%, respectively. The analytical procedure provides an acceptable degree of linearity, accuracy, and precision (Tab 1).

Tab 1 Recovery and precision of PMEA in dog plasma($\bar{x} \pm s$, n= 5)

Concentrations /mg·L^{-1}	Recovery /%	RSD /%	
		Within-day	Between-day
0.05	107.3±2.3	5.5	10.8
0.20	106.2±1.5	2.9	3.0
2.00	97.1±3.3	6.5	3.2
20.0	99.7±2.1	2.1	3.8

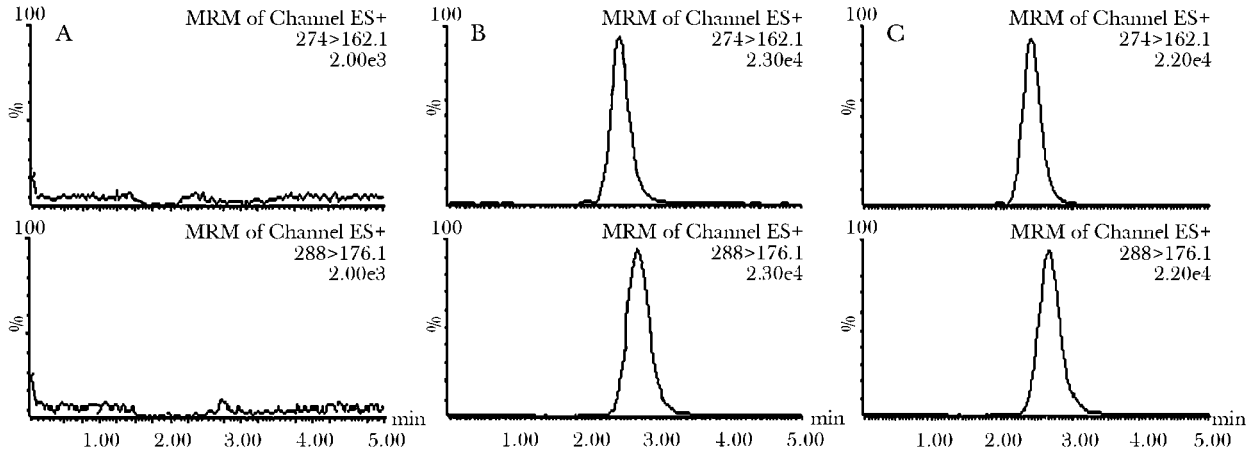

Fig 2　Typical chromatograms of PMEA-Na and 9-(3-phosphony-methoxypropyl) adenine (internal standard, IS) by multiple reaction monitoring scan mode

A: Blank dog plasma; B: Blank dog plasma spiked with standard; C: Dog plasma of 1 h after iv dose of 3.0 mg·kg^{-1} PMEA-Na; Peak 1: PMEA-Na; Peak 2: IS

2.2 Pharmacokinetics in dog　The concentration-time profiles for plasma PMEA-Na following intravenous administration of 1.0, 3.0 and 6.0 mg·kg^{-1} was shown in Fig 3. Pharmacokinetic parameters are given in table 2. The disposition of PMEA-Na was adequately described by a two-compartment model. At the dose level of 1.0, 3.0 and 6.0 mg·kg^{-1}, after the single dose studies, the areas under the plasma concentration-time curve (*AUC*) were 2.3±0.5, 8.2±1.3 and 18.5±1.3 mg·L^{-1}·h; the C_{max} were 3.4±0.8, 9.3±2.5, 19.6±4.9 mg·kg^{-1}. After the statistical analysis, both the *AUC* and C_{max} were dose-dependent over the dose range of 1.0 to 6.0 mg·kg^{-1}, and correlation coefficients both were 0.9992 (*P* values both were 0.026). The mean terminal half-life was ($t_{1/2}$) 3.9±1.8, 8.4±1.5 and 8.9±0.6 h. The $t_{1/2}$ of the 1.0 mg·kg^{-1} dosage group appeared to be less than the ones of others. That was probably owing to the constraints of the analytical method (PMEA-Na was not detectable beyond 8 or 12 h post-dosing at the dose group of 1.0 mg·kg^{-1}, whereas the drug was detectable at 24 h post dosing at the dose groups of 3.0 and 6.0 mg·kg^{-1}. The clearance (*CL*) was 0.44±0.09, 0.35±0.05 and 0.31±0.03 ml·h^{-1}·kg^{-1}, which exceeded the glomerular filtration rate in this species (0.160 ml·h^{-1}·kg^{-1}) indicating the possibility of active tubular secretion, which was similar with the result of previous studies.

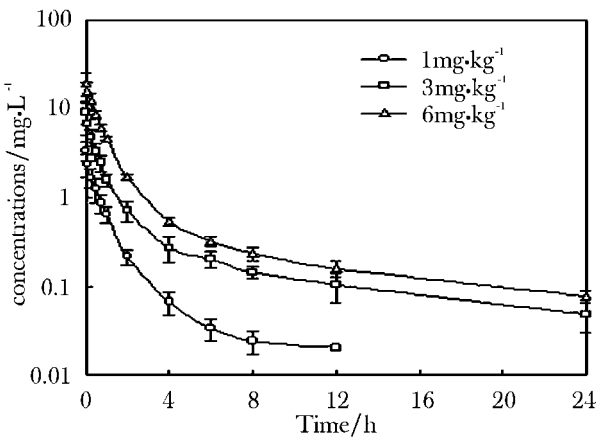

Fig 3　Mean plasma concentration-time curve of PMEA-Na after iv a single dose to beagle dogs ($\bar{x}\pm s$, $n=6$)

Tab 2　Pharmacokinetic parameters of PMEA-Na after iv a single dosage to dogs($\bar{x}\pm s$, $n=6$)

Dose /mg·kg^{-1}	C_{max} /mg·L^{-1}	AUC_{0-24} /mg·L^{-1}·h	$AUC_{0-\infty}$ /mg·L^{-1}·h	$t_{1/2}$ /h	*CL* /L·h^{-1}·kg^{-1}	Vd /L·kg^{-1}
1.0*	3.4±0.8	2.3±0.5*	2.3±0.5	3.9±1.8	0.44±0.09	2.5±1.0
3.0	9.3±2.5	8.2±1.3	8.8±1.6	8.4±1.5	0.35±0.05	4.3±0.6
6.0	19.6±4.9	18.5±1.3	19.2±1.5	8.9±0.6	0.31±0.03	4.2±0.6

* In this dosage group, PMEA was only detectable at 8 or 12 h post-dosing, so the *CL* values was also higher than the other dose group

3 DISCUSSIONS

The analytical methods of derivatization PMEA with chloroacetaldehyde and ion-pair liquid chromatography have been reported previously[3-6], however, lower limits of quantification (LOQ) in plasma, 40, 250 and 200 μg·L^{-1}. The reported method[7] was referred to analyze the sample with HPLC/MS/MS method and treat plasma

with protein precipitation combined with evaporation of the aqueous sample and reconstitution with mobile phase. The assay method was simple and sensitive without time-consuming in pre-treatment procedures; LOQ in dog plasma was 20 $\mu g \cdot L^{-1}$, and analysis time only had 5 min using isocratic liquid chromatography.

Data on the pharmacokinetics of PMEA in a number of animal species are available, including mice, rats, monkeys and human[3-6]. Plasma PMEA concentration generally declined in a biexponential manner following intravenous administration. In our experiment, the disposition of PMEA-Na was adequately described by a two-compartment model. The results suggest that the pharmacokinetic behavior of PMEA-Na was liner kinetic over the dose range of 1.0—6.0 $mg \cdot kg^{-1}$. The mean terminal half-life were longer than the previously examined values in mice, rat, rhesus monkeys and human (0.1, 3.1, 1.6 and 1.0 h) but similar with the terminal half-life of PMPA (9-[2-(phosphonomethoxy) propyl] adenine), a structural analog of PMEA adding a methyl side chain, intravenously in dogs (4.5±1.9 and 9.54±1.21 h at the dose level of 1.0 and 10.0 $mg \cdot kg^{-1}$, respectively)[8]. The terminal half-life may be dependent on the sensitivity and accuracy of the analyses method and design of sampling time point. The LOQ of methodology above PMPA was 25 $\mu g \cdot L^{-1}$, which was similar with ours.

In summary, the bioanalytical method of LC/MS/MS is sensitive, specific and suitable for pharmacokinetics study of PMEA-Na in beagle dogs.

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PMEA-Na 在 beagle 犬血浆中的液相色谱/质谱/质谱联用法测定及其药代动力学

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摘要 目的: 建立 LC/MS/MS 法测定犬血浆中 PMEA-Na 浓度, 进行其药代动力学研究。方法: 血浆样品经甲醇沉淀蛋白后, 采用多反应监测法测定其血药浓度。色谱柱为 Xterra MS 柱, 流动相为甲醇∶水∶甲酸(25∶75∶0.5), 流速为 0.25 $ml \cdot min^{-1}$。Beagle 犬分 3 个剂量组经静脉给药, 给药剂量分别为 1.0、3.0 和 6.0 $mg \cdot kg^{-1}$。药代动力学参数通过 DAS 软件计算获得。结果: PMEA-Na 线性范围: 0.02 ~ 20 $mg \cdot L^{-1}$ ($r = 0.999$); 最低检测浓度为 20 $\mu g \cdot L^{-1}$, 方法回收率为 97.1% ~ 107.3%, 日内日间变异分别小于 6.5%、10.8%。beagle 犬在 1.0, 3.0 与 6.0 $mg \cdot kg^{-1}$ 剂量下单次 iv PMEA-Na 后, 测得其 *AUC* 分别为 2.3±0.5, 8.2±1.3 and 18.5±1.3 $mg \cdot L^{-1} \cdot h$; $t_{1/2}$ 为 3.9±1.8, 8.4±1.5 and 8.9±0.6 h; *CL* 为 0.44±0.09, 0.35±0.05 and 0.31±0.03 $ml \cdot h^{-1} \cdot kg^{-1}$。结论: 本方法专属性强, 准确性好, 可用于 PMEA-Na 血药浓度测定和药代动力学研究。

关键词 PMEA-Na; LC/MS/MS; 血药浓度; 药代动力学