Determination of Peramivir and Its Toxicokinetics in Beagle Dogs

by

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Approved by _____
Supervisor

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碩士學位論文

測定 Beagle 犬體內的帕拉米韋及其毒代動力學研究

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University of Macau

ABSTRACT

DETERMINATION OF PERAMIVIR AND ITS TOXICOKINETICS IN BEAGLE DOGS

by Yang Jing

Thesis Supervisor: Professor Li Shaoping
Major: Chinese Medicinal Sciences

Peramivir as a new potent neuraminidase inhibitor was reported effectively inhibition of both influenza A and influenza B virus and also considered as a therapy for the influenza pandemic, such as H1N1, H5N1 and H3N2. *In vitro* and *in vivo* pharmacodynamics results show that peramivir is comparable or better than other two licensed neuraminidase inhibitors, oseltamivir carboxylate and zanamivir. Therefore peramivir was considered as a potent new drug for treatment of influenza. For drug registration, toxicokinetic analysis has generally been requested as a routine component of toxicological evaluation and safety assessment. This paper studies on the concomitant toxicokinetics of peramivir in beagle dog and provide useful information for its clinical safety trials.

The thesis consists of two chapters. **Chapter 1** is the introduction of the thesis including the pandemic hazard of influenza virus as well as its treatment, and the current progress of peramivir. In **Chapter 2**, a 30-day concomitant toxicokinetics study of peramivir in beagle dogs was carried out as part of the preclinical safety profile. Three groups of four dogs per gender were intravenously administrated once daily at three dose levels of 77.8, 233.3 and 700.0 mg/kg. Plasma samples were collected on two periods (1^{st} day and 30^{th} day). Reverse-phase HPLC method with 210 nm UV detection was established to determine the concentration of peramivir in plasma. Good inter/intra-batch precision (<9.1%) and recoveries (> 82.5%) on low-, mid-, and high-level were obtained. The toxicokinetic analysis was performed with

WinNonlin 5.2. C_{max} and AUC increased with increment of dose. At 77.8, 233.3 and 700.0 mg/kg, AUC_{0-24} were 301.3±45.8, 939.6±275.5, 5445.9±956.7 mg*hr/L and 293.0±40.6, 993.5±165.6, 4180.8±1102.0 mg*hr/L on 1st day and 30th day, respectively; There was no statistic significant difference in $MRT_{0-\infty}$ among different doses or between the two collecting terms.

Key words: *peramivir; toxicokinetics; influenza; neuraminidase inhibitor; beagle dog*

摘要

測定 Beagle 犬體內的帕拉米韋

及其毒代動力學研究

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帕拉米韋作為強有效的神經氨酸酶抑制劑能有效抑制流感病毒A和B而被認為可以控制諸如H1N1，H5N1及H3N2等病毒引起的流感傳播。體內及體外藥效學實驗顯示帕拉米韋的藥效至少不低於其他兩種臨床神經氨酸酶抑制劑：奧司他韋和紮那米韋。因此帕拉米韋被視為相當有潛力的新藥。在新藥註冊過程中毒代動力學分析是毒性評定和藥物安全評估中必不可少的部分。而本實驗為帕拉米韋在Beagle犬體內的毒代動力學研究並為臨床安全評價提供依據。

本論文包括兩章：第一章是綜述部分，包括：流感病毒的流行性危害及治療手段，和抗流感病毒藥帕拉米韋的研究進展。第二章為帕拉米韋 Beagle犬靜脈滴注反復給藥（一個月）毒性試驗的伴隨試驗。將24只健康Beagle犬隨機分成三組分別按劑量77.8 mg/kg、233.3 mg/kg和700.0 mg/kg靜脈滴注帕拉米韋，每天給藥一次，連續給藥一個月，分別於兩個週期即給藥第1天和第30天采血，採用HPLC-UV法測定帕拉米韋血藥濃度。該方法高、中、低三個濃度批間和批內變異（RSD）均小於10％；提取回收率>82%，顯示了良好的適用性。採用WinNonlin 5.2進行毒代動力學資料分析發現AUC與劑量成正相關：給藥第1天，低、中、高三個劑量組帕拉米韋AUC_{0-24}分別為301.3±45.8 mg*hr/L、939.6±275.5 mg*hr/L和5445.9±956.7 mg*hr/L，第30天，AUC_{0-24}分別為293.0±40.6 mg*hr/L、993.5±165.6 mg*hr/L和4180.8±1102.0 mg*hr/L。相同給藥週期（第1天或第30天）不同劑量組和不同給藥週期（第1天和第30天）相同劑量組相比較，帕拉米韋平均駐留時間（$MRT_{0-\infty}$）均無顯著性差異。

關鍵詞：帕拉米韋；毒代動力學；流行性感冒；神經氨酸酶抑制劑；Beagle犬

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LIST of Abbreviations

Abreviations	Full Name
HA	Haemagglutinin
NA	Neuraminidase
M2	Matrix 2
NANA	*N*-acetylneuraminic acid
DANA	2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid
T 705	Pyrazine
IMP	Inosine 5′-monophosphate
Neu5ac2en	Neuraminic acid
AUC	The area under the plasma concentration-time curve
C_{max}	Maximum concentration in plasma
MRT	Mean residence time
CPE	Cytopathic effect
UV	Ultra violet
MS	Mass spectrum
IM	Intramuscular
FDA	Food and Drug Administration
EUA	Emergency Use Authorization
LLOQ	Lower limit of quantification
ULOQ	Upper limit of quantification
QC samples	Quality control samples
i.v.	Intravenous

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