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Dose-dependent pharmacokinetics of bulleyaconitine A in rats

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This study was conducted to determine the pharmacokinetic characteristics of bulleyaconitine A (BLA) after oral gavage and intravenous administration of BLA at a single dose of 0.04, 0.12, 0.36 mg/kg (oral) or 0.02 mg/kg (i.v.) in male Sprague-Dawley rats. Plasma concentration profiles were analysed using a non-compartmental pharmacokinetic method. Following i.v. 0.02 mg/kg and oral administration 0.04, 0.12 or 0.36 mg/kg, the geometric mean C_{max} values were 19.97, 2.11, 5.11 and 11.47 ng/ml, respectively; the corresponding geometric mean $AUC_{0\text{-}t}$ values were 10.50, 3.19, 9.59 and 18.10 ng · h/ml, respectively. The median T_{max} values were 0.033, 0.167, 0.167 and 0.167 h, respectively. The terminal elimination half-lives ($t_{1/2}$) were 1.23, 2.48, 1.93 and 2.17 h, respectively. The results showed that C_{max} and $AUC_{0\text{-}t}$ increased with increasing doses of BLA. The increase in exposure with increasing dose was lower than expected under conditions of strict proportionality.

1. Introduction

Bulleyaconitine A (BLA, Fig. 1) is isolated from *Aconitum* plants and is classified as an "aconitine-like" alkaloid. BLA in solution (0.2 mg/2 ml; IM) or in tablet form (0.4 mg) has been prescribed for the treatment of chronic pain and rheumatoid arthritis in China. Aconitine can inhibit the function of neuronal Na^+ channels during repetitive pulses in a dose-dependent manner. The co-injection of BLA at ≤ 0.125 mM with lidocaine and epinephrine elicits complete cutaneous analgesia that lasts for up to 24 h without adverse effects (Wang et al. 2008; Rao et al. 2005).

The pharmacokinetics of BLA after an intramuscular dose of BLA (0.2 mg) in 10 healthy male Chinese volunteers has been evaluated. The peak plasma concentration (C_{max}) was 1.13 ng/ml, the time to reach C_{max} (T_{max}) was 0.90 h, and the $AUC_{0\text{-}t}$ was 5.16 ng · h/ml (Weng et al. 2005). However, the pharmacokinetics and the metabolism of BLA in rats have not been reported.

The purpose of this study was to characterise the pharmacokinetics of BLA after i.v. and oral administration at various doses in rats. In addition, the linearity of the pharmacokinetics parameters with respect to the dose was investigated.

2. Investigations and results

In this study, we evaluated the pharmacokinetic properties of BLA at different doses and for different modes of administration. The mean plasma concentration-time profiles of BLA for each dosing regimen are shown in Fig. 2. The linear regressions of $\ln(C_{max})$ and $\ln(AUC_{0\text{-}t})$ versus ln(dose) are shown in Fig. 3 and Fig. 4, respectively. The PK parameters were calculated and are summarised in the Table.

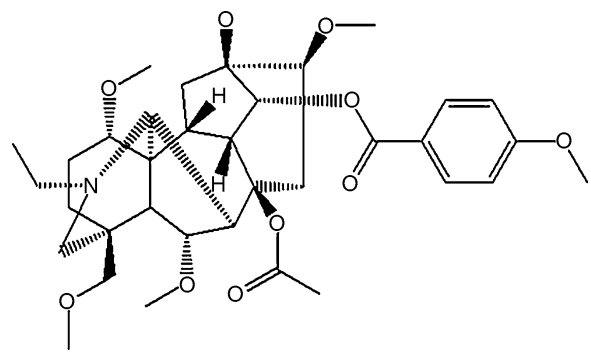

Fig. 1: Chemical structure of bulleyaconitine A

As shown in the Table, BLA was cleared rapidly in rats when the drug was given by intravenous administration, with an MRT (mean retention time) of 1 h. The Vd of BLA was more than 3 L/kg, which suggested that BLA was distributed widely in rats. The geometric means of the BLA C_{max} values after oral gavage of single doses at 0.04, 0.12, 0.36 mg/kg were 2.11, 5.41 and 11.47 ng/ml, respectively. The corresponding geometric mean $AUC_{0\text{-}t}$ values were 3.19, 9.59 and 18.10 ng · h/ml, respectively. The $t_{1/2}$ ranged from 1.93 to 2.48 hours. The increase in exposure with increasing dose was lower than expected under conditions of strict proportionality.

The proportionality coefficients for Ln C_{max} and Ln $AUC_{0\text{-}t}$ versus Ln(dose) were 0.770 (90% CI, 0.522-1.018) and 0.790 (90% CI, 0.629-0.951), respectively. These values indicated that

Table: Main pharmacokinetic parameters of BLA after a single dose of 0.04, 0.12 or 0.36 mg/kg administered by oral gavage and a dose of 0.02 mg/kg administered intravenously

Parameters	Oral gavage			Intravenous injection
	0.04 mg/kg (n = 6)	0.12 mg/kg (n = 6)	0.36 mg/kg (n = 6)	0.02 mg/kg (n = 6)
C_{max} (ng/ml)	2.11 (59.60)	5.41 (56.47)	11.47 (45.32)	19.97 (10.23)
AUC_{0-t} (ng · h/mL)	3.19 (23.94)	9.59 (37.70)	18.10 (31.42)	10.50 (5.34)
AUC_{0-inf} (ng · h/mL)	3.49 (23.51)	9.87 (37.40)	18.42 (31.49)	10.58 (5.13)
T_{max} Media	0.167	0.167	0.167	0.033
(range)h	(0.083−0.333)	(0.167−0.333)	(0.167−0.333)	0.033
$t_{1/2}$ (h)	2.48 (40.26)	1.93 (26.17)	2.17 (17.33)	1.23 (12.35)
$MRT_{(0-t)}$ (h)	1.75 (15.93)	1.93 (15.61)	1.71 (20.05)	0.99 (7.09)
Λz (1/h)	0.280 (36.67)	0.359 (30.73)	0.320 (14.69)	0.566 (12.42)
Vd/F (L/kg)	40.97 (50.56)	33.91 (52.46)	61.13 (39.63)	3.34 (14.62)
CL/F (L/h/kg)	11.47 (25.10)	12.16 (37.86)	19.54 (44.99)	1.89 (5.38)

All values are the geometric mean (%CV) unless otherwise specified.

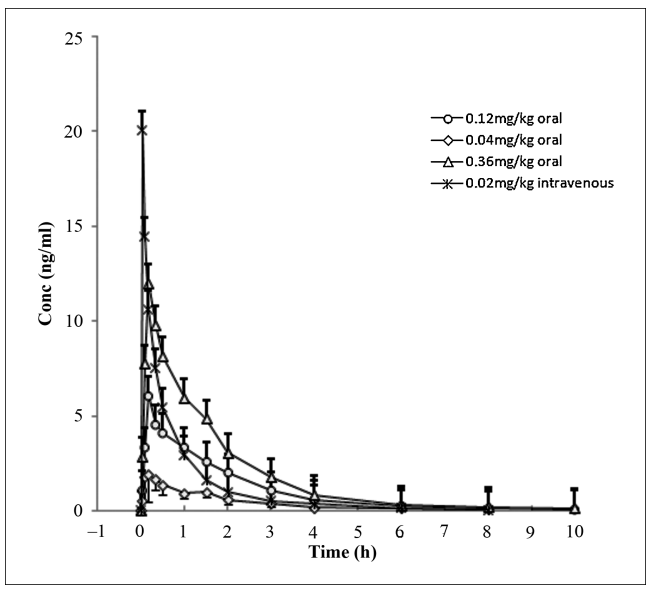

Fig. 2: Plasma concentration profiles for bulleyaconitine A in rats after oral doses of 0.04, 0.12, 0.36 mg/kg and intravenous of 0.02 mg/kg

there was a less-than-proportional increase in exposure with increasing dose. There were no significant differences in T_{max} or $t_{1/2}$ for different doses according to the nonparametric test and ANOVA, respectively.

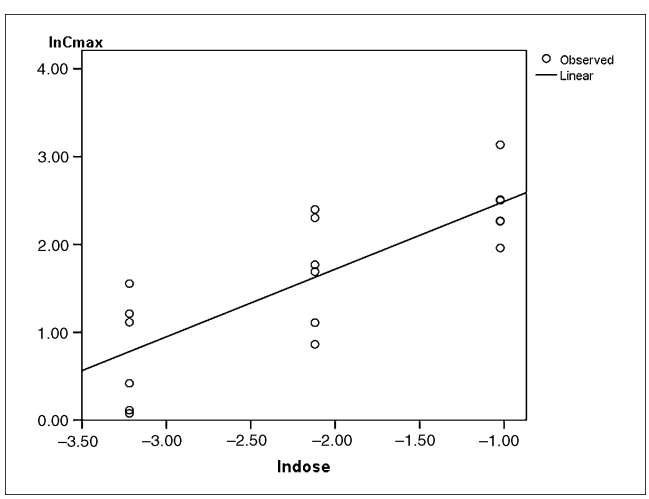

Fig. 3: Linear regression profile of Ln(C_{max}) versus Ln(dose) in rats after oral administration of 0.04, 0.12, 0.36 mg/kg of bulleyaconitine A

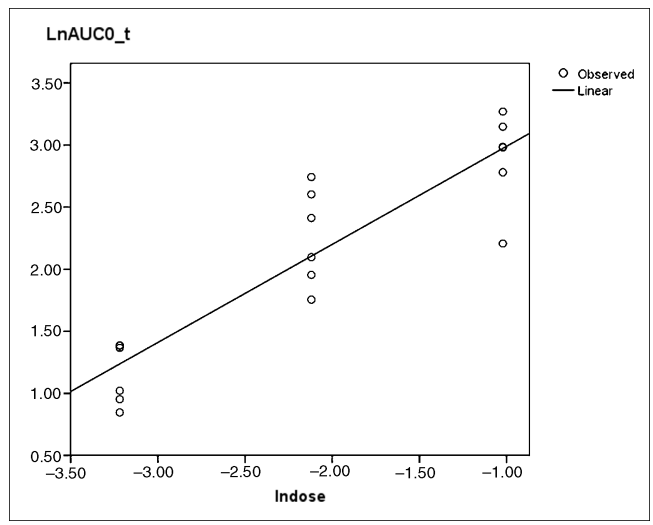

Fig. 4: Linear regression profile of Ln(AUC_{0-t}) versus Ln(dose) in rats after oral administration 0.04, 0.12, 0.36 mg/kg of bulleyaconitine A

3. Discussion

Linear regression analysis of the three oral BLA doses showed that the increase in exposure with increasing dose was lower than expected under conditions of strict proportionality, and a linear response to BLA was not obvious in the range of 0.04∼0.36 mg/kg. If it is linear at a range of higher dose, we could speculate it would also be linear at a range of lower dose. Based on these results, there is no evidence that the exposure of BLA will be linear below a dose of 0.04 mg/kg. Therefore, we cannot evaluate the absolute oral bioavailability of BLA by dose correction at a dose of 0.04 mg/kg. However, we may conclude that the oral bioavailability is quite low, which may be the result of extensive first-pass metabolism.

It is possible that the sample size was not sufficiently large in our study, and thus our results may not accurately reflect the real relationships, especially given the large individual variability exists after oral gavage administration. Therefore, the small sample size cannot be ruled out as a contributing factor to the lack of dose proportionality in the present study.

4. Experimental

4.1. Chemicals

BLA powder (purity>98.3%), BLA tablets were purchased from Yunnan HaoBang Pharmaceutical Co., Ltd. (Kunming, China). Mesaconitine (inter-

nal standard, purity > 99.3%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol was obtained from Merck (Darmstadt, Germany). Formic acid was supplied from Tedia (Fairfield, OH, USA). Diethyl ether was supplied from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Deionised water was obtained from a Milli–Q Advantage A10 system (Millipore, MA, USA). All solvents were of HPLC grade, and reagents were of the highest quality available.

4.2. Animals

Male Sprague-Dawley rats (body weight 240–306 g, 8–10 weeks of age) were purchased from Sino–British SIIPPR/BK Lab. Animal Ltd. (Shanghai, China). Animals were housed in an air-conditioned room at a temperature of 23 ± 2 °C, with a relative humidity of 50 ± 10%. Food and water were supplied *ad libitum*. Rats were fasted for 10 h before experiments but had free access to water during this time, and they were randomly divided into four groups prior to the experiment. All animal procedures involving were approved by the Fudan University Animal Care and Use Committee.

4.3. Study design and dose

The rats were cannulated with polyethylene tubing in the right jugular vein under light ether anaesthesia. Each rat was housed individually in a rat metabolic cage and allowed to recover from anaesthesia for 1 day before the study began. The rats were not restrained at any time during the study. A 0.9% NaCl injectable solution was used to flush each cannula to prevent blood clotting.

After an overnight fast, three groups of rats (n = 6) received BLA suspension (prepared in 0.5% CMC-Na aqueous solution) at doses of 0.04, 0.12 and 0.36 mg/kg, respectively, by oral gavage. One group of rats (n = 6) was administrated BLA solution (prepared in pH5.0 PBS solution) by an intravenous injection via the caudal vein at a dose of 0.02 mg/kg. Blood samples (0.25 ml) were collected via the jugular vein prior to (to serve as a control) and 2 min, 5 min, 10 min, 20 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h and 10 h after drug administration. Plasma was separated immediately by centrifugation and kept at -20 °C until analysis.

4.4. Analytical methods

The concentration of BLA was determined by LC-MS/MS (API 4000 QTRAP, AB SCIEX, Forster City, CA, Canada). Aliquots of 100 µl of plasma were spiked with 10 µl of the internal standard solution (mesaconitine, 10 ng/ml). The mixture was extracted with 2 ml of diethyl ether, followed by vortex mixing for 2 min and centrifugation at 120000 × *g* for 10 min at room temperature. The supernatant was transferred into a new glass tube and evaporated to dryness under a gentle stream of nitrogen gas at 35 °C. The residue was reconstituted with 100 µl of mobile phase, and a 5 µl aliquot was injected into the LC–MS/MS system.

The method was fully validated according to the bioanalytical method validation guidelines of the Chinese State Food and Drug Administration (SFDA). No interference peaks were observed in drug-free plasma, and both inter- and intra-batch accuracy were 92.0–102.0%, with a precision (CV%) of less than 6.1%. The calibration curve was linear over a concentration range of 0.02–20 ng/ml.

A new calibration curve was constructed using spiked blank rat plasma containing 0.02, 0.05, 0.2, 1, 4, 8 or 20 ng/ml of BLA when analysing each batch of samples, and quality control samples with concentrations of 0.05, 1.0 and 16 ng/ml were analysed along with the samples.

4.5. Pharmacokinetic and statistical analysis

To determine the pharmacokinetic parameters of BLA, the concentration-time data were analysed with a non-compartmental approach using WinNolin software (version 6.2.1, Pharsight, USA). The maximum plasma drug concentration (C_{max}) and the time to reach the maximum plasma drug concentration (t_{max}) values were taken directly from the detected concentration versus time data. The area under the curve ($AUC_{0\text{-}t}$) was calculated using the linear trapezoidal linear/log interpolation rule, with extrapolation to infinity ($AUC_{0\text{-}\infty}$) from the last detectable concentration using the terminal elimination rate constant (k_e) calculated by linear regression of the final log-linear part of the drug concentration-time curve. Plasma clearance (CL/F) was calculated from the equation $CL/F = dose/AUC_{0\text{-}\infty}$, and the apparent volume of distribution (Vd/F) was determined as $Vd/F = dose/(ke \times AUC_{0\text{-}\infty})$. The apparent elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = \ln 2/k_e$. All results were expressed as the geometric mean ± the standard deviation (S.D.). Bioavailability was calculated according to the following equation:

$$\text{Bioavailability(F)} = \frac{AUC_{0\text{-}t(p.o)}}{AUC_{0\text{-}t(i.v.)}} \times 100\%$$

Data analysis was performed using SPSS (version 16.0; SPSS Institute, USA). Analysis of variance (ANOVA) was used to analyse the logarithm of the AUC and the C_{max} of different doses of BLA using dose correction and to analyse $t_{1/2}$. A nonparametric rank sum test was used to analyse t_{max} to compare the PK parameters of different doses. $P < 0.05$ was considered statistically significant. At the same time, the ratios between the dose and the major PK parameters, AUC and C_{max}, were determined using the parameters for oral gavage, with the logarithm of the dose as the fixed factor and the intercepts of individual rats as the random factor to assess the overall average slope of the rats and to calculate 90% confidence interval (CI). If the 90% confidence interval of the slope for Log(AUC) or Log(C_{max}) versus Log (dose) ranges between 0.7 to 1.43, then there are linear relationships between the dose and the AUC and between the dose and C_{max}.

Conflict of interest statement: None of the authors of this paper have a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of this article.

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References

Wang CF, Gerner P, Schmidt B, Xu ZZ, Nau Carla, Wang SY, Ji RR, Wang GK (2008) Use of bulleyaconitine A as an adjuvant for prolonged cutaneous analgesia in the rat. Anesthesia Analgesia 107: 1397–1405.

Weng WY, Xu HN, Huang JM, Wang GQ, Shen T, Zhang JF (2005) A pharmacokinetic study of intramuscular administration of bulleyaconitine A in healthy volunteers. Biol Pharm Bull 28: 747–749.

Rao S, Sikdar SK (2000) Modification of alpha subunit of RIIA sodium channels by aconitine. Pflugers Arch 439: 349–355.