

## Pharmacokinetic behavior of 16-dehydropregnenolone after intramuscular administration in rats

Hong-Ying Yang<sup>1</sup>, Wen-Meng Zhang<sup>1</sup>, Wen-Wen Yang<sup>1</sup>, Ting Zhao<sup>2</sup>, Li-Xin Sun<sup>1\*</sup>

<sup>1</sup> School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China;

<sup>2</sup> Liaoning Institute of Pharmaceutical Industry, Shenyang 110015, China.

**Abstract:** The pharmacokinetics of 16-dehydropregnenolone (16-DHP), a sterols compound isolated from *Solanum lyratum* Thunb., was investigated in rats following a single intramuscular administration (40 mg/kg). The concentration of 16-DHP in rat plasma was determined by a high performance liquid chromatography (HPLC) method with UV detection. Levonorgestrel was used as the internal standard (IS). The pharmacokinetic parameters of 16-DHP were derived by non-compartmental method. After a single intramuscular administration, the maximum plasma concentration ( $C_{max}$ ) was  $(289 \pm 25)$  ng/mL, time to reach  $C_{max}$  ( $t_{max}$ ) was  $(0.38 \pm 0.14)$  h, the elimination half-life ( $t_{1/2}$ ) was  $(2.5 \pm 1.1)$  h, the area under the plasma concentration-time curve from time zero to the time of the last measurable concentration ( $AUC_{(0-t)}$ ) was  $(544 \pm 73)$  ng·h/mL. The results indicated that 16-DHP was absorbed quickly and eliminated rapidly in rats after the intramuscular injection.

**Keywords:** 16-dehydropregnenolone; intramuscular administration; pharmacokinetics; high performance liquid chromatography

### 1 Introduction

*Solanum lyratum* Thunb. is a popular medicinal plant in East Asian countries and widely used for the treatment of various kinds of cancer and other diseases [1]. 16-dehydropregnenolone (16-DHP), a sterols compound isolated for the first time from the active part of *Solanum lyratum* Thunb., shows efficient anti-tumor activity on HeLa cell line in our previous study [2].

16-DHP is an efficient inhibitor of  $17\alpha$ -hydroxylase and  $15\alpha$ -reductase [3]. A patent declared that 16-DHP could significantly decrease blood lipid in healthy volunteers or patients with hyperlipidemia [4].

16-DHP has been developed as an oral hypolipidaemic agent by Central Drug Research Institute (CDRI), Lucknow. There were some limited data published on the pharmacokinetics of 16-DHP in animals. Suryawanshi *et al.* [5] reported the pharmacokinetics of 16-DHP and its metabolites in rabbit plasma after oral administration. Singh *et al.* [6] described an HPLC-UV method for the measurement of 16-DHP in rat biological matrices after oral and intravenous administration to rats. Intramuscular administration is one of the common routes to administer sterols compounds, which possess the similar structure to 16-DHP, to rats parenterally. Compared with other routes of administration, a large enough amount of the drug can be readily

deposited in muscle with little pain and local irritation. In addition, possible degradation and metabolism could be avoided by the intramuscular administration [7]. Thereby, intramuscular administration could be a potential route of dosing 16-DHP in clinical use. However, pharmacokinetic information after intramuscular administration of 16-DHP is not available up until now.

The purpose of this study was to investigate the pharmacokinetic profile of 16-DHP in rats after intramuscular administration. The concentration of 16-DHP was determined by a desirable HPLC method with levonorgestrel as the internal standard (IS).

### 2 Materials and methods

#### 2.1 Animal pharmacokinetic study

Six male Sprague-Dawley rats (240–260 g) were purchased from the Experimental Animal Center of Shenyang Pharmaceutical University (SCK-2007-004). They were housed in a room with controlled temperature and humidity and allowed to free access to food and water. They were fasted overnight and allowed free access to water before drug administration. They did not receive any drug treatment for at least 30 days preceding the study. The protocol of the animal study was in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China.

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\* Corresponding author. E-mail: sunlixin67@yahoo.com

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## 2.2 Experimental design

16-DHP, prepared at 20 mg/mL in propylene glycol-alcohol-normal saline (3:4:3, v/v), was given via intramuscular injection as a bolus dose of 40 mg/kg. Blood samples were collected from venous plexus of eye socket in heparinized tubes before and 0.08, 0.17, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8 h after the dosing. Plasma samples were obtained after centrifugation at 3 000 rpm for 10 min and stored at  $-20^{\circ}\text{C}$ .

## 2.3 Sample preparation

To an aliquot of 200  $\mu\text{L}$  plasma sample, 20  $\mu\text{L}$  of acetonitrile and 20  $\mu\text{L}$  of IS (300 ng/mL) were added, and the mixture was extracted with 2 mL of n-hexane for 4 min. After centrifugation ( $12\,750 \times g$ ) for 10 min, the upper organic layer was separated and transferred to another tube. The extraction procedure was repeated and the combined supernatant was evaporated to dryness at  $40^{\circ}\text{C}$  under a gentle stream of nitrogen. The residue was reconstituted with 100  $\mu\text{L}$  of mobile phase. A 20  $\mu\text{L}$  aliquot of the resulting solution was injected into the HPLC system for analysis.

## 2.4 Analytical method

16-DHP (purity,  $>99.0\%$ , Figure 1A) was synthesized at School of Pharmacy, Shenyang Pharmaceutical University. Levonorgestrel (IS, Figure 1B) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile of HPLC grade was obtained from Shandong Yuwang Chemical Factory (Shandong, China). Purified water was purchased from Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China). 16-DHP standard solutions and IS solution were prepared in acetonitrile and stored at  $4^{\circ}\text{C}$  until used.

The HPLC system (Shimadzu, Japan) consisted of a Shimadzu LC-6A pump, an SPD-6A ultraviolet detector and a Shimadzu SCL-6B system controller. Data were acquired and processed by Anastar chromatography software (Tianjin Automatic Science Instrument Co., Ltd.). Separation was performed on a reversed-phase Diamonsil  $\text{C}_{18}$  column (250 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ , Dikma Technologies, Beijing) at the temperature of  $30^{\circ}\text{C}$ . The isocratic mobile phase consisted of acetonitrile-10 mM ammonium acetate (49:51, v/v) at a flow rate of 1.0 mL/min. The detector was set at 238 nm, and the injection volume was 20  $\mu\text{L}$ .

## 2.5 Method validation

The specificity of the method was evaluated by analyzing the blank plasma from six different sources of rats to test interference at the retention times of the analyte and internal standard. The linearity of the assay method was deter-

mined by analyzing spiked calibration samples at concentrations of 20, 40, 80, 200, 400, 800 ng/mL (each concentration in duplicate) on three consecutive days. Samples were quantified using the ratio of the peak area. Peak area ratios of 16-DHP to that of IS were plotted against concentrations, and standard curves were established using weighted ( $1/x^2$ ) least squares linear regression. The precision and accuracy of the method were evaluated by determination of QC samples at three concentration levels of 40, 200 and 640 ng/mL in six replicates on three validation days. The lower limit of quantification (LLOQ) was defined as the lowest concentration in the linear calibration curve. The extraction recovery of 16-DHP was evaluated by comparing the mean peak areas of the regular prepared QC samples ( $n=6$ ) at 40, 200 and 640 ng/mL with the mean peak areas of spiked-after-extraction samples. The stabilities of 16-DHP in rat plasma were evaluated by analyzing three replicates of plasma samples at the concentrations of 40, 200 and 640 ng/mL. The spiked plasma samples were analyzed after storage at  $4^{\circ}\text{C}$  for 24 h, at  $-20^{\circ}\text{C}$  for 45 days and after three complete freeze/thaw cycles.

## 2.6 Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using non-compartment model by Drug and Statistics (DAS, version 2.0). The values of maximum plasma concentration ( $C_{\text{max}}$ ) and time to reach  $C_{\text{max}}$  ( $t_{\text{max}}$ ) were calculated from the measured data. The area under the plasma concentration-time curve from time zero to the time of the last measurable concentration ( $\text{AUC}_{(0-t)}$ ) was calculated by the linear trapezoidal method. The terminal elimination rate constant ( $k_e$ ) was estimated by linear least squares regression of the terminal portion of the plasma concentration-time curve. The

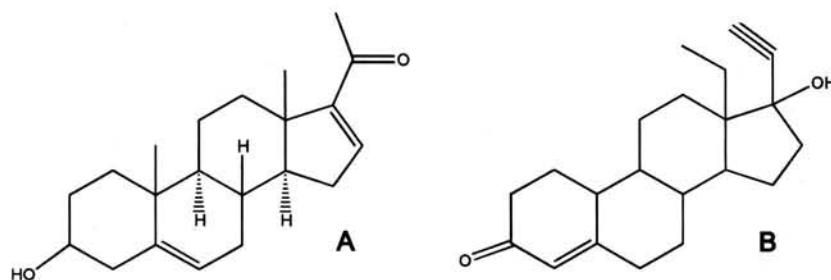


Figure 1 Structures of 16-DHP (A) and IS (levonorgestrel, B).

area under the plasma concentration-time curve from zero to infinity ( $\text{AUC}_{(0-\infty)}$ ) was calculated using the trapezoidal rule with extrapolation to infinity with  $K_e$ . The elimination half-life ( $t_{1/2}$ ) was calculated as  $0.693/k_e$ .

## 3 Results and discussion

### 3.1 Chromatographic conditions

Wavelength of 238 nm which is the maximum absorption of 16-DHP was chosen for UV detection in this assay.



The response of 16-DHP was low and the chromatographic peak shape is abnormal when using the mobile phase consisting of acetonitrile and water. Formic acid, phosphoric acid and ammonium acetate were attempted as the modifier, and ammonium acetate was selected because it could improve the response of the analyte and achieve good peak shape. The mobile phase was finally optimized as acetonitrile-10 mM ammonium acetate buffer (49 : 51, v/v) at a flow rate of 1.0 mL/min.

### 3.2 Internal standard

In HPLC assay, internal standards are used for the accurate quantification of concentrations. Levonorgestrel gave similar chromatographic profile and extraction recovery comparing with that of 16-DHP, and was finally selected as the internal standard.

### 3.3 Method validation

Figure 2 shows the typical chromatograms of the blank plasma sample, blank plasma sample spiked with 16-DHP (LLOQ, 20 ng/mL) and IS, and a rat plasma sample obtained after a single dose of 40 mg/kg. No interfering endogenous substances were observed at the retention times of 16-DHP and IS.

The method presented excellent linearity over the concentration range of 20 – 800 ng/mL with the correlation coefficient of 0.9951. The typical equation of the calibration curve was  $y = 7.700 \times 10^{-3} x + 1.690 \times 10^{-1}$  ( $r = 0.9951$ ), where  $y$  represents the ratio of 16-DHP peak area to that of IS, and  $x$  represents the plasma concentration of 16-DHP. The LLOQ was 20 ng/mL with RSD values below 8.9%, and RE values were between -11.9% and 9.3% ( $n = 6$ ).

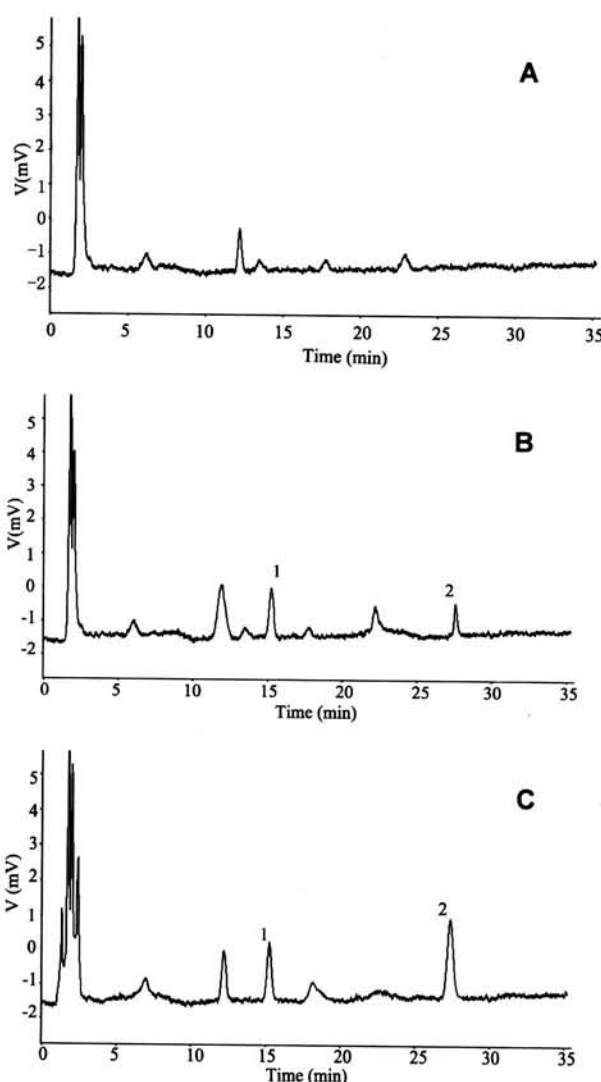
The precision and accuracy of the method were evaluated with QC samples at concentrations of 40, 200, and 640 ng/mL. The results are shown in Table 1. The intra- and inter-day precisions of the QC samples were less than 3.7% and 14.5%, respectively. The accuracy was less than -8.1%.

**Table 1** Precision (RSD) and accuracy (RE) of the HPLC method to determine 16-DHP in rat plasma ( $n = 6$ )

| Concentrations of 16-DHP (ng/mL) | RSD (%)   |           | RE (%) |
|----------------------------------|-----------|-----------|--------|
|                                  | Intra-day | Inter-day |        |
| 40                               | 3.3       | 8.7       | 5.9    |
| 200                              | 3.4       | 14.5      | 1.6    |
| 640                              | 3.7       | 12.9      | -8.1   |

The mean extraction recoveries of 16-DHP from plasma at three concentrations were  $(60.4 \pm 3.4)\%$ ,  $(74.4 \pm 1.4)\%$  and  $(67.5 \pm 5.1)\%$  ( $n = 6$ ). The extraction recovery of the IS was  $(72.1 \pm 2.3)\%$ .

The RE values for all stability samples were within  $\pm 10.5\%$ . It indicated that 16-DHP was stable in these storage conditions.



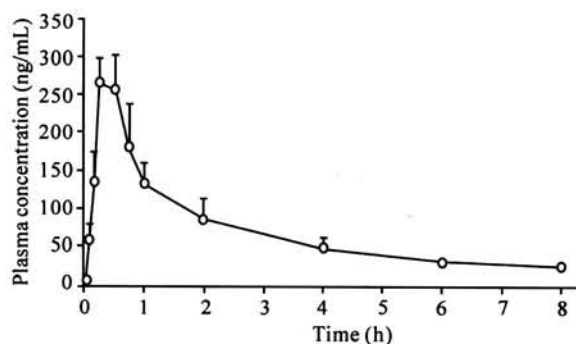
**Figure 2** Typical chromatograms of blank plasma (A), blank plasma spiked with 16-DHP (LLOQ, 20 ng/mL) and IS (B), and plasma sample obtained from a rat after intramuscular administration of 16-DHP (C). 1, IS; 2, 16-DHP.

### 3.4 Pharmacokinetic study

The validated analytical method was applied to the assay of 16-DHP in rat plasma after a single dosing by intramuscular injection into male Sprague-Dawley rats with 40 mg/kg. Mean plasma concentration-time profile after intramuscular injection of 16-DHP is presented in Figure 3. The pharmacokinetic parameters of 16-DHP derived by non-compartmental analysis using DAS 2.1 program are summarized in Table 2.

There were a few studies on pharmacokinetics of 16-DHP. Singh *et al.* [6] reported the pharmacokinetic properties of 16-DHP following a single intravenous dose (18 mg/kg) in rats. Suryawanshi *et al.* [5] exhibited the pharmacokinetic data of 16-DHP in rabbit after oral administration (40 mg/kg). After intramuscular administration of 16-DHP, the time to reach peak concentration [ $t_{max}$ ,  $(0.38 \pm$

0.14)h] was shorter than that obtained after oral administration in rabbit ( $t_{max}$ , 0.75 h) [6], suggesting that 16-DHP is absorbed more quickly after intramuscular injection in rats. The clearance ( $CL_z/F$ ) of 16-DHP in rats [(67 ± 13) L/(h·kg)] was greater than that obtained in rabbit (4.91 L/h) [6], and the value of elimination half-life [ $t_{1/2}$ , (2.5 ± 1.1)h] of 16-DHP was shorter than the corresponding value reported in rabbit ( $t_{1/2}$ , 6.0 h). These differences are probably due to the differences in metabolism with routes of administration of the drug. As total clearance is the genuine kinetic parameter controlling drug exposure, this fact also explains the low plasma levels detected,  $AUC_{(0-t)}$  [(544 ± 73)ng·h/mL] and the short  $MRT_{(0-t)}$  [(2.2 ± 0.4)h] in plasma following intramuscular administration.



**Figure 3** Mean plasma concentration time profile in rats after intramuscular administration of 16-DHP.

**Table 2** Pharmacokinetic parameters of 16-DHP in rat plasma after a single dose of 40 mg/kg of 16-DHP in six rats

| Pharmacokinetic parameters | Mean ± SD   |
|----------------------------|-------------|
| $C_{max}$ (μg/L)           | 289 ± 25    |
| $t_{max}$ (h)              | 0.38 ± 0.14 |
| $t_{1/2}$ (h)              | 2.5 ± 1.1   |
| $AUC_{(0-t)}$ (ng·h/mL)    | 544 ± 73    |
| $AUC_{(0-∞)}$ (ng·h/mL)    | 621 ± 116   |
| $MRT_{(0-t)}$ (h)          | 2.2 ± 0.4   |
| $MRT_{(0-∞)}$ (h)          | 3.4 ± 1.2   |
| $CL_z/F$ [L/(h·kg)]        | 67 ± 13     |
| $V_z/F$ [L/kg]             | 229 ± 69    |

16-DHP exhibited potential anti-tumor activity in our previous study, and chronic toxicity studies demonstrated that this drug is free from any untoward effects and possesses a suitable therapeutic window [5]. It would appear to be a potential therapeutic tool for the treatment of certain kinds of tumor in the future. Considering the structure of 16-DHP, intramuscular injection may be a probable administration route, although further studies are still needed.

#### 4 Conclusion

The pharmacokinetics of 16-DHP in rats after intramuscular administration was investigated using a specific and precise HPLC method. According to the pharmacokinetic data obtained, it is concluded that 16-DHP is absorbed quickly and eliminated rapidly in rats after intramuscular injection.

#### Acknowledgments

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