

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

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**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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## 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 ( $C_{\max}$ ) and time to reach  $C_{\max}$  ( $t_{\max}$ ) 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

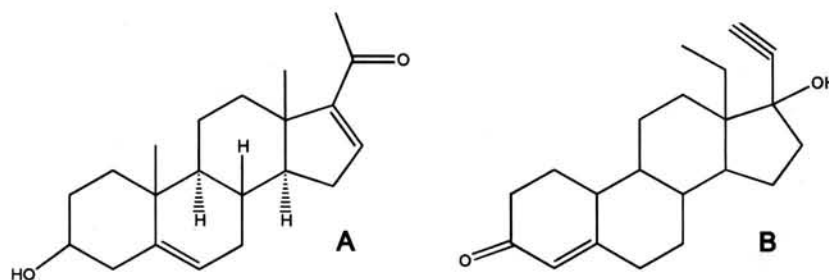


Figure 1 Structures of 16-DHP (A) and IS (levonorgestrel, B).

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.

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