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DISTRIBUTION AND ELIMINATION OF GRISEOFULVIN RESIDUE IN RABBIT TISSUES

GAO Shu-xia, YANG Li-ping, ZHANG Xiu-ling, SUN Hai-tao, JIANG Wen-xue*

Institute of Animal Science and Veterinary Medicine, Shangdong Academy of Agricultural Sciences; Shandong Provincial Key Laboratory of Animal Disease Control and Breeding, N0.8, Sangyuan Rd, Licheng District, 250100, Jinan, China
*Corresponding author: jwx0127@163.com

ABSTRACT

The distribution and elimination of griseofulvin residue in rabbit tissues were studied in order to provide basis for the safety assessment of griseofulvin used for prevention and treatment of rabbit fungal dermatopathy. Forty-two rabbits (70 days old) were fed for 14 days with pelleted feed containing 0.8 g griseofulvin/kg. The liver, kidney, muscle, skin and brain were collected from 6 rabbits per drug withdrawal day (1, 3, 5, 7, 9, 12 and 21). The concentrations of griseofulvin in rabbit tissues were determined by HPLC. Results showed that after continuous administration, the griseofulvin concentration in liver tissue was the highest (134.61 ng/g), followed by kidney tissue (54.09 ng/g). The griseofulvin residue in brain was not detected. Along with the drug withdrawal time extension, the griseofulvin concentration in muscle, liver, kidney and skin tissue was gradually eliminated. At the 21st day of drug withdrawal, the griseofulvin residue in liver and kidney tissue could still be detected (12.36 ng/g and 3.39 ng/g, respectively). The elimination rate of griseofulvin in the liver tissue was found to be the fastest. We suggest that griseofulvin should be used with caution in the rabbit farming practice.

Key words: Griseofulvin, rabbit, distribution, residue.

INTRODUCTION

Griseofulvin (*7-Chloro-2',4,6-trimethoxy-6'-methylspiro(benzofuran-2[3H],1'-[2]cyclohexene)-3,4'-dione*) is a metabolic product of *Penicillium griseofulvum*. It has fungistatic activity and is used for the treatment of dermatophytosis. It is eutherapeutic in anti-infection of *Trichophyton, Microsporum* and *Epidermophyton*. A report suggested that griseofulvin can be used for prevention and treatment of rabbit fungal dermatopathy (Wang Yao-xian, 2004).

It was reported that taking griseofulvin had toxic and side effects to human body in some cases. It was reported that there were 6 cases of malignant tumors after taking large doses of griseofulvin for treating dermatophytosis for a long time,but the 6 patients had no other carcinogenic factors to trace back (Hu Jian-jia *et al.*, 1986). In another paper it was reported that some pregnant women taking griseofulvin in the first 3 months of pregnancy gave birth to defective fetus (Rosa *et al.,* 1987). And in yet another paper it was reported that a porphyria cutanea tarda case because of taking large dose of griseofulvin for a long time (Zhang Meng-xia, 1984).

In view of the toxic effects of griseofulvin to human health, the purpose of this paper is to study the distribution and elimination of griseofulvin residue in rabbit tissues after griseofulvin administration, and to provide basis for the safety assessment of griseofulvin used for prevention and treatment of rabbit fungal dermatopathy.

MATERIALS AND METHODS

Animals and experimental design

Forty-two New Zealand White rabbits (2 kg average live weight, 70 days old) were fed *ad libitum* with pelleted feed (with griseofulvin 0.8 g/kg) for 14 days consecutively. Six rabbits were slaughtered at each drug withdrawal day (1, 3, 5, 7, 9, 12 and 21). Samples of liver, kidney muscle, skin and brain tissue were collected, packed and stored at -20 ^{0}C till analysis. Other 6 rabbits (2 kg average live weight, 70 days old) received the same diet but without griseofulvin as Control group. The same tissue samples were collected from Control group.

Chemical and materials

Griseofulvin were purchased from Wuhan Yuancheng Gongchuang Technology Co., Ltd.. Griseofulvin reference substance and propranolol hydrochloride were purchased from Alfa Aesar. HPLC grade acetonitrile and methanol were purchased from Labscience. HPLC grade acetic acid was purchased from Shanghai ANPEL Scientific Instrument Co., Ltd.. GR grade methylene dichloride was were purchased from Guangzhou Howei Chemical Co., Ltd..

Preparation of Standard Solutions

The accurately weighted griseofulvin reference substance was dissolved in methanol to a final concentration of 497 μg/mL to prepare griseofulvin stock solution. And the stock solution was diluted with methanol to prepare griseofulvin working solution. The concentrations were from 10 ng/mL to 2485 ng/mL.

The accurately weighted propranolol hydrochloride was dissolved in methanol to a final concentration of 389 μg/mL to prepare propranolol hydrochloride stock solution. And the propranolol hydrochloride stock solution was diluted with methanol to prepare propranolol hydrochloride working solution. The concentration was 116.7 ng/mL.

Equipment

Agilent 1200 series HPLC system (consisting of G1329A column oven, G1311A autosampler, G1322A pump and Agilent 6410 triple quadrupole mass spectrometer); XW-80A Vortex; Milli Q A10 ultrapure water purification system; TG16-WS high speed centrifuge; Biofuge stratos high speed refrigerated centrifuge; Haier DW-86L386 ultra-low temperature refrigerator.

Liquid Chromatography and Mass Spectrometric Condition

The mobile phase consisted of acetonitrile:water (0.1% acetic acid) (65:35, v:v). The flow rate of the mobile phase was 0.4mL/min. The chromatographic column was ZORBAX SB-C18 pre-column (4.6 mm×12.5 mm, 5μm) and ZORBAX RX-C18 column(2.1 mm×150 mm, 5μm). The column temperature was 30 °C. The sample volum was 2μL. The collection time was 3.0 min.

The multiple reaction monitoring (MRM) mode was used for quantitive analysis. A triple quadrupole mass spectrometer and ESI were employed. The data was collected under the ESI(+) mode. The mass spectrometric parameter: capillary 4000 V, drying gas 10.0 L□min, neb press 40.0 psi, gas temperature 350 °C. Quadrupole 1 and quadrupole 3 were maintained at unit resolution. Helium was used for collision gas. The collision energy was 20V. Under the ESI(+) mode the ion pair parameter of griseofulvin was (353.0→165.0) and propranolol Hydrochloride (260.1→116.2) , respectively.

Sample Preparation

Tissue samples were homogenated and stored at -20 °C and thawed at room temperature before analysis. The propranolol hydrochloride working solution 10 μL and methylene chloride 5mL were added to the samples. The samples were then adequately vortexed for 1min and were centrifuged at 3200 r/min for 10 min. The organic phase were transferred to a 10mL tube, and dried with N_2, and

then resuspended with 100 μL methanol, transfered to a 1.5 mL tube (for brain tissue, the samples were resuspended with 1mL methanol, transfered to a 1.5 mL tube, centrifuged at 12000 r/min for 10min, then transfered to another tube, dried with N_2, and then resuspended with 100 μL methanol), centrifuged at 12000 r/min for 10 min, the eluate were then transfered to a brown vial, and placed in an autosampler rack for injection.

Calibration Curve

The homogenated control tissue samples were mixed with griseofulvin working solutions to prepare calibration samples at concentration of 0, 1.08, 3.24, 12.97, 25.94, 51.87, 65.125, 103 and 249 ng/mL. and propranolol hydrochloride working solution 10 μL and methylene chloride 5 mL were added to the calibration samples. The subsequent proceeding was the same as sample preparation above. The peak area ratio (Y) of the analyte (griseofulvin) to internal standard (propranolol Hydrochloride, Hiren *et al*., 2007) and the griseofulvin concentration (X) was used for making the calibration curves.

Limit of Quantity

The lower concentration griseofulvin working solutions, propranolol hydrochloride working solution 10 μL and methylene chloride 5 mL were added to control tissue samples. The subsequent proceeding was the same as sample preparation above. If the signal of the determined lowest concentration to noise ratio (S / N) is 10:1, the lowest concentration would be the limit of quantity.

Linearity, precision and recovery

The linearity of the method was determined using linear regression analysis.

Intra-day and inter-day precision were evaluated at three concentration levels. Each level had five replicates. The samples were quantified for four days consecutively. The proceeding was the same as sample preparation above. The quantified concentrations were used to calculate intra-day and inter-day precision and compared with their nominal concentration to calculate recovery of the method.

RESULTS AND DISCUSSION

Method development and method validation

In view of the toxic and side effects of griseofulvin to human health and the use of griseofulvin to prevent and treat rabbit fungal dermatopathy, it's important to study the distribution and elimination of griseofulvin residues in rabbit tissues after its administration. To obtain a good extraction solvent, different solvents were tried. And methylene chloride worked well and was used for the study. According to references, propranolol hydrochloride was selected as the internal standard.

Distribution and concentration of griseofulvin residue in rabbit tissues

After 14 days continuous administration, the griseofulvin concentration in muscle, kidney and liver tissues within the range of 1.08 to 249 ng/mL, and the griseofulvin concentration in brain, skin tissues within range of 1.08 to 51.87 ng/mL, the response peak area and concentration was linear correlation, and the correlation coefficient (r) was greater than 0.99. The limit of quantity of griseofulvin in each tissue was the concentration of 1.08 ng/mL. The recoveries of griseofulvin in tissues was 81% to 94%.The intra-day coefficient of variation was 4.23% to 12.4%, and the inter-day coefficient of variation was 4.46% to 14.01%. The method was conformance to the testing requirement of drug in biological samples, and could be used for the quantitive analysis of griseofulvin in the rabbit tissues.

HPLC quantitative analysis results of the 1st day tissue samples after drug withdrawal showed that, except the brain tissue, the all other tissues had griseofulvin distribution. The griseofulvin concentration in liver tissue was the highest (134.61 ng/g); followed by the griseofulvin concentration in kidney tissue (54.09 ng/g); and the griseofulvin concentration in muscle and skin tissue was 32.51 ng/g and 22.03 ng/g, respectively; the griseofulvin concentration in brain were the lowest, the

detection value was lower than the limit of quantity. Griseofulvin in rabbit tissue distribution and residue concentration was shown in Table 1.

Table 1: Distribution and residue concentration of griseofulvin in rabbit tissues after continuous administration (ng/g).

Tissue	Number	Residue concentration of griseofulvin
Kidney	6	54.09±30.76
Liver	6	134.61±54.46
Muscle	6	32.51±28.52
Skin	6	22.03±24.01
Brain	6	ND

ND = not detected (lower than the limit of quantity).

The Elimination Regularity of Griseofulvin in rabbit tissues

HPLC quantitative analysis results of different days tissue samples after drug withdrawal showed that, along with the time extension, the griseofulvin concentration in muscle, liver, kidney and skin tissue were gradually decreased, and the elimination of griseofulvin in liver was the fastest. To the 21st day of drug withdrawal, the griseofulvin residue in liver and kidney tissue could still be detected. The griseofulvin concentration in liver tissue was 12.36 ng/g and in kidney tissue was 3.39 ng/g, respectively. The griseofulvin residues in other tissues were not detected. (See, Table 2).

Table 2: Concentration of griseofulvin residue in rabbit tissues of different withdrawal days (ng/g)

Withdrawal days	Number	Kidney	Liver	Muscle	Skin	Brain
1	6	54.09±30.76	134.61±54.46	32.51±28.52	22.03±24.01	ND
3	6	37.47±14.53	110.43±7.73	9.67±6.14	21.08±14.90	ND
5	6	30.13±9.43	100.39±25.78	2.70±1.47	4.52±3.82	ND
7	6	27.30±14.88	53.95±57.96	1.51±1.14	3.26±1.57	ND
9	6	24.46±19.36	50.1±41.20	1.80±2.60	4.93±4.85	ND
14	6	5.61±4.57	29.46±15.50	1.10±0.58	2.53±2.86	ND
21	6	3.39±1.64	12.36±8.30	ND	ND	ND

ND = not detected (lower than the limit of quantity).

CONCLUSIONS

After 14 days continuous administration, the griseofulvin residue in liver, kidney, muscle and skin could be detected, and the griseofulvin residue concentration in tissues from highest to lowest was liver, kidney, muscle and skin in turn. The griseofulvin residue in brain was not detected. After drug withdrawal, the griseofulvin concentration in muscle, liver, kidney and skin tissue was gradually eliminated along with the time extension. To the 21st day of drug withdrawal, the griseofulvin residue in liver and kidney tissue could still be detected.

In rabbit production, a few farms (households) tend to use griseofulvin for preventing and treating of rabbit fungal dermatopathy. On one hand, griseofulvin cannot be used as veterinary medicine, on the other hand, it was reported that taking griseofulvin had toxic and side effects for human health.
Our results showed that using 0.8 g/kg griseofulvin in rabbit production, the residue remained in products (muscles concentration of griseofulvin residue = 1.10±0.58 ng/g). Therefore, we suggest that griseofulvin should be used with caution in the rabbit farming practice, lowering its concentration and applying a withdrawal of at least 21 days.

REFERENCES

Cao Ji-yue, Lu Cong-xiao. 2005. Veterinary Pharmacokinetics. *M. China Agriculture Press, 176-217.*

Hiren N.M., Arvind G.J., Mallika S., Pranav S. 2007. Electrospray ionization LC–MSs/MS validated method to quantify griseofulvin in human plasma and its application to bioequivalence study. *Journal of Chromatography B, 850 (2007) 318–326.*

Hu Jian-jia, Zhu Yong-fu, Zhang Zhong-yi. 1986. 6 cases report of malignant tumors after taking large dose of griseofulvin. *J.Cancer, 1:115-116.*

Rosa F.W., Hernandez C., Carlo W.A. 1987. Griseofulvin Teratology, Including Two Thoracopagus Conjoined Twins. *Lancet, 1(8525), 171.*

Wang Yao-xian 2004. Using griseofulvin tablet to treat rabbit rabbit fungal dermatopathy effects well. *Chinese Journal of Rabbit Farming, 6:34-35.*

Zhang Meng-xia. 1984. One case report of griseofulvin induced porphyria cutanea tarda. *J. of Hunan Medical College, 9(3):315.*