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Acute and subchronic toxicity of arprinocid in Sprague–Dawley rats

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ABSTRACT

We subjected Sprague–Dawley rats to an acute and 13-week subchronic oral toxicity of arprinocid, a nucleoside analogue used as a coccidiostat, according to toxicological guidelines as part of its safety assessment. In the acute study, arprinocid was administered once by oral gavage to rats at doses ranging from 292.4 to 506.0 mg/kg b.w. The calculated LD_{50} was 442.9 mg/kg b.w. in males and 378.7 mg/kg b.w. in females. In the subchronic study, male and female rats were fed with diets supplemented with 0, 25, 187.5 or 500 ppm arprinocid for 13 weeks. Significantly lower body weights were noted in the 500 ppm group females. The mean body weights of the 500 ppm group females were 12.9% lower than that of the controls. Significant differences in haematological and biochemical parameters as well as organ weights were detected between the 500 and 187.5 ppm groups. Histopathological observations revealed that 500 and 187.5 ppm arprinocid could induce hepatic steatosis and focal hepatocellular necrosis. Slight protein cast in some renal tubules and tubular regeneration were observed in the high dose group of both genders. The dietary no-observed-adverse-effect level (NOAEL) of arprinocid in rats for 13 weeks is 25 ppm (approximately 1.7 mg/kg b.w./day).

1. Introduction

Arprinocid or 9-(2-chloro-6-fluoro-phenylmethyl)-9H-purine-6-amine (CAS No. 55779-18-5, $C_{12}H_9ClFN_5$, Fig. 1) is a nucleoside analogue applied as a coccidiostat in chickens and turkeys to prevent and treat coccidiosis. Arprinocid treatment protects broilers against the effects of *Eimeria acervulina*, *Eimeria mivati*, *Eimeria necatrix*, *Eimeria maxima*, *Eimeria brunetti* and *Eimeria tenella* (Ruff et al., 1978). Arprinocid inhibits hypoxanthine transport in the parasite (Wang et al., 1979), and the anticoccidial effect of arprinocid on chicks is due to a metabolite (Latter and Wilson, 1979).

Arprinocid exerts diverse toxicities. This drug causes potential developmental and reproductive risks to treated animals (Atef et al., 1989; Dilov et al., 1983; EU, 1981; Keshavarz and McDougald, 1982; Siebentritt and Kosters, 1984). In addition, a long-term toxicological study involving experimental animals has revealed that arprinocid is teratogenic in rats and mice, posing hepatotoxicity and nephrotoxicity at high doses (EU, 1981). Accordingly, the Commission of the European Community prohibited the use of arprinocid as food additive. However, insufficient toxicological information or published standard repeated dose animal toxicity data about arprinocid are available. Therefore, the present study aims to investigate the oral acute and subchronic toxicity of arprinocid in Sprague–Dawley rats. A no-observed-adverse-effect level (NOAEL) of exposure was also confirmed. The study provides useful information for the subsequent research and new drug exploration of arprinocid. The study was approved by the Ethical Committee of Shanghai Veterinary Research Institute and was conducted in compliance with Good Laboratory Practice guidelines at the Experimental Animal Center of Shanghai Veterinary Research Institute (Shanghai, China).

2. Materials and methods

2.1. Test materials

Arprinocid (molecular weight: 277.68 gmol, purity: 99.1%, lot number: 20121115) was obtained from Laboratory of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Nanjing Agricultural University (Jiangsu Province, PR China). Arprinocid standard (purity: 99.99%, lot number: 120915) was purchased from Witega Laboratorien Berlin-Adlershof GmbH (Berlin, Germany).

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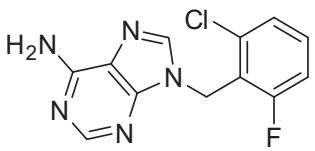

Fig. 1. Structure of arprinocid.

2.2. Acute oral toxicity study

2.2.1. Animals

Male and female specific pathogen-free Sprague–Dawley rats weighing 180–220 g were obtained from Shanghai Sippr-BK Laboratory Animal Co., Ltd. (Certificate No. 2009009). The animal rooms were maintained at a relative humidity of 50% ± 15%, a temperature of 22 ± 3 °C and a 12-h light/dark cycle. The animals were acclimated to the laboratory environment for 1 week prior to study initiation, and each animal was examined for any gross signs of disease or injury. The animals were maintained in accordance with the NIH Publication 85-23 'Guide for the Care and Use of Laboratory Animals' (NRC, 1996). All the animal protocols used in our study were approved by the Shanghai Veterinary Research Institutional Animal Care Committee.

2.2.2. Experimental design

To obtain the LD_{50} of arprinocid, the experiments and their intervals were designed according to the method provided by the Chinese Toxicology Assessment Procedures and Methods for Food Safety (ChineseStandard, 2003) and the Organization for Economic Cooperation and Development (OECD, 2001). An oral acute study for calculating LD_{50} was performed according to modified Karber's method. In this method, the dosage levels of arprinocid were determined through a pre-testing. After 7 d periods of quarantine and acclimatisation, healthy rats of either sex were randomly divided into seven groups (Table 1) of 10 animals each (5 males and 5 females). The animals were fasted overnight prior to conducting the experiment but had free access to water. The drug was suspended in 0.5% sodium carboxymethylcellulose solution and exposed to a single dose (1 mL/100 g b.w.) by oral gavage.

A detailed clinical observation was conducted for all rats throughout the study. Body weights were recorded on the day of treatment and on test days 4, 7, 10, 13 and 16. Necropsies were carried out as soon as possible after death on all rats which died during the course of study. At the end of the study, all surviving animals were sacrificed.

For acute oral toxicity, LD_{50} values and 95% confidence intervals (A) were calculated as follows:

$$LD_{50} = \lg^{-1}\left[X_m - i\left(\sum p - 0.5\right)\right] \quad (1)$$

$$A = \lg^{-1}\left(\lg LD_{50} \pm 1.96 \times i \times \sqrt{\frac{\sum p(1-p)}{n}}\right) \quad (2)$$

where X_m – logarithm of maximum dose; i – the logarithm difference between the two adjacent doses; $\sum_P$ – the sum of the mortality of animals; n – number of animals per group.

2.3. Subchronic toxicity study

2.3.1. Animals

Male and female specific pathogen-free Sprague–Dawley rats were obtained from Shanghai Sippr-BK Laboratory Animal Co. Ltd. (Certificate No. 2009009). After 10 d of acclimation to the laboratory environment, the rats were stratified by body weight and randomly assigned to treatment groups. At the start of the study, the rats were approximately 5 weeks old with males weighing between 111.5 and 134.3 g and females weighing between 97.3 and 108.4 g. Individual body weights of groups were within ±20% of the mean for each sex. All rats were housed five per group per sex in shoebox cages in rooms with a temperature of 22 ± 3 °C, a relative humidity of 50% ± 15% and a 12-h light/dark cycle.

2.3.2. Feed and water

The basal diet Slacom Certified Rodent LabDiet M01-F (Shanghai SLAC Laboratory Animal Co., Ltd.) was used as the control diet and in the preparation of the arprinocid/dietary admixes (test diet). Feed and tap water were provided *ad libitum*, except when the animals were fasted.

2.3.3. Experimental design

This study was designed and conducted in general accordance with the FDA Redbook 2000 testing guidelines (FDA, 2003) and the OECD Guidelines for the Testing of Chemicals (OECD, 1998). Before the subchronic toxicity study, we conducted a 22-day pilot study to select dose levels. In the subchronic toxicity study, after a 10 d acclimation period, groups of 10 rats of each sex were fed with diets supplemented with 0, 25, 187.5 or 500 ppm arprinocid (approximately equivalent to 0, 2, 15 and 40 mg/kg b.w./day) for approximately 13 weeks followed by a recovery period of 2 weeks. The dosage of 500 ppm was selected as the high dose because dose level 750 ppm arprinocid was found to severely inhibit growth of the rats in the pilot study. Dose level 50 ppm arprinocid was found to cause abnormality in some hematology and biochemistry parameters compared with the control group and dose level 25 ppm arprinocid was used as the minimal dose of the subchronic toxicity study. Animals were maintained in accordance with the NIH Publication 85–23 'Guide for the Care and Use of Laboratory Animals' (NRC, 1996).

2.3.4. Preparation of diets

An appropriate amount of the drug was thoroughly mixed with a small amount of basal diet and then further mixed with the rest

Table 1
Grouping and outcomes of acute toxicity study.

Group No.	Dose (mg/kg b.w.)		Number of rat dead/dosed		Death time							
	Male	Female	Male	Female	Male				Female			
					1–48 h	49–72 h	3–6 day	7∼day	1–48 h	49–72 h	3–6 day	7∼day
1	506.0	432.6	5/5	5/5	0	0	1	4	0	1	4	0
2	467.8	400.0	2/5	2/5	0	1	1	0	0	0	0	2
3	432.6	369.9	2/5	1/5	0	0	1	1	0	0	1	0
4	400.0	341.9	1/5	1/5	0	0	1	0	0	0	0	1
5	369.9	316.2	1/5	1/5	0	0	1	0	0	0	1	0
6	341.9	292.4	0/5	0/5	0	0	0	0	0	0	0	0
7	0	0	0/5	0/5	0	0	0	0	0	0	0	0