Preclinical Transplacental Transfer and Pharmacokinetics of Fipronil in Rats

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ABSTRACT

Fipronil, a widely used insecticide and pesticide, with its toxic metabolite fipronil sulfone was detected in fipronil-contaminated eggs as a result of inappropriate use. However, little is known about whether fipronil and fipronil sulfone transfer into fetus through the blood-placenta barrier. Our objectives were to investigate the transplacental transfer and the pharmacokinetics of fipronil and fipronil sulfone in rats. Male and female (with 13 days of gestation) Sprague-Dawley rats were used in pharmacokinetics and transplacental transfer experiments, respectively. Biologic samples were collected at each time point after fipronil intravenous or oral administration. To monitor fipronil and fipronil sulfone in the plasma, placenta, amniotic fluid, and fetus, a validated liquid chromatography tandem mass spectrometry method was developed. After fipronil administration in male rats, the oral bioavailability decreased, whereas the biotransformation increased as the dose increased, revealing an enhancement of first-pass effect and a fast metabolism in vivo. The results of fipronil transplacental transfer in pregnant rats demonstrated that the concentration of fipronil and fipronil sulfone varied in the following order, respectively: placenta > plasma > fetus > amniotic fluid and plasma > placenta > fetus > amniotic fluid. This is the first direct evidence that fipronil and fipronil sulfone cross the blood placental barriers and enter the fetus. The amount of fipronil distributed to the fetus was greater than that of fipronil sulfone in the short term, but by contrast, pharmacokinetic data showed that the latter stayed longer in the body. These findings provide constructive information for public health alarm.

SIGNIFICANCE STATEMENT

Fipronil and fipronil sulfone interfere with the GABAergic system. Fipronil can cause thyroid dysfunction, which may affect brain growth and nerve development. Although we knew that fipronil and fipronil sulfone could enter eggs, there was no direct evidence that they would enter fetuses. This research provided evidence on the pharmacokinetics and transplacental transfer of fipronil and fipronil sulfone, confirming our hypothesis.

Introduction

Fipronil, a member of the phenyl-pyrazole chemical family, is commonly used as an insecticide and pesticide to eliminate fleas, lice, and ticks (Tingle et al., 2003). It is a GABA-gated channel and glutamate-gated chloride channel antagonist (Cole et al., 1993; Horoszok et al., 2001). Because of its low resistance potential, high selective toxicity to arthropods (Cole et al., 1993), and long persistence in the environment (Bobé et al., 1998), fipronil occupies approximately 10% of the global pesticide market. Although fipronil is selectively toxic, it still exhibits adverse effects on multiple target organisms and threatens human health. Human exposure may lead to acute poisoning, including headaches, dizziness, sweating, nausea, vomiting, agitation, and seizures (Mohamed et al., 2004; Lee et al., 2010). The US Environmental Protection Agency also reported that fipronil shows acute toxicity, carcinogenicity, neurotoxicity, endocrine disruption, reproductive toxicity, and developmental toxicity (JMPR, 1997). Fipronil has been classified as a class II moderately hazardous pesticide by the World Health Organization. In addition, fipronil sulfone, the major metabolite of fipronil, is more toxic than fipronil itself in the GABA- and glutamate-activated chloride channel systems (Haimz et al., 1998; Zhao et al., 2005). Previous pharmacokinetic and metabolism studies have indicated that fipronil primarily converted into fipronil sulfone, which persisted for a much longer time and was stored mainly in adipose tissue and the adrenal glands within the body (Mohamed et al., 2004; Cravedi et al., 2013).

Although there are only a few studies on this subject, fipronil seems to affect the reproduction and development of the fetus. The developmental toxicity studies of fish demonstrated that fipronil exposure in the embryonic stage results in deforming and sublethal effects in Japanese Medaka (Wagner et al., 2017) and impairs the development of spinal
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in a standard solution (500 µl of blood was drawn into heparin-rinsed tubes with a paraffin film wrap as a stock solution (10 µg/ml), which was further diluted to give a series of working standard solutions. The internal standard (1 µg/ml) was prepared by diluting sorafenib, which was dissolved in acetonitrile and stored in several individual Eppendorf tubes wrapped with paraffin. All of the solutions were stored at −20°C.

Calibration curves were prepared by adding the working standard solutions into blank male rat plasma to give calibration concentrations of 1, 5, 10, 50, 100, and 500 ng/ml; in blank pregnant rat plasma, amniotic fluid, placenta homogenates, or fetus homogenates, the calibration concentrations were 2.5, 5, 10, 50, 100, and 500 ng/ml. The calibration curve was constructed from the ratio of the peak areas of fipronil or fipronil sulfone and the internal standard to the nominal concentration of fipronil or fipronil sulfone. Linearity was evaluated by the correlation coefficient ($r^2$), and a value at least 0.995 was considered to be acceptable.

Six replications of the calibration curve were performed on the same day (intraday) and over six consecutive days (interday) to evaluate the precision and accuracy. Accuracy describes the closeness of the mean results [observed concentration (Cobs)] of this method to the true concentration [nominal concentration (Cnom)]. Accuracy, quantified as relative error (RE), was calculated as RE (%) = [(Cobs − Cnom)/Cnom] × 100%. Precision, quantified as the correlation of variation (CV), was calculated as follows: CV (%) = [S.D./Cobs] × 100%. The relative error and coefficient of variation were maintained within ±15%, except for the lower limit of quantification (LLOQ), which was not permitted to exceed ±20%.

The matrix effects and recovery were calculated by three sets of samples. Fipronil and fipronil sulfone were evaluated at 1, 50, and 500 ng/ml in male samples and 2.5, 50, and 500 ng/ml in female samples, and sorafenib was evaluated at 50 ng/ml. For set 1, working solutions of fipronil, fipronil sulfone, and internal standard were diluted with acetonitrile. For set 2, blank plasma or tissue homogenate was processed as described under sample preparation without the drug to obtain blank matrix, followed by the addition of the working solution and internal standard, giving postextraction spiked fipronil and fipronil sulfone samples. For set 3, working solution and blank plasma or tissue homogenate were mixed and subjected to sample preparation, obtaining pre-extraction spiked fipronil and fipronil sulfone samples. Then, all samples were injected into the LC-MS/MS system for analysis.

The matrix effect was determined by comparing the peak area ratio of the postextraction spiked samples (set 2) to that of the standard solution samples (set 1). The recovery was quantified as the peak area ratio of the pre-extraction spiked samples (set 3) to that of the postextraction spiked samples (set 2).

Experimental Animals and Drug Administration. The animal experimental protocol listed below was reviewed and approved by the institutional animal care and use committee (approval number 1070525) by the Institutional Animal Experimentation Committee of the National Yang-Ming University and was consistent with the guidelines of the National Research Council.

Male Sprague-Dawley rats (230 ± 20 g) and female Sprague-Dawley rats (350 ± 30 g) with 13 days of gestation were used in the bioavailability and transplacental transfer experiments, respectively, and were purchased from the National Yang-Ming University Animal Center, Taipei, Taiwan. Laboratory rodents diet 5001 (PMI Feeds, Richmond, IN) was used as food. Rats were housed with a 12-hour light/dark photoperiod cycle and given ad libitum access to water.

Rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). A polyethylene tubing filled with heparinized saline (100 U/ml) was inserted into the right jugular vein emerging from the back of the neck and guided through a protective cap for fixation. After surgery, the rats were allowed to rest and recover in a clean cage overnight before drug administration. After the stabilization period, fipronil was administered (1 mg/kg, i.v.; 3, 10, or 30 mg/kg, oral) to the rats (n = 6 for each group). Collection of the blood samples was divided into two sets of time points. One set was 5, 15, 30, 45, 60, 90, 120, 150, 180, 240, and 360 minutes after intravenous administration of fipronil, and the other was 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, and 24 hours after oral administration of fipronil. At each time point, 200 µl of blood was drawn into heparinized Eppendorf tubes and then centrifuged at 13,000 rpm for 10 minutes at 4°C to obtain plasma. Plasma was stored at −20°C until analysis.

Pregnant rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and remained anesthetized as needed throughout the experimental period. The left
femoral vein was catheterized with polyethylene tubing for drug administration. The laparotomy incision was covered using gauze immersed in warm saline. To investigate the transplacental transfer of fipronil and fipronil sulfone, fipronil was administered intravenously via the femoral vein by bolus injection at a dose of 10 mg/kg (n = 6). Biologic samples, including maternal blood, placenta, amniotic fluid, and fetus from one single uterus of a dam, were collected at each time point at 15, 30, 60, 120, 180, 240, 300, and 360 minutes after drug administration. Collected placenta and fetus samples were weighed immediately, and all samples were stored at −20°C until further sample preparation.

**Sample Preparation.** The placenta or fetus was homogenized with a 2-fold amount of 0.9% normal saline (w/v) using a Polytron PT 2100 homogenizer (Kinematica, Lucerne, Switzerland). The homogenate was centrifuged at 13,000 rpm for 5 minutes at 4°C. The supernatant was collected and stored at −20°C. Biologic samples (50 µl) were mixed with 10 µl of internal standard (sorafenib 1 µg/ml in acetonitrile) and 140 µl of acetonitrile for protein precipitation. The mixture was vortexed for 5 minutes and then centrifuged at 13,000 rpm for 10 minutes at 4°C. The supernatant was filtered through a 0.22-µm filter. An aliquot (4 µl) of the filtrate was analyzed using LC-MS/MS.

**Pharmacokinetic Parameters Analysis and Statistics.** Pharmacokinetic parameters were calculated using WinNonlin Standard Edition version 5.3 (Pharsight Corp., Mountain View, CA) with an intravenous bolus input and an extravascular input noncompartmental model for the intravenous and oral groups, respectively. In addition, an intravenous bolus input noncompartmental model was employed to obtain the pharmacokinetic parameters for the transplacental transfer experiment. All data are presented as means ± S.D.. ANOVA was used to evaluate differences using IBM SPSS Statistics 24.0 (IBM Corp., Armonk, NY), and a value of P < 0.05 was taken as statistically significant.

**Results**

**Optimization of LC-MS/MS.** An LC-MS/MS method was developed to determine the fipronil, fipronil sulfone, and internal standard analytes. By optimizing the different collision energies, there was good sensitivity at m/z 434.95 → 329.95 for fipronil with a collision energy of 18 V, m/z 450.95 → 414.95 for fipronil sulfone with a collision energy of 16 V, and m/z 463.10 → 194.10 for the internal standard with a collision energy of 15 V (Supplemental Fig. 1). After modifying the LC conditions, the experimental results revealed that the sharpest peaks and best retention times occurred when a reversed-phase C18 minibore column was used to separate the analytes from the biologic matrix with an isocratic elution system consisting of acetonitrile/methanol (6:1, v/v) and water (68:32, v/v). Under these conditions, the retention times of fipronil, fipronil sulfone, and sorafenib were 3.2, 4.2, and 2.9 minutes, respectively (Supplemental Fig. 2).

The typical LC-MS/MS chromatograms of the blank biologic samples, including male rat plasma, pregnant rat plasma, placenta, amniotic fluid, and fetus, presented no obvious endogenous interference in the biologic samples. By optimizing the different collision energies, the analyte selectivity (Supplemental Fig. 2, A2–6, B2–3, C2–3, and D2–3).

**Calibration Curves and Linearity.** Linearity was achieved for the calibration curves, which were derived from the peak area ratios of fipronil and the internal standard. The linear range for the calibration curves in male rat plasma was 1–500 ng/ml, and it was 2.5–500 ng/ml in pregnant rat plasma, placenta, amniotic fluid, and fetus homogenate. The correlation coefficient of all calibration curves was greater than 0.995. The LLOQ for the male samples was 1 ng/ml, and the LLOQ for the female samples was 2.5 ng/ml.

**Matrix Effect and Recovery Evaluation.** Matrix effects and recoveries were used to assess ion suppression or enhancement and loss in sample preparation. By using the postextraction fortification method, the average matrix effects of fipronil in both male and pregnant rat plasma, placenta, amniotic fluid, and fetus samples were 94.55% ± 7.23%, 98.71% ± 5.42%, 98.73% ± 6.32%, 90.96% ± 2.28%, and 99.61% ± 3.49%, respectively. Additionally, the average matrix effects of fipronil sulfone in both male and pregnant rat plasma, placenta, amniotic fluid, and fetus samples were 92.12% ± 7.54%, 96.93% ± 4.49%, 96.20% ± 2.02%, 84.94% ± 1.73%, and 93.01% ± 3.06%, respectively. Comparing the pre-extraction and postextraction spiked solutions, the mean extraction recoveries of fipronil in both male and pregnant rat plasma, placenta, amniotic fluid, and fetus samples were 98.57% ± 9.23%, 95.03% ± 2.90%, 97.95% ± 4.72%, 96.47% ± 1.75%, and 101.2% ± 2.36%, respectively. The average recoveries of fipronil sulfone in both male and pregnant rat plasma, placenta, amniotic fluid, and fetus samples were 99.03% ± 11.83%, 103.0% ± 2.24%, 99.47% ± 2.46%, 102.2% ± 4.27%, and 105.8% ± 1.87%, respectively (Supplemental Table 1).

The results above demonstrated that the amniotic fluid samples had the most severe ion suppression in the analysis of fipronil and fipronil sulfone. However, all the matrix effects and extraction recoveries of the samples were less than ±20%, meaning that the sample preparation had no significant effect on fipronil, fipronil sulfone, or the internal standard in the biologic samples.

**Intraday and Interday Precision and Accuracy.** In intraday assays, the precision ranged from 0.49% to 9.24%, and the accuracy ranged from −10.81% to 15.11% for fipronil analysis (Supplemental Table 2). The precision ranged from 0.66% to 9.62%, and the accuracy ranged from −7.24% to 8.31% for fipronil sulfone analysis (Supplemental Table 3). In interday assays, the precision was in the range of 0.46%–10.51%, and the accuracy was in the range of −4.11% through 15.44% for fipronil analysis (Supplemental Table 2). The precision was in the range of 0.96%–15.98%, and the accuracy was in the range of −12.92% through 18.76% for fipronil sulfone analysis (Supplemental Table 3).

The precision and accuracy values were within ±15%, and the LLOQ values were less than ±20%, which was considered to be in the acceptable experimental concentration range. This result indicated that the method was considered acceptable and reproducible.

**Pharmacokinetics of Fipronil and Fipronil Sulfone.** The pharmacokinetics of fipronil and fipronil sulfone were assessed after intravenous administration 1 mg/kg of fipronil and oral administration at low, medium, and high doses of fipronil (3, 10, and 30 mg/kg). The maximum plasma concentrations (Cmax) of fipronil for the three oral doses from low to high were 8.81 ± 1.91, 23.76 ± 9.21, and 47.62 ± 20.16 ng/ml, respectively, and the area under the plasma concentration–time curve from time zero to the time of the last measurable concentration (AUClast) of fipronil for the doses from 3 to 30 mg/kg were 72.26 ± 16.80, 172.4 ± 48.02, and 454.3 ± 186.2 h ng/ml, respectively. The Cmax values of fipronil sulfone for the doses of 3, 10, and 30 mg/kg were 18.74 ± 11.56, 56.92 ± 18.28, and 152.5 ± 35.62 ng/ml, respectively; and the AUClast values of fipronil sulfone for the low, medium, and high doses were 286.7 ± 146.4, 1052 ± 414.1, and 3005 ± 671.6 h ng/ml, respectively. The pharmacokinetic data demonstrated that the Cmax and AUC were proportional to the administered oral dose of fipronil and fipronil sulfone and had a significant difference (P < 0.001) (Table 1).

By comparing intravenous administration and oral administration of fipronil using the formula oral bioavailability (F, %) = (AUCoral / doseoral) / (AUCiv / doseiv) × 100%, the results were 19.28 ± 4.48%, 13.80 ± 8.75% through 12.92% for fipronil and 84.94% through 96.93% for fipronil sulfone analysis (Supplemental Table 2). The precision was in the range of 0.46%–10.51%, and the accuracy was in the range of −4.11% through 15.44% for fipronil analysis (Supplemental Table 2). The precision was in the range of 0.96%–15.98%, and the accuracy was in the range of −12.92% through 18.76% for fipronil sulfone analysis (Supplemental Table 3).
The elimination half-life (and the biotransformation results with the pharmacokinetic data of doses of oral administration (kilogram, respectively, which significantly increased from low to high fipronil in the placenta, amniotic fluid, and fetus were all approximately fipronil sulfone was approximately 4 hours. In addition, the Tmax of respectively, and there was a significant dose-dependent decrease fipronil administration (3, 10, and 30 mg/kg, oral), respectively (Table 1).

The biotransformation ratio for oral administration was more than 4-fold greater than that of the intravenous administration. These results indicated that the elimination of fipronil sulfone partially penetrated the blood-placental barrier to reach approximately 29% for fipronil sulfone, suggesting that both fipronil and fipronil sulfone partially penetrated the blood-placental barrier to reach approximately 29% for fipronil sulfone. Both pharmacokinetic parameters suggested that the elimination of fipronil sulfone might be slow.

Transplacental Transfer of Fipronil Sulfone. This validated LC-MS/MS method was used to determine the biodistribution of fipronil and fipronil sulfone in the plasma, placenta, amniotic fluid, and fetus after fipronil administration (1 mg/kg, i.v.) in pregnant rats. The concentration of fipronil gradually decreased after fipronil administration (10 mg/kg, i.v.). Then, the concentration of fipronil sulfone gradually increased to reach an average Cmax of 60.52 ng/ml at the Tmax of 4.17 hours in the pregnant rat plasma. A similar phenomenon occurred in the placenta, amniotic fluid, and fetus, for which the Tmax of fipronil sulfone was approximately 4 hours. In addition, the Tmax of fipronil in the placenta, amniotic fluid, and fetus were all approximately 2 hours. These results suggest that the biodistributions of fipronil and fipronil sulfone for placental transfer were approximately 2 and 4 hours, respectively (Fig. 2; Table 2).

For fipronil, the Cmax and the AUClast in the plasma and placenta were both significantly higher than that of amniotic fluid and fetus, respectively (P < 0.001). The AUCmax of the placenta also significantly exceeded the AUCmax of the amniotic fluid and fetus (P < 0.001). In terms of fipronil sulfone, the Cmax and the AUClast in the plasma were significantly higher than that of amniotic fluid and fetus (P < 0.001). The Cmax of the placenta was significantly higher than the Cmax of the amniotic fluid (P = 0.001) (Table 2).

The AUClast values of fipronil in the pregnant rat plasma and placenta were 2859 ± 1438 and 3411 ± 1097 h ng/ml, respectively. The AUClast values of fipronil sulfone in the pregnant rat plasma and placenta were 287.7 ± 162.2 and 215.5 ± 87.45 h ng/ml, respectively (Table 2). Comparing the AUClast of the plasma to the placenta for both fipronil and fipronil sulfone, it can be found that the AUClast of fipronil sulfone was lower in the plasma than in the placenta, whereas the AUClast of fipronil sulfone was higher in the plasma than in the placenta.

The ratio of AUCfetus/AUCplasma was defined as the mother-to-fetus pass transformation ratio of the analyte. The AUCfetus values of the fetus in fipronil and fipronil sulfone were 1085 ± 268.8 and 84.06 ± 23.83 h ng/ml, respectively (Table 2). The average ratio of AUCfetus/AUCplasma for fipronil was approximately 38%, whereas this ratio was approximately 29% for fipronil sulfone, suggesting that both fipronil and fipronil sulfone partially penetrated the blood-placental barrier to reach the fetus.

Discussion

The pharmacokinetic data in our study demonstrated that the Cmax and AUC of both fipronil and fipronil sulfone are proportional to the administered oral dose of fipronil, suggesting a linear pharmacokinetic phenomenon. A previous study mentioned that the first-pass effect of hepatic metabolism in the oral route was very important for fipronil (Roques et al., 2012), which is consistent with our findings. First, the oral bioavailability is only 12%–19%. Second, the oral biotransformation is about four to seven times higher than the intravenous one. These pharmacokinetic phenomena demonstrate that compared with intravenous administration, the concentration of fipronil dramatically falls.

### Table 1

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Fipronil (1 mg/kg, i.v.)</th>
<th>Fipronil (3 mg/kg, Oral)</th>
<th>Fipronil (10 mg/kg, Oral)</th>
<th>Fipronil (30 mg/kg, Oral)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_{1/2} (h)</td>
<td>2.66 ± 0.56</td>
<td>12.16 ± 5.21</td>
<td>9.16 ± 11.28</td>
<td>4.44 ± 1.19</td>
<td>0.207</td>
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<tr>
<td>Tmax (h)</td>
<td>1.75 ± 0.88</td>
<td>0.83 ± 0.00</td>
<td>8.11 ± 1.91</td>
<td>2.17 ± 0.75</td>
<td>0.098</td>
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<tr>
<td>Cmax (ng/ml)</td>
<td>100.5 ± 39.65</td>
<td>8.18 ± 1.91</td>
<td>23.76 ± 9.21</td>
<td>47.62 ± 20.16</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>AUClast (h ng/ml)</td>
<td>125.0 ± 20.66</td>
<td>72.26 ± 16.80</td>
<td>172.4 ± 48.02</td>
<td>454.3 ± 186.2</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>AUC_{i.h} (ng/ml)</td>
<td>153.8 ± 17.90</td>
<td>119.4 ± 16.19</td>
<td>204.6 ± 42.84</td>
<td>481.3 ± 197.1</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CL/F (l/h per kilogram)</td>
<td>6.58 ± 0.82</td>
<td>25.50 ± 3.46</td>
<td>50.84 ± 11.29</td>
<td>70.36 ± 24.28</td>
<td>0.001*</td>
</tr>
<tr>
<td>MRT_{i.h} (h)</td>
<td>3.34 ± 0.88</td>
<td>17.32 ± 6.63</td>
<td>12.98 ± 11.94</td>
<td>8.30 ± 1.39</td>
<td>0.177</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>19.28 ± 4.84</td>
<td>13.80 ± 3.84</td>
<td>12.12 ± 4.97</td>
<td>12.16 ± 3.9</td>
<td>0.055*</td>
</tr>
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</table>

MRT_{i.h}, mean residence time from time zero to infinity.

*P < 0.05 compared with the 3, 10, and 30 mg/kg, oral, fipronil groups.

**P < 0.05 compared with the 3 and 30 mg/kg, oral, fipronil group.

†P < 0.05 compared with the 10 and 30 mg/kg, oral, fipronil group.

‡P < 0.05 compared with the 10 and 30 mg/kg, oral, fipronil group.

### 3.84% and 12.12 ± 4.97% for the 3, 10 and 30 mg/kg doses of fipronil, respectively, and there was a significant dose-dependent decrease (P=0.035). The low oral bioavailability should be due to the first-pass effect. The biotransformation ratio (AUC_{fipronil sulfone} / AUC_{fipronil}) × 100% was 104.8 ± 14.88% for fipronil intravenous administration (1 mg/kg). However, the biotransformation ratios for oral administration were 400.8% ± 197.4%, 608.4% ± 197.8%, and 730.4% ± 257.7% after fipronil administration (3, 10, and 30 mg/kg, oral, respectively) (Table 1). The biotransformation ratio for oral administration was more than 4-fold greater than that of the intravenous administration. These results indicated that fipronil metabolism was closely related to the hepatobiliary system and the gastrointestinal tract.

The clearance (CL/F) of fipronil for the three orally administered doses were 25.50 ± 3.46, 50.84 ± 11.29, and 70.36 ± 24.28 l/h per kilogram, respectively, which significantly increased from low to high doses of oral administration (P = 0.001) (Table 1). Combining the CL/F and the biotransformation results with the pharmacokinetic data of the elimination half-life (t_{1/2}) and the mean residence time from time zero to infinity, which both decreased according to the dose, revealed that fipronil was metabolized vary rapidly to the metabolite fipronil sulfone in vivo and that the transforming speed was elevated by the dose. Moreover, the time to reach the maximum plasma concentration after drug administration (T_{max}) of fipronil sulfone was 4.00 ± 1.10, 6.33 ± 3.20, and 14.00 ± 2.19 hours by the oral administration of 3, 10, and 30 mg/kg of fipronil, respectively, which increased significantly by the dose (P < 0.001) (Table 1). The t_{1/2} of fipronil sulfone increased up to the plateau concentration (Fig. 1). Both pharmacokinetic parameters suggested that the elimination of fipronil sulfone might be slow.

Transplacental Transfer of Fipronil and Fipronil Sulfone. This validated LC-MS/MS method was used to determine the biodistribution of fipronil and fipronil sulfone in the plasma, placenta, amniotic fluid, and fetus after fipronil administration (10 mg/kg, i.v.) in pregnant rats. The concentration of fipronil gradually decreased after fipronil administration (10 mg/kg, i.v.). Then, the concentration of fipronil sulfone gradually increased to reach an average Cmax of 60.52 ng/ml at the Tmax of 4.17 hours in the pregnant rat plasma. A similar phenomenon occurred in the placenta, amniotic fluid, and fetus, for which the Tmax of fipronil sulfone was approximately 4 hours. In addition, the Tmax of fipronil in the placenta, amniotic fluid, and fetus were all approximately 2 hours. These results suggest that the biodistributions of fipronil and fipronil sulfone for placental transfer were approximately 2 and 4 hours, respectively (Fig. 2; Table 2).
in the systemic circulation after oral administration, whereas the concentration of fipronil sulfone greatly rises. In addition, the average bioavailability of doses 3, 10, and 30 mg/kg is about 19.28%, 13.80%, and 12.12%, respectively, and the average bio-transformation is about 400.8%, 608.4%, and 730.4%, respectively (Table 1). As the dose increases, the trends of both the above are reverse but similar in magnification, reflecting the possibility of a first-pass effect enhancement. Fipronil is metabolized through three major mechanisms (Caboni et al., 2003). The first step is the oxidation step, which leads to fipronil sulfone formation via cytochrome P450 (Scharf et al., 2000). In humans, CYP3A4 is the major isoform of cytochrome P450 that metabolizes fipronil, and its activity toward fipronil is five times higher than that of CYP2C19 (Tang et al., 2004). The second step is the reduction step, which produces fipronil sulfide. The third step is the hydrolysis step, which forms fipronil amide. Considering the interspecies differences, although the main metabolite of fipronil is fipronil sulfone in both humans and rats, and the Michaelis constant ($K_m$) values of the liver microsomes are similar, there is a 3.8-fold-higher rate of fipronil sulfone formation in rat liver microsomes than in human liver microsomes (Tang et al., 2004).

In a previous pharmacokinetic study of rats, after administration of fipronil and fipronil sulfone separately via intravenous and oral administration, the results indicated that fipronil sulfone was substantially converted from fipronil and lasted longer than fipronil (Roques et al., 2012). Compared with the previous study, a dose-dependent (3, 10, and 30 mg/kg) study design is applied in the current study and obtained consistent results. In addition, from the biotransformation and the half-life of fipronil sulfone reaching the plateau concentration, it is confirmed that fipronil is rapidly metabolized in the organisms and the elimination process of fipronil sulfone may be slow. The half-lives of fipronil in this study at 3 and 30 mg/kg were 12.16 and 4.44 hours, respectively. A previous report used radiolabeling to detect and measure the elimination half-lives of fipronil at 4 and 40 mg/kg in Charles River CD rats. The

Fig. 1. Concentration-time curves of (A) fipronil and (B) fipronil sulfone in male rat plasma after fipronil administration at the doses of 1 mg/kg, i.v. (●); 3 mg/kg, p.o. (▲); 10 mg/kg, p.o. (▼); and 30 mg/kg, p.o. (△). Data are expressed as means ± S.D. (n = 6).

Fig. 2. Concentration-time curves of (A) fipronil and (B) fipronil sulfone in pregnant rat plasma (●), placenta (○), amniotic fluid (▲), and fetus (△) after 10 mg/kg fipronil intravenous administration. Data are expressed as means ± S.D. (n = 6).
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TABLE 2
Pharmacokinetic data for the plasma, placenta, amniotic fluid, and fetus of fipronil (10 mg/kg, i.v.) in pregnant rats

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Plasma</th>
<th>Placenta</th>
<th>Amniotic Fluid</th>
<th>Fetus</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Fipronil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>0.25 ± 0.00</td>
<td>2.25 ± 1.60</td>
<td>2.00 ± 1.26</td>
<td>2.29 ± 2.52</td>
<td>0.125</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>1060 ± 443.3$^{a,b}$</td>
<td>8201 ± 282.6$^{c,d}$</td>
<td>182.7 ± 57.79</td>
<td>286.4 ± 92.49</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>$AUC_{\text{tot}}$ (ng/ml)</td>
<td>2859 ± 1435$^{a,b}$</td>
<td>3411 ± 1097$^{c,d}$</td>
<td>787.4 ± 242.9</td>
<td>1085 ± 268.8</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>$AUC_{\text{c}}$ (b μg/ml)</td>
<td>4468 ± 1933</td>
<td>6642 ± 3319$^{c,d}$</td>
<td>1253 ± 386.0</td>
<td>2204 ± 1006</td>
<td>0.002*</td>
</tr>
<tr>
<td>Fipronil sulfone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>4.17 ± 1.60</td>
<td>4.16 ± 1.69</td>
<td>4.83 ± 0.23</td>
<td>4.83 ± 1.17</td>
<td>0.619</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>60.52 ± 32.2$^{a,b}$</td>
<td>52.80 ± 17.78$^{c}$</td>
<td>8.84 ± 3.97</td>
<td>22.43 ± 5.79</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>$AUC_{\text{tot}}$ (b ng/ml)</td>
<td>287.7 ± 162.$^{a,b}$</td>
<td>215.5 ± 87.45</td>
<td>54.72 ± 39.96</td>
<td>84.06 ± 23.83</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*P < 0.05 compared with the plasma and amniotic fluid group.
$P < 0.05$ compared with the plasma and fetus group.
$P < 0.05$ compared with the placenta and amniotic fluid group.
$P < 0.05$ compared with the placenta and fetus groups. *P < 0.05 compared among the plasma, placenta, amniotic fluid and fetus groups.

**Figure 1**

![Graph showing the pharmacokinetic parameters of fipronil and fipronil sulfone in pregnant rats](image_url)

Data are expressed as means ± S.D. ($n = 6$).

The distribution study for fipronil supports the above discourse. The highest levels of fipronil residue were found in abdominal fat, followed by the adrenals. The intermediate levels were observed in the liver, pancreas, thyroid, and ovaries, and the lowest levels were in the muscle, brain, heart, and cardiac blood (JMPR, 1997). Excretion research can also provide some explanation for these phenomena. Feces not only appears to be the main route of excretion, but fipronil itself can also be detected from it, whereas it is not found in any other excrement (JMPR, 1997; Cravedi et al., 2013). In urine, the secondary route of excretion, there are several metabolites found, including detrifluoromethylsulfophenyl fipronil and its derivative (Cravedi et al., 2013).

Among most of the literature on organ distribution of fipronil, the placenta, amniotic fluid, and fetus distribution has not been mentioned. Our research provided important information that in decreasing order, the fipronil concentration is from the placenta, plasma, fetus to amniotic fluid, but the fipronil sulfone concentration is from the plasma, placenta, fetus to amniotic fluid after fipronil administration in the pregnant rat. This result was supported by previous research showing that fipronil sulfone was detected in the serum of mother-neonate pairs (Kim et al., 2019). At the same time, we put forward a different view that long-term exposure to fipronil may not lead to detection of the parent compound in the fetus, which is related to the half-life of fipronil itself (Tang et al., 2004), but in a short period of time, fipronil can still enter the fetus. Considering the developmental and reproductive toxicity of fipronil (JMPR, 1997; Udo et al., 2014; de Barros et al., 2016, 2017), it is still a potential menace. In addition, the concentration distribution could be explained by the scheme of an in vivo animal model for the transplacental transfer of oseltamivir and oseltamivir carboxylic acid in our previous study (Lin et al., 2012). Fipronil and fipronil sulfone penetrate the placenta from the maternal blood through the blood-placental barrier, enter the fetus, and finally, spread to the amniotic fluid, thus causing a sequential decrease in drug concentration.

Regarding the inconsistency in concentration distribution order of fipronil and fipronil sulfone, the affinity of the drugs and placental tissue may have a certain effect. Some drugs have been found to bind to and accumulate in placental tissue ex vivo, causing a depot phenomenon (Ala-Kokko et al., 2000). This might be due to the uptake of the lipophilic drug by the syncytiotrophoblast in the placenta (Sastry, 1999). If the drug has a high affinity for placental tissue, it cannot be easily released from the placenta into fetal circulation, especially for lipophilic drugs. In our research, the higher concentration of fipronil found in the placenta compared with maternal plasma may have been affected by this phenomenon.

Various reasons contributed to the mother-to-fetus transfer ratio of fipronil and fipronil sulfone being 38% and 29%, respectively, which indicates an incomplete transfer (Griffiths and Campbell, 2014). This can be explained by the physicochemical properties and the mechanism of the drug, which passively diffuses through the blood-placental barrier (Syme et al., 2004; Griffiths and Campbell, 2014). The molecular mass of fipronil and fipronil sulfone is less than 500 Da for each, and both are lipophilic, causing a higher opportunity to cross the blood-placental barrier. However, lipophilic drugs with a good protein binding rate usually have more difficulty entering the fetus. According to a previous report, fipronil binds to fatty acid site 1 of human serum albumin in humans (Ascenzi et al., 2018). Moreover, the ATP-binding cassette (ABC) transporters of P-glycoprotein and breast cancer resistance protein are abundantly expressed in the syncytiotrophoblast layers of placenta and in the fetal brain, liver, spleen, and intestine throughout gestation to protect the fetus from drugs in the maternal circulation (Han et al., 2018). In the fipronil-resistant strain of *Plutella xylostella* larvae, *ABC*2 gene is upregulated, but *ABCB1* gene is downregulated (Qi et al., 2016). It seems that P-glycoprotein/ABCB1 and breast cancer resistance protein/ABCG2 could impact fipronil transportation into placenta and fetus. Our data suggest that both fipronil and fipronil sulfone partially penetrate into placenta, amniotic fluid, and fetus (Table 2).

On the other hand, the discrepancy in the mother-to-fetus transfer ratio between fipronil and fipronil sulfone is a complicated issue. The main metabolic enzymes of fipronil in humans are described as follows: CYP3A4 exists in the fetal liver after 9 weeks of gestation and in the placenta, whereas CYP2C19 presents in the fetal liver after 12 weeks but not in the placenta (Hakkola et al., 1996, 1998; Hines, 2008). However, the fetoplacental metabolism has little contribution to the overall pharmacokinetics of drugs because of the small organ sizes and low

Former obtained 183 hours in males and 245 hours in females; the latter got 135 hours in males and 171 hours in females (JMPR, 1997). The former obtained 183 hours in males and 245 hours in females; the latter got 135 hours in males and 171 hours in females (JMPR, 1997). The former obtained 183 hours in males and 245 hours in females; the latter got 135 hours in males and 171 hours in females (JMPR, 1997). The former obtained 183 hours in males and 245 hours in females; the latter got 135 hours in males and 171 hours in females (JMPR, 1997).
cytochrome P450 content of the fetus and placenta. Similarly, studies on the above two enzymes in rat fetus and placenta are also inadequate. It is speculated that the fipronil sulfone in the fetus is mostly transmitted by the maternal blood, thus showing that the lipophilic properties of drugs are the main factors contributing to this inequality. Drugs with high lipophilicity are more likely to cross the blood-placental barrier (Dickinson et al., 1989). The octanol-water partition value of fipronil (4.0) is greater than the octanol-water partition value of fipronil sulfone (3.8), inducing a lower mother-to-fetus transfer ratio for fipronil sulfone (European Food Safety Authority (EFSA), 2006).

There have been many studies on the transplacental transfer of insecticides and pesticides. In animals, ivemeticon can be detected in fetal blood after maternal or fetal intravenous administration to sheep (Pérez et al., 2008). Through collected carcasses, polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, and organochlorine pesticides can be found in the blubber of ringed seal fetuses (Brown et al., 2001; Ma et al., 2014; Zhang et al., 2018). Although fipronil is not common in analytical epidemiology, and most studies are about pesticides can be found in the blubber of ringed seal fetuses (Brown et al., 2001; Ma et al., 2014; Zhang et al., 2018). Although fipronil is not common in analytical epidemiology, and most studies are about pesticides can be found in the blubber of ringed seal fetuses (Brown et al., 2001; Ma et al., 2014; Zhang et al., 2018). Although fipronil is not common in analytical epidemiology, and most studies are about pesticides can be found in the blubber of ringed seal fetuses (Brown et al., 2001; Ma et al., 2014; Zhang et al., 2018). Although fipronil is not common in analytical epidemiology, and most studies are about pesticides can be found in the blubber of ringed seal fetuses (Brown et al., 2001; Ma et al., 2014; Zhang et al., 2018). Although fipronil is not common in analytical epidemiology, and most studies are about.


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