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Single and multiple-dose pharmacokinetics of tepoxalin and its active metabolite after oral administration to rabbits (*Oryctolagus cuniculus*)

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The anti-inflammatory agent, tepoxalin, was administered to eight healthy 6-month-old female New Zealand white rabbits once daily at an oral dose of 10 mg/kg. Blood samples were obtained immediately before and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h postadministration on days 1 and 10. Tepoxalin and its active metabolite, RWJ 20142, concentrations were determined in plasma by use of high-performance liquid chromatography with mass spectrometry. C_{max} of the parent compound was reached between 3 and 8 h of drug administration, with a harmonic mean $t_{1/2}$ of 3.6 h. Peak tepoxalin plasma concentrations were 207 ± 49 ng/mL. After oral administration, the metabolite RWJ 20142 achieved C_{max} in plasma 2–8 h after administration, with a $t_{1/2}$ of 1.9–4.8 h (harmonic mean 2.8 h). Peak plasma concentrations of RWJ 20142 on day 1 were 2551 ± 1034 ng/mL.

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Tepoxalin is a nonsteroidal anti-inflammatory drug (NSAID) with multiple targets. As a dual inhibitor of cyclooxygenase (COX) and 5-lipoxygenase (LOX), tepoxalin inhibits the synthesis of inflammatory prostaglandins and leukotrienes and other inflammatory mediators, such as tumor necrosis factor, interleukin-2, and interleukin-6 (Argentieri *et al.*, 1994; Ritchie *et al.*, 1995; Goossens & Berthomme, 2001; Papich, 2004).

Eight 6-month-old female New Zealand white rabbits (*Oryctolagus cuniculus*) were obtained from a private breeder. Rabbits were deemed healthy based on physical examination, hematocrit, and total protein. During a 2-week acclimation period and subsequent treatment periods, rabbits were fed grass hay and water *ad libitum* as well as alfalfa-based pellets (Bunny Basics 15/23; Oxbow Hay Company, Murdock, NE, USA). The Kansas State University Institutional Animal Care and Use Committee approved the study protocol.

A 50 mg, rapidly disintegrating tepoxalin tablet (Zubrin, Schering-Plough, Union, NJ, USA) was dissolved in 1 mL of tap water within a 3-mL syringe. Tepoxalin was administered to rabbits at a dose of 10 mg/kg once every 24 h for 10 days at 7 AM.

Heparinized blood samples (2 mL) were obtained from the central ear artery or the lateral/medial saphenous vein immediately before and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h after administration of tepoxalin on days 1 and 10. Blood samples were then obtained immediately before drug administration and at 3 h postadministration on days 3, 5, and 7. Plasma was obtained after centrifugation for 10 min at 1000 ***g*** and stored at approximately at −70 °C until analyzed.

Plasma concentrations of tepoxalin and its active metabolite, RWJ 20142, were determined by use of validated high-performance liquid chromatography/mass spectroscopy (HPLC/MS) assay (Burinsky *et al.*, 1996; Waldman *et al.*, 1996). The extraction of tepoxalin and its metabolite from rabbit plasma used a 200 μL aliquot. To this aliquot was added 20 μL of 20 μg/mL celecoxib, and then 1 mL of methyl-*t*-butyl ether (MTBE) was added. The sample was vortexed for 1 min and centrifuged for 10 min at 1000 ***g***. The supernatant was transferred to a fresh 1.5 mL microcentrifuge tube, dried under nitrogen in a 30 °C water bath, and reconstituted with 100 μL of mobile phase. Samples were assayed within 30 days of collection. At least 30 days of stability had been demonstrated for both compounds.

The limit of quantification for this method was 1 ng/mL for both tepoxalin and its metabolite RWJ 20142, and a linear standard curve, weighted $1/x$, from 1 to 5000 ng/mL was used for quantification of both compounds. Using quality control samples of 10, 100, and 1000 ng/mL the intraday variation obtained for tepoxalin was 7.0%, 9.0%, and 7.2%, intraday

Table 1. Pharmacokinetic parameters of tepoxalin (10 mg/kg) after once daily oral administration for 10 days in eight female New Zealand white rabbits

Parameters	Tepoxalin day 0	Tepoxalin day 10	RWJ 20142 day 0	RWJ 20142 day 10
$AUC_{0-\infty}$ (h·ng/mL)	1498 ± 375	1618 ± 285	18 078 ± 8347	37 739 ± 20 648
$AUMC_{0-\infty}$ (h^2·ng/mL)	13 367 ± 7586	9339 ± 1804	135 834 ± 63 445	302 746 ± 219 254
C_{max} (ng/mL)	207 ± 49	371 ± 114	2551 ± 1034	5958 ± 2979
t_{max} (h)	5.4 ± 1.6	4.0 ± 0.32	5.4 ± 2.2	4.3 ± 1.0
$t_{1/2}$ (h)	3.6 hm	2.7 hm	2.8 hm	3.0 hm
MRT (h)	8.4 ± 2.7		7.6 ± 1.6	

Values are presented as the mean ± standard deviation. C_{max}, maximum concentration; t_{max}, time of maximum concentration; AUC_{∞}, area under the concentration–time curve; $t_{1/2}$, half-life; *MRT*, mean residence time; h, hours; hm, harmonic mean.

accuracy was 8.6%, 4.8%, and 1.2%; interday variation was 12.8%, 9.4%, and 10.0%, and the interday accuracy was 2.9%, 4.9%, and 1.2%. For the tepoxalin metabolite RWJ 20142, at the same quality control sample concentrations, the intraday variation was 7.2%, 9.4%, and 6.4%: intraday accuracy was 4.6%, 4.9%, and 2.5%, interday variation was 14.1%, 10.4%, and 13.9%; and the interday accuracy was 4.6%, 2.3%, and 2.2%. At the quality control concentrations, recoveries for tepoxalin were 63%, 62%, and 63% and for its metabolite were 77%, 63%, and 63%.

Pharmacokinetic parameters were determined for each rabbit by use of noncompartmental analysis with a commercial software program (WinNonlin®, version 4.0.1; Pharsight, Mountain View, CA, USA) (Gibaldi & Perrier, 1982; Riviere, 1999). Maximum concentration (C_{max}) of tepoxalin and RWJ 20142 in plasma and the time C_{max} (t_{max}) occurred were read directly from the data. The terminal rate constant (λ) was determined from the slope of the terminal phase of the plasma concentration curve that included a minimum of three points. The apparent half-life ($t_{1/2}$) was calculated by use of $t_{1/2} = 0.693/\lambda$ after the 1st and 10th doses and is reported as a harmonic mean. The area under the curve (*AUC*) and the area under the moment curve (*AUMC*) following the 1st and 10th doses were calculated by use of trapezoidal rule with extrapolation to infinity and without extrapolation to infinity to allow for appropriate comparison between the 1st and 10th dose.

Pharmacokinetic values following the 1st and 10th dose were compared by use of the nonparametric Wilcoxon matched-pairs signed-rank test. Differences were considered significant when $P < 0.05$. Values for the various pharmacokinetic parameters are reported as mean ± SD, median, and range (Table 1).

After the first dose, plasma concentrations of tepoxalin reached a maximum of 207 ± 49 ng/mL at 3–8 h (Fig. 1). The mean C_{max} of tepoxalin in plasma was reached at 5.4 ± 1.6 h on day 1. Tepoxalin could still be detected in plasma at 24 h postadministration at low concentrations (<10 ng/mL). The area under the concentration–time curve (AUC_{∞}) measured 1498 ± 375 h·ng/mL. On day 1, the harmonic mean terminal phase half-life ($t_{1/2}$) of tepoxalin was 3.6 h (range 1.7–9.2 h).

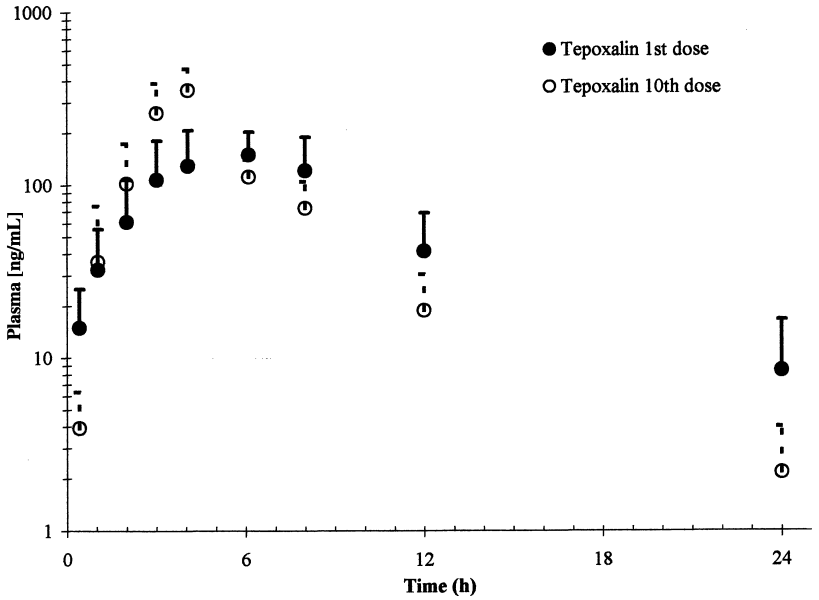

Fig. 1. Mean ± SD concentration vs. time curves for tepoxalin in plasma after administration of tepoxalin over 24 h (10 mg/kg, PO, q 24 h) in New Zealand white rabbits ($n = 8$).

Plasma concentrations of RWJ 20142 also increased after oral administration of tepoxalin on day 1 (Fig. 2). Peak concentrations of RWJ 20142 were reached at 5.4 ± 2.2 h on day 1. Metabolite concentrations increased for up to 8 h. Like its parent compound, RWJ 20142 could be quantitated in plasma at 24 h postadministration at relatively low concentrations (<100 ng/mL). Plasma concentrations of the metabolite were over 10× higher than those of tepoxalin. The *AUC* of RWJ 20142 measured 18 078 ± 8347 h·ng/mL. The half-life of RWJ 20142 ranged from 1.9 to 4.8 h (harmonic mean 2.8 h).

During day 10 of tepoxalin administration, plasma drug concentrations rose quickly, reaching a maximum of 371 ± 114 ng/mL within 3–4 h of drug administration (Fig. 1). The mean peak concentrations of tepoxalin in plasma were reached at 4.0 ± 0.3 h on day 10. Tepoxalin was detected in plasma at 24 h postdrug administration at extremely low concentrations (approximately 2 ng/mL). The area under the concentration–time curve (AUC_∞) measured 1618 ± 285 h·ng/mL. The mean half-life ($t_{1/2}$) of tepoxalin was 2.7 h on day 10. Again, there was wide variability in values obtained for the $t_{1/2}$ of tepoxalin, which ranged from 1.7 to 13 h.

Plasma concentrations of RWJ 20142 increased for up to 6.7 h. Peak concentrations of RWJ 20142 were reached at 4.3 ± 1.0 h on day 10. Like its parent compound, RWJ 20142 could also be quantitated in plasma at 24 h postadministration at a little over 100 ng/mL. As seen on day 1 of the study, plasma concentrations of the metabolite far exceeded those of the parent compound measuring over 15× higher than those of tepoxalin. The *AUC* of RWJ 20142 measured 33 739 ± 20 648 h·ng/mL. The half-life of RWJ 20142 ranged from 2.2 to 8.4 h (harmonic mean 3.0).

In rabbits, the parent compound, tepoxalin, reaches peak concentrations in plasma within 5 h (5.4 ± 1.6 h), which is slower than the time to C_{max} seen in dogs and humans (2.3 ± 1.4 and 2.3 ± 1.0 h, respectively). Maximum plasma concentrations were much higher in rabbits for tepoxalin and RWJ 20142 (207 ± 49 and 2551 ± 1034 ng/mL, respectively). The half-life for tepoxalin in rabbits (2.7 h) was similar to that seen in dogs (1.6 h) and humans given tepoxalin at 0.45 mg/kg (1.7 h). However, the half-life for the active metabolite, RWJ 20142, was much shorter in rabbits (3 h) when compared with dogs (13.4 h) and humans (14.5 h) given tepoxalin at 0.45 mg/kg (Depré *et al.*, 1996; Waldman *et al.*, 1996; Zubrin Technical Monograph, 2005).

The pharmacokinetics of tepoxalin in the rabbit appear different from that seen in the dogs and humans. Pharmacokinetic studies in dogs suggest that tepoxalin absorption may be enhanced by administration with a meal or within 1–2 h after a meal. Peak plasma levels of tepoxalin were significantly lower when tepoxalin was given to fasted dogs (Zubrin Technical Monograph, 2005). Nevertheless, when one compares gastric emptying in a fed rabbit whose stomach is never completely empty, with that of a fed carnivore whose stomach does empty completely, this delay or 'incompleteness' of gastric emptying in rabbits may affect tepoxalin pharmacokinetics. However, this difference unlikely to be clinically relevant for rabbit therapy. Rabbits in this study were also fed a high-fiber diet; cellulose or fiber may also affect tepoxalin pharmacokinetics.

If drug concentrations for therapeutic efficacy in the rabbit are similar to those measured in dogs and humans, then oral administration may be tentatively suggested at every 8–12 h. Clinical efficacy studies are needed to confirm that two to three times daily drug administration is indicated in the rabbit. In humans receiving 35–300 mg tepoxalin, *AUC* increased in a dose-dependent, but not dose-proportional, manner suggesting that oral absorption of tepoxalin may be saturable (Waldman *et al.*, 1996).

Plasma concentrations do not necessarily reflect therapeutic efficacy. Tepoxalin and its active metabolite are highly protein bound in the canine, and higher drug concentrations may be achieved at sites of inflammation than one can predict based on

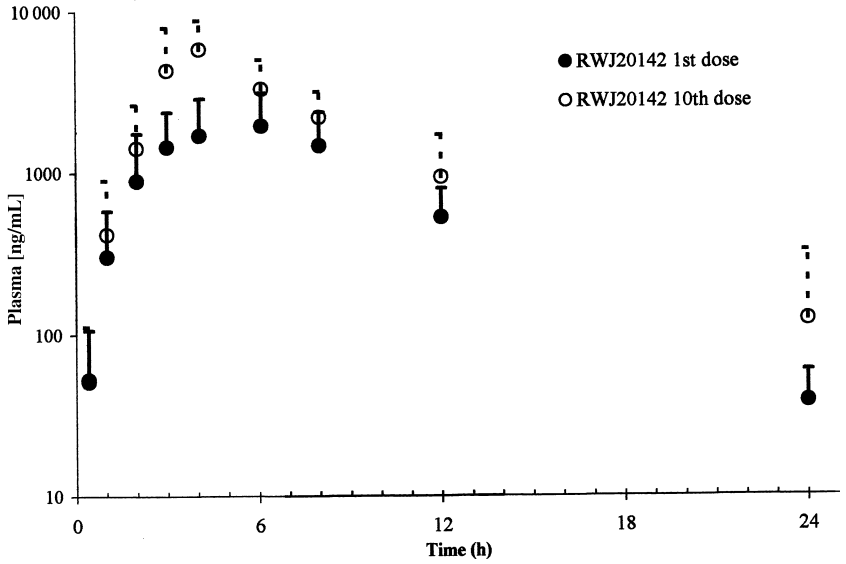

Fig. 2. Mean ± SD concentration vs. time curves for active metabolite of tepoxalin, RWJ 20142, in plasma after administration of tepoxalin over 24 h (10 mg/kg, PO) in rabbits (n = 8).

plasma concentrations (Zubrin Technical Monograph, 2005). No adverse effects were detected in the rabbits in our study and little renal or hepatic toxicity has been reported in canine trials (Zubrin Technical Monograph, 2005). However, safety trials in the rabbit are indicated if higher dosages or more frequent drug administration is indicated. More research is also needed on the bioavailability of tepoxalin in the rabbit.

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