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Radezolid demonstrates favorable safety compared to linezolid in a three-month rat toxicology study

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Abstract

Background: To evaluate the long-term safety of radezolid (RX-1741; RDZ), an oxazolidinone in clinical development, a 3-month rat toxicology study was undertaken. As a comparator, linezolid (LNZ) was dosed in a concurrent study; doses were chosen to closely approximate exposures of either projected (RDZ) or known (LNZ) clinical doses. Both studies had one-month interim sacrifices and toxicokinetic determinations; the radezolid study also had a one-month recovery arm.

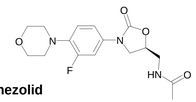
Methods: Rats (M and F) were dosed by oral gavage QD with radezolid at 10, 50 and 200 mg/kg/day, or with linezolid at 40 and 100 mg/kg/day. Animals were weighed and monitored for food consumption weekly, and observed daily.

Results: Radezolid was well-tolerated at all dose levels, with no clinical observations, no changes in food consumption, and only modest decreases in body weight gains. All rats in the radezolid groups completed the study. In the high-dose linezolid groups, rats exhibited decreased body weight gain and food consumption, with associated clinical observations that were considered test article-related and adverse; these rats were sacrificed early on Day 75 of the study. These rats had decreased red cell mass, and reticulocyte and neutrophil counts that correlated with a decrease in bone marrow cellularity.

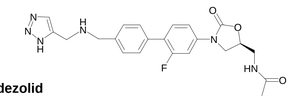
Conclusions: No test-article-related changes in food consumption or clinical signs were observed at any dose level for radezolid, resulting in a No Observed Adverse Effect Level (NOAEL) of 200 mg/kg/day. This dose led to an $AUC_{0\text{-}24}$ of 74 μg*hr/mL (M) and 105 μg*hr/mL (F) on Day 90. For linezolid, rats in the high-dose groups exhibited decreased body weight and food consumption with associated clinical and hematological effects; these groups were terminated on Day 75. The NOAEL for linezolid was 40 mg/kg/day, which resulted in an $AUC_{0\text{-}24}$ of 169 μg*hr/mL (M) and 147 μg*hr/mL (F) on Day 90.

Introduction

Radezolid (RDZ) is a next-generation oxazolidinone in clinical development, designed to expand upon the favorable properties of linezolid (LNZ) (shown below) by improving ribosome binding, and thus enhance activity against pathogens of clinical interest (1). In order to be of maximum benefit in the clinic, however, a new oxazolidinone would need to have improved safety relative to the currently-marketed LNZ, which has been shown to cause myelosuppression during long-term dosing (2). A three-month rat toxicology study was undertaken to evaluate the long-term dosing potential of radezolid in comparison to LNZ. Radezolid was dosed via oral gavage daily for three months, with a 1-month scheduled interim sacrifice and a 1-month recovery. LNZ was also administered daily via oral gavage for three months with a 1-month scheduled sacrifice but no recovery arm. The doses chosen for RDZ were 10, 50 and 200 mg/kg/day, and for LNZ, 40 and 100 mg/kg/day; Table 1 shows rat pharmacokinetic data for these compounds to support these dose selections. In rats dosed with LNZ, the 40 mg/kg/day dose correlates with efficacious exposures in humans, whereby doses of 500 – 600 mg achieve AUCs of 100 μg*hr/mL (3, 4); the 100 mg/kg/day dose corresponds to an exposure ~2.5-fold over that. For radezolid, the 50 mg/kg/day dose in rats approximates the predicted exposure of 20 – 40 μg*hr/mL needed for efficacy at a dose of 450 mg, whereas the 200 mg/kg/day dose corresponds to an approximately 5-fold higher exposure.



Linezolid



Radezolid

Table 1: Pharmacokinetics in rats to support dose selections for linezolid (dosed at 30 mg/kg PO) and radezolid (dosed at 50 mg/kg PO)

Compound	C_{max} (μg/mL)	$AUC_{0\text{-}24}$ (μg*hr/mL)	$AUC_{0-\infty}$ (ng*hr/mL)	Vol dist. (mL/kg)	Clearance (mL/hrkg)	$T_{1/2}$ (hr)	T_{max} (hr)
Linezolid	16.23	96.73	118.74	1280	269	2.4	1.2
Radezolid	8.58	38.36	41.29	10062	1231	6.0	1.0

Methods

Toxicology study: Rats (M and F) were dosed by oral gavage QD with RDZ at 10, 50 and 200 mg/kg/day, or with LNZ at 40 and 100 mg/kg/day, in a dose volume of 10 mL/kg. Both compounds were dosed in a vehicle of 0.5% Tween 80 (JT Baker) and 0.5% methylcellulose (1500 cps) (MP Biomedical) in Sterile Water for Injection, USP (Baxter Healthcare Corp.). Animals were weighed and monitored for food consumption weekly, and observed daily. Toxicokinetics were performed in groups separate from the main study animals; blood samples were collected into tubes containing lithium heparin as the anticoagulant and plasma was harvested and prepared for bioanalysis as described below. For RDZ-dosed animals, blood was sampled from main study animals for hematology, coagulation and clinical chemistry on Days 30 and 94 of the dosing phase and Day 28 of recovery phase; urine was also sampled on those days. For the LNZ-dosed animals, blood and urine were collected during Week 5 of the dosing phase (for interim sacrifice) and from all surviving animals at the scheduled termination sacrifice.

Bioanalytical methods: Acetonitrile was used to precipitate protein components of plasma and tissue samples. MS analysis was performed using a Sciex API3200 mass spectrometer with electrospray positive ionization in the multiple reaction monitoring mode. An ion transition of m/z 439 to m/z 341 was set for radezolid analysis. For chromatographic separations, a Waters Acquity UPLC system, typically equipped with a reversed-phase column, was used for 3min run. The linear dynamic range of this LC/MS/MS method was 0.020 to 10.0 μg/L.

Pharmacokinetic analyses: Pharsight WinNonLin (v. 5.2) was used to determine pharmacokinetic parameters.

Results

Summary of linezolid findings:

- Male and female rats administered 100 mg/kg/day were sacrificed early on Day 75 due to body weight and food consumption decreases and associated clinical observations (Figure 1)
- Females dosed with 100 mg/kg/day exhibited decreased red cell count, hematocrit, reticulocyte count and hemoglobin concentration from Day 30 onward. Hematological results correlated with microscopic findings of decreased splenic extramedullary hematopoieisis and decreased cellularity of sternal and femoral bone marrow (Figure 2)
- No significant changes in coagulation or urinalysis
- Male rats dosed at 100 mg/kg/day had minimally higher myeloid:erythroid (M:E) ratios, indicating some decrease in erythropoiesis
- Toxicokinetic analysis showed that exposures generally increased with increasing dose level and were dose-proportional, and gender differences were less than two-fold. In addition, values for C_{max} and $AUC_{0\text{-}24}$ were similar on Days 1, 29, 58, 75 and 90, suggesting no accumulation in rats after multiple dosing (Table 2; dose proportionality ratios are in the final column).

Figure 1: Body-weight graphs of linezolid- and radezolid-dosed rats
(A) Linezolid-dosed rats: Red lines represent male rats; blue lines represent female rats. Squares: vehicle-dosed controls; triangles: 40 mg/kg; circles: 100 mg/kg.
(B) Radezolid-dosed rats: Red lines represent male rats; blue lines represent female rats. Squares: vehicle-dosed controls; diamonds, 10 mg/kg; triangles, 50 mg/kg; circles: 200 mg/kg.

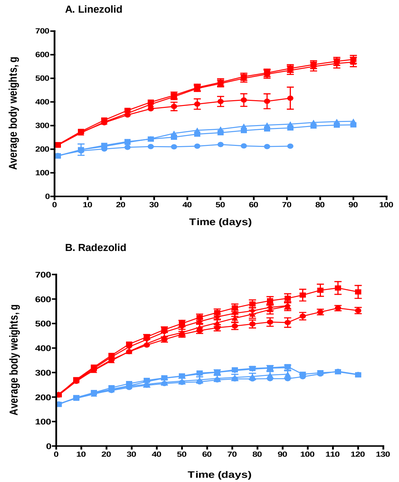


Table 2: Toxicokinetic parameters of linezolid in rat plasma

Day	Dose Level (mg/kg/day)	Sex	C_{max} (μg/mL)	$AUC_{0\text{-}24}$ (μg*hr/mL)	$t_{1/2}$ (hr)	$AUC_{0\text{-}24}$ ratio
1	40	M	19.6	12.8	1.32	1.0
		F	16.9	120.1	1.98	1.0
	100	M	25.2	346.3	NA	2.7
		F	39.2	322.3	5.97	2.7
29	40	M	29.4	143.0	1.96	1.0
		F	24.9	147.6	2.62	1.0
	100	M	41.4	434.4	8.53	3.0
		F	36.3	422.3	NA	2.9
58	40	M	18.1	144.9	2.83	1.0
		F	20.0	139.6	2.54	1.0
	100	M	24.7	351.7	NA	2.4
		F	26.0	315.1	9.69	2.3
75	100	M	40.3	614.5	NA	1.0
		F	32.8	489.5	NA	1.0
90	40	M	27.4	169.5	2.64	3.6
		F	22.5	147.2	3.18	3.3

Table 3: Toxicokinetic parameters of radezolid in rat plasma

Day	Dose Level (mg/kg/day)	Sex	C_{max} (μg/mL)	$AUC_{0\text{-}24}$ (μg*hr/mL)	$t_{1/2}$ (hr)	$AUC_{0\text{-}24}$ ratio
1	10	M	0.96	8.19	NA	1.0
		F	0.84	8.52	NA	1.0
	50	M	3.54	27.7	2.87	3.4
		F	3.45	21.58	3.15	2.5
	200	M	6.52	46.50	6.04	5.7
		F	6.79	44.45	4.99	5.2
29	10	M	2.26	11.72	4.45	1.0
		F	3.01	13.69	2.43	1.0
	50	M	8.65	51.83	3.83	4.4
		F	10.98	58.46	4.79	4.3
	200	M	17.23	101.87	3.55	8.7
		F	21.33	125.29	4.92	9.2
58	10	M	2.16	13.16	3.81	1.0
		F	2.51	13.26	3.37	1.0
	50	M	5.46	42.36	3.60	3.2
		F	7.72	44.85	4.38	3.4
	200	M	10.68	77.99	3.74	5.9
		F	18.10	96.21	4.03	7.3
90	10	M	2.53	12.70	3.83	1.0
		F	2.28	15.25	3.41	1.0
	50	M	6.31	46.85	3.83	3.7
		F	9.15	50.09	4.08	3.3
	200	M	10.11	74.30	4.14	5.8
		F	15.83	105.14	4.23	6.9

Results

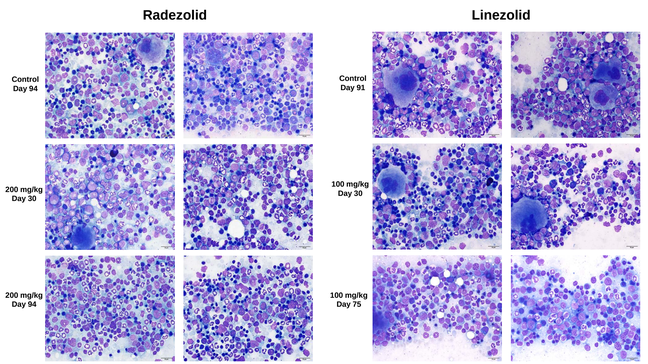


Figure 2: Photomicrographs of bone marrow smears from radezolid- and linezolid-dosed rats. Samples from high-dose groups only (radezolid at 200 mg/kg/day and linezolid at 100 mg/kg/day) are shown.

Left panel: Radezolid-dosed rat bone marrow smears; top: control animals at three-month sacrifice; middle: radezolid-dosed rats at one-month interim sacrifice; bottom: radezolid-dosed rats at three-month sacrifice. Left panel: male rats; right panel: female rats.

Right panel: Linezolid-dosed rat bone marrow smears; top: control animals at three-month sacrifice; middle: linezolid-dosed rats at one-month interim sacrifice; bottom: linezolid-dosed rats at terminal sacrifice. Left panel: male rats; right panel: female rats.

Summary of radezolid findings:

- Radezolid had no effect on clinical observations, mortality or organ weights at interim, terminal or recovery sacrifices at any dose level studied
- Changes in body weight were minimal and not considered adverse due to their small magnitude, lack of similar changes in food consumption, and improvement during recovery phase (Figure 1)
- At Day 30 and 94 of dosing phase and Day 28 of recovery phase, no changes were observed with respect to hematology, coagulation, clinical chemistry or urinalysis results in any animal at any dose
- No changes in M:E ratios or cell sequence maturation seen at any dose at any time point (Figure 2)
- Toxicokinetic analysis showed that exposures generally increased with increasing dose level but were less than dose-proportional, and gender differences were less than two-fold. In addition, values for C_{max} and $AUC_{0\text{-}24}$ were in general two-fold higher on Days 29, 58 and 90 than on Day 1, suggesting accumulation in rats after multiple dosing (Table 3; dose proportionality ratios are in the final column).

Conclusions

- Radezolid had no effect on clinical observations, mortality or organ weights at interim, terminal or recovery sacrifices at any dose level studied, resulting in a No Observed Adverse Effect Level (NOAEL) of 200 mg/kg/day. This dose led to an $AUC_{0\text{-}24}$ of 74 μg*hr/mL (M) and 105 μg*hr/mL (F) on Day 90.

- For linezolid, rats in the high-dose groups (100 mg/kg/day) exhibited decreased body weight and food consumption with associated clinical and hematological effects; these groups were terminated on Day 75. The NOAEL for linezolid was 40 mg/kg/day, which resulted in an $AUC_{0\text{-}24}$ of 169 μg*hr/mL (M) and 147 μg*hr/mL (F) on Day 90.

References

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