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Absence of a Pressor Response to Oral Tyramine in Conscious Telemeterized Rats Treated with the Novel Oxazolidinone TR-701: Comparison to Linezolid

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ABSTRACT

BACKGROUND TR-701, a phosphate prodrug of TR-700, is a novel once-a-day oral oxazolidinone antibiotic in Phase 2. TR-700 is potently active against all relevant gram-positive and fastidious gram-negative pathogens. Other oxazolidinones, such as linezolid, inhibit monoamine oxidase (MAO), an enzyme located in the gut (MAO_A) and brain (MAO_B) and responsible for metabolizing vasoactive amines to inactive products. This study was designed to assess whether TR-701 potentiated the tyramine-induced pressor response in rats, an accepted model for evaluating the human risk potential of MAO inhibition, and in an *in vitro* human cell-based assay of MAO inhibition.

METHODS Male Sprague-Dawley rats implanted with radiotelemetry devices were administered single doses of TR-701 orally via gavage at 0 to 150 mg/kg followed 2 hours later by 15 mg/kg oral tyramine. The positive control group was similarly treated with linezolid at 50 mg/kg, a dose comparable to that used in the clinic. Blood pressure was monitored at 5 min intervals and plasma levels of the active (TR-700) determined in satellite groups. TR-701 also was assessed for inhibition of MAO_A and MAO_B in an established human cell-based assay.

RESULTS TR-701 had no effect on the pressor response of tyramine at doses up to 150 mg/kg, which generated TR-700 plasma exposures 33-fold greater than the targeted human therapeutic exposure in Phase 2. As expected, linezolid produced the characteristic pressor response (>30 mm Hg increases in mean arterial pressure) following tyramine administration. In addition, in a human cell-based assay, TR-701 was inactive as an inhibitor of MAO_A and MAO_B (IC50 >10 µM).

CONCLUSIONS The absence of a tyramine pressor response to high-dose oral TR-701 using a validated animal model suggests that this oxazolidinone may have a significant safety advantage over linezolid for patients consuming tyramine containing foods or who have been prescribed vasoconstrictors or SSRIs

INTRODUCTION

TR-701, a phosphate prodrug of TR-700, is a novel oral oxazolidinone antibiotic currently in Phase 2 clinical trials. Other oxazolidinones such as linezolid competitively inhibit human monoamine oxidase-A (MAO_A), which is responsible for the inactivation of vasoactive amines. Tyramine is a vasoactive amine and constituent of many foods, including cheese. When consumed in a normal diet, tyramine has no effect on blood pressure. In the presence of MAO inhibitors, tyramine present in food can lead to potentially dangerous increases in blood pressure. This study evaluated the safety of TR-701 by assessing the potential acute MAO inhibitory effects of TR 701 *in vivo* using the tyramine challenge assay and *in vitro* using human MAO activity measures.

The principle behind the tyramine challenge assay is that oral administration of high doses of tyramine results in a marked increase in blood pressure due to a release of stored norepinephrine which occurs as a result of tyramine uptake and concentration in adrenergic neurons. Inhibition of MAO prevents metabolism of tyramine, resulting in an increase in the levels of circulating tyramine, thereby causing a transient potentiation of the pressor response.

METHODS

***In Vitro* Human MAO Activity**
TR-701 (prodrug), TR-700 (active moiety) or positive controls (linezolid, clorgyline, R(-)-deprenyl) were incubated with human recombinant MAO_A or MAO_B or with cell preparations from humans (placenta for MAO_A; platelets for MAO_B). The IC50 values were compared to free plasma levels to understand the relative importance of these *in vitro* findings.

Tyramine Challenge Assay in Rats
TR-701 in vehicle, sterile distilled water, was administered as single oral doses (by gavage) at dose levels of 10, 20, 40, 60, 80, 100 and 150 mg/kg to 3-6 Crl:CD(SD) rats (per group) implanted with radiotelemetry probes. The vehicle and positive control article, linezolid (also known to as Zyvox), were administered on a comparable regimen. Tyramine at 15 mg/kg was administered as a single oral gavage dose 2 hours after TR-701 dose administration.

Heart rate (derived from arterial waveforms), arterial pressure (systolic, diastolic and calculated mean) and body temperature were collected for a 30-second period every 5 minutes for approximately 1 hour prior to dose administration and for at least 2 hours following each tyramine challenge. Change from baseline (prior to tyramine) and maximal change post tyramine were measured. To assess plasma exposure to TR-700, blood samples were collected from 3 rats per time point at approximately 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours post-dosing. Plasma levels from this study were compared to human plasma levels at efficacious doses to generate human safety margins.

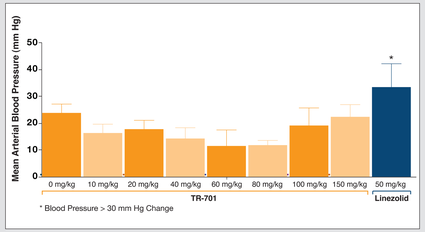
A significant blood pressure effect was determined to be a dose of TR-701 or linezolid that induced a ≥30 mm Hg increase in mean arterial pressure following oral tyramine challenge.

Table 1. Effect of TR-701 and TR-700 on Human Monoamine Oxidase Activity

Compound	MAO_A		MAO_B	
	Recombinant[1] IC_{50} (µM)	Placenta[2] IC_{50} (µM)	Recombinant[1] IC_{50} (µM)	Platelets[3] IC_{50} (µM)
TR-701	NC	>10	NC	>10
TR-700	2.44	6.95	2.83	7.66
Linezolid	19.0	NC	0.691	NC
Cloryline	0.0012	0.026	NC	NC
Deprenyl	NC	NC	0.0095	0.011

NC = Not Conducted
1 Human recombinant MAO enzyme using 50 µM kynuramine, 60-min incubation at 37°C with 15 min preincubation and monitoring conversion to 4-hydroxyquinoline.
2 Human placenta preparations using 150 µM kynuramine, 30-min incubation at 30 °C and monitoring conversion to 4-hydroxyquinoline.
3 Human platelet preparations using 500 µM benzylamine, 45-min incubation at 37 °C and monitoring conversion to benzaldehyde.

Figure 1. Effects of TR-701 and Linezolid on Tyramine-induced Mean Arterial Pressure Change



TR-701 and linezolid were administered as single oral doses to 3-6 rats/group implanted with radiotelemetry probes; tyramine (15 mg/kg) was administered 2 hr postdose, and heart rate and arterial pressure collected for a 30-sec period every 5 min at least 2 hr following tyramine challenge. Values are mean ± standard error for peak blood pressure change after tyramine.

RESULTS SUMMARY

In Vitro Human MAO Activity

The TR-701 prodrug was inactive as an inhibitor of MAO_A and MAO_B (Table 1). TR-700 (active moiety) was weakly active as an inhibitor and was similar to linezolid on a molar basis. However, since gut MAO_A plays a major role in metabolism of dietary amines and because TR-701 (not TR-700) is the form absorbed in the gastrointestinal tract, TR-700 is not expected to be an important contributor to a pressor response caused by dietary or orally administered monoamines. The well known MAO inhibitors clorgyline and R(-)-deprenyl were shown to be potent inhibitors.

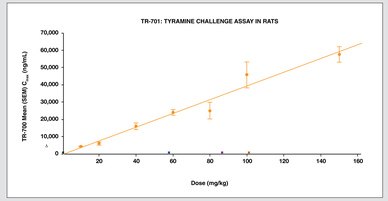
Tyramine Challenge Assay in Rats

TR-701 had no effect on the pressor response of tyramine at doses up to 150 mg/kg (Figure 1). As expected, 50 mg/kg linezolid produced a characteristic potentiation of the pressor response with mean arterial pressure increasing >30 mm Hg following tyramine administration. Plasma exposures of TR-700 after oral administration of TR-701 show linear pharmacokinetics in rats up to the highest dose (Figures 2 and 3).

Human Safety Margins

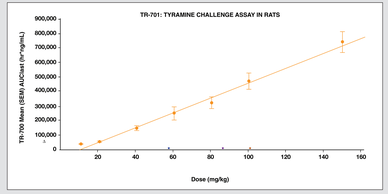
Safety margins generated from *in vivo* results are shown in Table 2. TR-701 showed significant advantage over linezolid. No plasma margin exists for linezolid's pressor response following oral tyramine in rats relative to the human efficacious plasma levels. In contrast, large safety margins (>30-fold) exist for TR-701 at plasma levels where no pressor response was seen after oral tyramine in rats relative to the efficacious plasma levels in humans.

Figure 2. TR-700 C_{max} vs. Dose



Plasma levels of TR-700 (active moiety) obtained after a single oral administration of TR-701 (prodrug) to male rats. Results show linear, dose proportional C_{max} levels up to the highest dose of 150 mg/kg.

Figure 3. TR-700 $AUC_{0\text{-}24\ h}$ vs. Dose



Plasma levels of TR-700 (active moiety) obtained after a single oral administration of TR-701 (prodrug) to male rats. Results show linear, dose proportional exposure up to the highest dose of 150 mg/kg.

SAFETY MARGINS

Table 2. TR-701 Safety Margins for MAO Inhibition: Comparison to Linezolid

	In Vivo MAO Inhibition (Pressor Response to Oral Tyramine in Rats)			
	Rat TR-700 C_{max} (µg/mL)[a]	Rat TR-700 AUC (µg/mLxh)[a]	Margin (animal/human) C_{max}	Margin (animal/human) AUC
TR-701 (150 mg/kg; NOEL)	57.6	739	32-fold[b]	33-fold[b]
Linezolid (50 mg/kg; pressor response observed)	15.6[c]	135[c]	0.8-fold[c]	0.9-fold[c]

a Plasma levels after single dose of TR-701 or linezolid.
b Margins (animal/human) based on estimated plasma levels of TR-700 in humans at the anticipated therapeutic dose of 200 mg/day (total C_{max}=1.8 µg/mL; total AUC 22.5 µg/mL•hr).
c Margins (animal/human) based on steady state plasma levels of linezolid in humans at the known therapeutic dose of 600 mg BID (total C_{max}=19.0 µg/mL; total AUC 147 µg/mL•hr).
Abbreviations: NOEL = no-observed effect level.

CONCLUSIONS

- TR-701 prodrug has no effect on the *in vitro* inhibition of MAO up to the highest tested concentration of 10 µM
- Although TR-700 (active moiety) inhibited MAO similarly to linezolid on a molar basis, TR-700 is only formed after absorption and therefore is not available to inhibit gastrointestinal MAO_A, the most important location for metabolism of dietary or orally administered monoamines
- TR-701 at doses up to 150 mg/kg did not potentiate the pressor response elicited by an oral tyramine challenge using a validated animal model
- Linezolid at 50 mg/kg significantly potentiated (>30 mm Hg change) the pressor response of oral tyramine
- **These nonclinical *in vitro* and *in vivo* results indicate that TR-701 will have a significant safety advantage over linezolid for patients consuming tyramine containing foods or who have been prescribed orally administered vasoconstrictors or SSRIs**
- **Mechanism for TR-701 safety advantage over linezolid may be related to the prodrug approach, which allows the active moiety TR-700 to by-pass gastrointestinal MAO**