Linezolid, a Novel Oxazolidinone Antibiotic: Assessment of Monoamine Oxidase Inhibition Using Pressor Response to Oral Tyramine

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The primary objective of this study was to compare the effects of oral linezolid with moclobemide and placebo on the pressor response to oral tyramine. Secondary objectives were to determine possible mechanisms of the effect based on changes in the pharmacokinetics of tyramine and to evaluate alternative methods for quantifying the pressor effect. Subjects received linezolid (625 mg bid orally), moclobemide (150 mg tid orally), or placebo for up to 7 days. Using the oral tyramine dose producing a > 30 mmHg increase in systolic blood pressure (SBP) ($PD_{>30}$), a positive pressor response was defined as a $PD_{>30}$ index (pretreatment/treatment ratio of $PD_{>30}$) of ≥ 2. There were 8/10, 11/11, and 1/10 responders with linezolid, moclobemide, and placebo, respectively. Responses returned to baseline within 2 days of drug discontinuation. The ratio of mean greatest SBP and heart rate at the time of greatest SBP (GSBP/HR) increased linearly with tyramine dose both pretreatment and during treatment with linezolid and moclobemide. During treatment, responses to tyramine when subjects took linezolid or moclobemide were significantly different from placebo. Both drugs significantly decreased tyramine oral clearance compared with placebo. Urinary excretion of catecholamines and metabolites was consistent with MAOI activity of the drugs, but results were variable. The MAOI activity of linezolid is similar to that of moclobemide, a drug used clinically without food restrictions. Restrictions to normal dietary intake of tyramine-containing foods are not warranted when taking linezolid.

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The oxazolidinone antibiotics are a new synthetic class of antibacterial agents that selectively inhibit bacterial protein synthesis. Linezolid ((S)-N-[[3-[3-Fluoro-4-(4-morpholinyl) phenyl]-2-oxo-5-oxazolidinyl]methyl]-acetamide) is in clinical development for the treatment of infections caused by a variety of gram-positive bacteria.[1]

The oxazolidinones inhibit monoamine oxidase (MAO), one of the primary enzymes responsible for the catabolism of catecholamines. In humans, MAO occurs in two forms, MAO-A and MAO-B. MAO-A preferentially deaminates serotonin (5-HT) and norepinephrine; MAO-B preferentially deaminates phenylethylamine, benzylamine, and, in man, dopamine. Linezolid, a derivative of the template compound of the oxazolidinone series, has been screened using in vitro and in vivo test systems for MAOI activity. The results of these studies suggest that linezolid is a weak, competitive (reversible) inhibitor of human MAO-A.[2]

Tyramine, a constituent of many foods, including cheese, is an indirectly acting sympathomimetic substance in vivo. However, it is a good substrate for MAO. In untreated subjects, moderate doses of oral tyramine are almost completely deaminated presystemically. Any tyramine reaching adrenergic nerve endings are deaminated intraneuronally. Thus, in the amounts consumed in a normal diet, tyramine exerts no significant sympathomimetic effects; only after high oral doses does sufficient tyramine reach the site of action to exert a pressor effect. In contrast, when MAO is inhibited, more tyramine is able to reach the systemic cir-

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culation, its intraneuronal deamination is also reduced, and the displaced norepinephrine is not deaminated prior to release. These three factors contribute to a potentiation of the tyramine pressor effect. Significant and potentially dangerous increases in blood pressure may occur following exposure to tyramine in amounts commonly encountered in a normal diet, the so-called "cheese effect." These may be avoided by adhering to a diet restricted in tyramine.

MAOIs have been in clinical use for many years for the treatment of depression and Parkinson's disease. The first MAOIs available were nonselective and irreversible; recovery of normal MAO function following cessation of treatment takes approximately 2 weeks as new MAO must be synthesized. More recently, MAOIs selective for one or the other isoform have been developed, such as moclobemide, an antidepressant and reversible MAO-A inhibitor. Comparison of these drugs on the pressor effects of oral tyramine indicated that those that both affected MAO-A and were irreversible were the most effective in potentiating the tyramine pressor response.[3,4] In view of the evidence that linezolid has in vitro MAOI activity, it is essential to determine its potential interaction with dietary tyramine, which may cause clinically significant pressor responses.

The interaction of linezolid with the over-the-counter drugs pseudoephedrine, phenylpropanolamine, and dextromethorphan has also been investigated and reported separately.[5]

Tyramine pressor studies are an accepted method of studying MAOI activity.[3,6-10] These assess the potential for clinical effects of increased sympathomimetic activity following oral exposure to an indirectly acting sympathomimetic agent. The most widely reported and clinically relevant measure of sensitivity to tyramine is the $PD_{>30}$, the actual dose of tyramine associated with a 30 mmHg increase in systolic blood pressure (SBP). Subjects receive increasing doses of tyramine at intervals, with close monitoring of the blood pressure response. The test is terminated when the $PD_{>30}$ is reached. The test is then repeated at the end of a period of treatment with study medication to determine the new dose of tyramine that is associated with the same pressor response. The $PD_{>30}$ index is the ratio of the actual dose of tyramine required to increase blood pressure by 30 mmHg before the start of study treatment to the tyramine dose required to cause the same increase in systolic blood pressure during study treatment.

MAOIs can affect sensitivity to oral tyramine both by an increase in systemic bioavailability of tyramine and by an increase in its effect at the site of action.[11] The primary objective of this study was to compare the effects of oral linezolid with moclobemide and placebo on the pressor response to oral tyramine. Secondary objectives were to compare the effects of oral linezolid, moclobemide, and placebo on the pharmacokinetics of oral tyramine and the urinary excretion of catecholamines and catecholamine metabolites.

It has been suggested that recording the ratio of greatest SBP/heart rate (GSBP/HR) may be a less variable measure of sensitivity to tyramine than the $PD_{>30}$.[11] However, its clinical relevance is less clear and is not widely reported. It was also investigated in this study so that its utility as an endpoint in possible future studies could be evaluated.

Moclobemide, a reversible MAOI drug, has been extensively evaluated in tyramine pressor tests. The results of these studies have been used to quantify moclobemide's potential for drug-food interactions. Moclobemide was therefore used as a positive control in this study.

SUBJECTS AND METHODS

Definitions

$PD_{>30}$: minimum dose of tyramine associated with an increase in SBP of more than 30 mmHg.

Treatment $PD_{>30}$ index: ratio of $PD_{>30}$ before the start of study treatment and during study treatment.

Posttreatment $PD_{>30}$ index: ratio of $PD_{>30}$ before the start of study treatment and after study treatment.

Subjects and Ethical Approval

All subjects were healthy, nonsmoking, male volunteers, who gave written informed consent. The study was approved by the Pinewood Independent Ethics Committee and was conducted by BIOS (Consultancy & Contract Research) Ltd. (BIOS) at the BIOS Clinical Pharmacology Unit, North Hampshire Hospital, Basingstoke, United Kingdom. Subjects were between ages 19 and 43 years and within 15% of predicted ideal weight according to the 1983 Metropolitan Life table. Subjects who entered the treatment period had a pretreatment $PD_{>30} > 200$ mg and $\leq$ 800 mg tyramine. Subjects were excluded if they had a HR < 40 bpm, SBP > 140 mmHg, and/or diastolic blood pressure (DBP) > 90 mmHg at rest.

Subjects had not received any investigational drug within 4 months or any known enzyme-inducing agents (e.g., barbiturates or similar drugs) or enzyme-inhibiting agents (e.g., cimetidine) for the period from 1 month before the start of the pretreatment period un-

til the final visit. No other drugs were to have been taken within 1 week of the start of or during any study period, unless under exceptional circumstances.

Study Design

Dosing Regimens

The highest dose of linezolid to be used in clinical practice is expected to be 600 mg twice daily (bid). In this study, subjects received 625 mg bid because of constraints in available tablet size. Similar doses have been well tolerated in previous studies.[1] The dose regimen of moclobemide was the midrange of that recommended by the data sheet.[12]

The tyramine dosing regimen was selected according to the results of a pilot study. A maximum of three doses of tyramine was given on any day, with a minimum of 2 hours between each dose. Dose escalation was stopped when the $PD_{>30}$ was reached or if there had been no significant change in sensitivity to oral tyramine during the treatment and posttreatment periods compared with the pretreatment period. A $PD_{>30}$ index ≥ 2 was believed to indicate an important change in sensitivity. Therefore, no further dose escalation was necessary if the subject had an increase in SBP of < 30 mmHg at a dose of tyramine that gave a $PD_{>30}$ index of ≤ 1.75 and the next scheduled dose of tyramine would result in a ratio of ≤ 2. Blood samples were taken at intervals throughout the study for assay of linezolid, moclobemide, and tyramine.

Sample Size

In a pilot study, 4 of 4 subjects who received moclobemide and 2 of 4 subjects who received linezolid were responders to tyramine. Response was defined as a PD_{30} index (pretreatment dose/treatment dose required to increase blood pressure 30 mmHg) > 2. The sample size estimate for this study was therefore based on a predicted response in the linezolid group of 50% but was to be reestimated using the data from approximately the first 24 subjects before treating the remainder. Following blinded analysis of the first 28 subjects, it was concluded that additional subjects would not be necessary as a high proportion of responders had been observed.

Pretreatment Period

A 12-hour urine collection was made from the evening of Day –1 to the morning of Day 1. Tyramine pressor testing was carried out for assessment of each subject's pretreatment $PD_{>30}$ on Days 1 to 3, with up to three tyramine doses given per day until the $PD_{>30}$ was reached. Tyramine doses were given in escalating order as follows: 200, 300, 400, 500, 600, 700, and 800 mg.

Treatment Period

Eligible subjects were randomized to one of three treatment groups. A partially blind, parallel-group study design was employed, with each group of subjects receiving linezolid (625 mg) or matching placebo bid, administered orally in a double-blind manner, or moclobemide (150 mg three times a day [tid]) in an open-label manner, for 4 to 7 days. Treatment Days 1 to 3 were conducted as outpatients.

A 12-hour urine collection was made from the evening of Day 3 to the morning of Day 4.

Tyramine pressor tests were conducted on treatment Days 4 to 7 after steady-state plasma concentrations of study medication had been achieved. Up to three tyramine doses were given on any one day, if required. Doses were given in escalating order as follows: 25, 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, and 450 mg until the study endpoint was reached.

Posttreatment Period

The posttreatment period commenced approximately 16 hours after the final dose of study medication. Urinary excretion of catecholamines and metabolites was measured from the evening of Day 1 to the morning of Day 2.

Repeat pressor testing was carried out on posttreatment Days 2 and 3, beginning approximately 49 hours after the final dose of study medication, to assess the time course of recovery of any change in sensitivity to oral tyramine observed during the treatment period. Tyramine doses were given in escalating order as described for the treatment period, with the starting dose the same as the $PD_{>30}$ during the treatment period. Up to three doses were given per day, if required. Pressor testing was not conducted if a $PD_{>30}$ index had not been attained during treatment.

There were two follow-up visits approximately 4 and 11 days after discharge for further safety assessments.

Study Procedures

Subjects fasted from midnight prior to any laboratory safety test or tyramine pressor test until 2 hours after the final tyramine dose. Standardized meals were given during admission periods at 15:00 to 15:30 hours (2 hours after the latest possible administration of tyramine) and 19:00 hours. A light snack was provided before lunch if tyramine testing had finished and also at 22:00 hours. Breakfast was given during nonfasting periods. No other food was permitted. Water was allowed,

but no other beverages were taken during fasting periods. Caffeinated products and foods containing potential substrates for MAO were prohibited from 48 hours prior to and during the pretreatment and treatment periods and until the morning of Day 2 of the posttreatment period. Alcohol was prohibited from 48 hours prior to the pretreatment period until the final visit, and strenuous activity was prohibited for 48 hours prior to each visit and throughout each admission.

Blood and urine samples were collected for laboratory safety analysis during all periods of the study. Each subject underwent a physical examination prestudy. In addition, 12-lead ECGs, vital signs, oral temperature, and respiratory rate were recorded during all periods of the study. Safety was also examined by recording adverse events on each day of each study period.

Excessive sympathetic stimulation arising during tyramine pressor testing was treated by intravenous administration of phentolamine (boluses of 1-2 mg at intervals of 1-2 minutes, with the dose adjusted according to response). Phentolamine was also administered for the following indications: an increase in SBP of > 60 mmHg from baseline or to an absolute value of > 180 mmHg, a reduction in HR of more than 5 bpm below baseline and to below 40 bpm, severe headache, and clinically significant changes in ECG.

Pharmacodynamic Measurements

Blood pressure and HR measurements commenced at 3-minute intervals prior to each administration of tyramine. When stable (three consecutive SBP values within 5 mmHg of the mean value), three readings were taken for the baseline determination. From 5 minutes after tyramine dosing, SBP and HR were recorded at 3-minute intervals or more frequently, if indicated clinically, for at least 2 hours. The GSBP, HR at the GSBP, and the time at which this occurred were recorded. Vital signs were closely monitored for a significant clinical response (increase in SBP ≥ 30 mmHg compared with predose baseline). In addition, ECG monitoring was continued during tyramine pressor testing up until 5 hours after the last dose of tyramine on any study day.

Twelve-hour urine collections were made during each study period as described earlier to measure the urinary excretion of catecholamines (norepinephrine, epinephrine, dopamine) and metabolites (3-methoxy-4-hydroxy-mandelic acid [VMA], 3-methoxy-4-hydroxyphenylethylene-glycol [MHPG], homovanillic acid [HVA], metanephrine, and normetanephrine).

The following pharmacodynamic variables were determined: mean baseline SBP, mean baseline HR, SBP/HR at baseline, GSBP/HR, maximum increase in SBP (i.e., GSBP – mean baseline SBP), $PD_{>30}$, and urinary excretion of catecholamines and metabolites.

Pharmacokinetic Measurements

Blood samples for linezolid or moclobemide assay were collected before the first tyramine test during the pretreatment period, at the same time as each tyramine sample during the treatment period, and before the first tyramine pressor test on each day of the posttreatment period.

Linezolid and moclobemide C_{av} values were determined to assess equivalent exposures during treatment days. Average plasma concentration (C_{av}) was calculated as ($C_{av} = AUC_{0\text{-}t}/t$). The $AUC_{0\text{-}t}$ (t = time to perform tyramine testing) was determined by trapezoidal rule for Days 4, 5, and 6 of the treatment period.

Blood samples for tyramine assay were collected before the tyramine dose and 30 minutes after the dose. Additional samples were also collected at the time of the GSBP and 30 minutes after the GSBP for the highest tyramine dose administered in each treatment period.

Tyramine pharmacokinetics was determined by a population pharmacokinetic analysis (NONMEM).[13] A one-compartment model was assumed fitting the log of the tyramine concentrations. Due to the fast absorption rate and limited sampling in the absorption phase, an intravenous bolus model was assumed for dose introduction. Interindividual variability was modeled on Cl and V_d and employed an exponential model. Each treatment group was modeled separately due to the large differences in pharmacokinetics between placebo and the two drug treatment groups. Individual subject parameters were generated by the post hoc function. The pharmacokinetic variables assessed for tyramine were oral clearance (Cl) and apparent volume of distribution (V_d).

Analytical Methods

Plasma samples were analyzed for linezolid and moclobemide using sensitive and selective high-performance liquid chromatographic (HPLC) methods with ultraviolet (UV) detection.[14] Tyramine was quantified using an HPLC system coupled with a triple quadrupole mass spectrometer (LC/MS/MS).[15] All specimens were stored at ≤ –20°C from collection until analysis.

Moclobemide levels were reported using a method developed and validated at the Institut for Klinische Pharmakologie–Bobenheim, Grunstadt, Germany. To 0.500 mL plasma was added the internal standard (IS),

4-iodo-N-[2-(4-morpholinyl)ethyl] benzamide hydrochloride (Ro 11-9900). The sample was made alkaline prior to solid-phase extraction, eluted with dichloromethane, and dried under nitrogen. The residue was reconstituted in 0.05 M sulfuric acid and injected onto the gradient HPLC system with UV detection at 240 nm. Retention times were approximately 4.0 and 6.0 minutes for moclobemide and the IS, respectively. Mean recoveries for moclobemide were > 77% over the calibration range. Linear regressions were calculated from peak height ratios plotted against the concentrations of the calibration standards with $1/Y^2$ weighting. The linear calibration range was from 10.7 to 2996 ng/mL. Results below the lowest calibration standard were reported as BLQ. During the analysis of study samples, seven calibration standards and three QC standards were run on 6 different days. Calibration standards (CS) were spiked into plasma each day of analysis while the QC standards had been previously prepared, and aliquots were created and frozen until time of analysis. Precision (CV%) of the CS was ≤ 5.3%, and accuracy was from 97.7% to 103%. Interday precision and accuracy were also monitored using QC standards with target concentrations of 20.6, 203, and 2421 ng/mL. Interday precision for the QC standards was ≤ 8.3%, and accuracy was from 103% to 111%.

Five runs were required to report linezolid concentrations found in the plasma specimens collected during the study. Eight calibration standards had CV% that were ≤ 5.5%, with mean accuracy between 95.8% and 107%. Interday accuracy and precision were further monitored by analysis of three linezolid quality control standards with target concentrations of 0.0400, 4.00, and 15.0 μg/mL. Quality control standard accuracy was from 99.2% to 103% with interday precision (CV%) for all QC standards ≤ 4.0%.

Eleven analytical runs were required to report the tyramine concentrations found in the plasma specimens. Coefficients of variation were used to express the precision of the back-calculated CS. The eight CS points had CV% that were ≤ 6.1%, with mean accuracy between 96.2% and 105%. Interday accuracy and precision were further monitored by analysis of three tyramine QC standards with target concentrations of 4.00, 40.0, and 200 ng/mL. Interday precision (CV%) for the three QC standards was ≤ 6.2% with assay accuracy from 100% to 106%.

Urine catecholamine levels were reported using high-performance liquid chromatography (HPLC) methods.[16] The linear ranges for norepinephrine, epinephrine, and dopamine were 7.9 to 1216 μg/24 hours, 8.0 to 1218 μg/24 hours, and 6.1 to 1161 μg/24 hours, respectively. Established urine quality control pools indicate CVs all < 7%.

Data Analysis

All statistical analyses were performed using SAS.[17] All statistical tests were two-tailed, and *p*-values of ≤ 0.05 were considered significant.

Differences in Days 4, 5, and 6 of the treatment period for linezolid and moclobemide C_{av} were compared using analysis of variance (ANOVA) procedures to confirm the achievement of steady-state conditions. Tyramine pharmacokinetic parameters were compared between treatment periods by a one-way ANOVA.

The chi-square test or Fisher's exact test was used to test for treatment group differences in contingency tables for the pharmacodynamic parameters. For continuous variables, one-way ANOVA with treatment group as the independent variable was used to test for treatment group differences. Fisher's least significant difference procedure was used for multiple comparisons in ANOVA. If the overall treatment effect was significant, then pairwise comparison of treatment means (adjusted means) was done using contrasts, each at the 0.05 level. Analysis of covariance (ANCOVA) and regression analysis were used in exploratory analyses to determine the sensitivity of GSBP/HR to tyramine. For vital signs, laboratory assays, and urinary catecholamines and metabolites, the changes from pretreatment values were tested for statistical significance using the paired *t*-test. The percentage of responders, defined as subjects with $PD_{>30}$ index ≥ 2, was calculated for each treatment group, and the percentages were compared for treatment difference.

GSBP/HR was the ratio of the GSBP to the corresponding HR following a tyramine dose. This ratio was calculated for each tyramine dose during the pretreatment, treatment, and posttreatment periods. ANCOVA and regression analysis were used to determine the relationship of the GSBP/HR ratio and tyramine dose. Variables that were considered as independent variables in the analyses included the tyramine dose, baseline SBP, heart rate, and DBP. Height, weight, and body surface area were used as covariates in preliminary analyses but were found not to be significant; these results are therefore not presented.

Regression of the ratio of GSBP to HR (GSBP/HR) and tyramine concentration at the GSBP following the highest tyramine dose administered in a treatment period were conducted to assess further the relationship between GSBP/HR and tyramine exposure.

RESULTS

Subject Disposition

Forty-one subjects entered the study and were analyzed for safety. Eight subjects did not continue to the treatment period due to excess response to tyramine in pretreatment (2), personal reasons (1), and early termination of the study (5). Thirty-three subjects were randomized and treated with study medication; 11 subjects were assigned to each treatment group. All 33 treated subjects were included in the pharmacokinetic analysis. Of these, 2 subjects (from the linezolid and placebo groups) did not complete the treatment period and were excluded from the pharmacodynamic analyses. Two further subjects (from the moclobemide and placebo groups) did not complete the posttreatment period. Thus, 29 subjects successfully completed the whole study.

Safety

No serious adverse events were experienced during the study, and there were no withdrawals due to adverse events. Of the 41 subjects, 23 subjects experienced a total of 39 adverse events. Nine events occurred during the pretreatment period (including dysautonomia, hypertension, headache, and palpitations), 20 during the treatment period, and 10 during the posttreatment period (2 following tyramine only). More adverse events were reported during the treatment period by subjects receiving moclobemide (11) compared with linezolid (5) and placebo (4). Four events (headache, dysautonomia, palpitation, and feeling unwell) were classed as being possibly caused by moclobemide and 3 events (2 of dysautonomia and 1 of hypertension) as being possibly caused by linezolid. Three adverse events during the posttreatment period were classed as being possibly caused by linezolid (elevated ALT [128 IU/L] and elevated AST [107 IU/L] in 1 subject and elevated ALT [149 IU/L] in another subject; all resolved to acceptable limits). Three adverse events were ongoing at the end of the study: abdominal bloating, iron deficiency anemia, and nasal stuffiness; all were classed as unrelated to investigational medication, and follow-up of these events was not considered necessary.

Four subjects in the linezolid group and 3 in the moclobemide group received phentolamine to treat excessive pressor responses to oral tyramine during the treatment period; 1 subject in the placebo group received phentolamine during the posttreatment period.

There were no other clinically significant laboratory results with the exception of a high creatine kinase (6001 IU/L) in 1 subject who had received only tyramine. It subsequently returned to within normal limits, and no further follow-up was required. There were some statistically significant increases in the postpressor HR (supine position) of the linezolid and placebo subjects, especially in the placebo group. Some statistically significant increases in the standing HR were found in the posttreatment period for the linezolid and placebo groups. Few statistically significant changes were found for SBP, respiration, and temperature. There were no changes in ECGs or vital signs during the course of the study that were considered to be clinically significant.

Pharmacodynamics

Treatment Period $PD_{>30}$

Ten subjects in the linezolid group completed the treatment period; 9 experienced an increase in SBP of ≥ 30 mmHg after tyramine doses ranging from 100 to 200 mg. The pretreatment tyramine doses that produced similar increases in SBP ranged from 300 to 600 mg. The mean $PD_{>30}$ index of the 9 subjects was 3.48; 8 subjects had $PD_{>30}$ indices ranging from 2 to 5.0, and 1 subject had a $PD_{>30}$ index < 2. In poststudy analysis, it was decided to classify the patient with the $PD_{>30}$ index value of 2.0 as a responder. Therefore, the response rate based on attainment of a $PD_{>30}$ index ≥ 2 was 8 out of 10 for linezolid treatment.

Similar results were obtained with moclobemide. The pretreatment $PD_{>30}$ values ranged from 300 to 800 mg, while the treatment $PD_{>30}$ values ranged from 75 to 250 mg. All 11 subjects in the moclobemide group had $PD_{>30}$ indices > 2, with a mean of 4.97 (range: 3.0-8.0).

Only 1 subject in the placebo group had a $PD_{>30}$ index of > 2, resulting in a response rate of 1 of the 10 subjects who completed treatment. The difference in the proportion of responders in the three treatment groups was statistically significant ($p = 0.001$).

Posttreatment Period $PD_{>30}$

Eight of the 9 linezolid-treated subjects evaluated in the posttreatment period had a $PD_{>30}$ index of ≤ 2. The remaining subject had a value of 2.5, which had decreased from a value of 5.0 in the treatment period. Nine of the 10 moclobemide subjects who completed the posttreatment period had posttreatment $PD_{>30}$ indices ≤ 2. The remaining subject had a value of 2.3, which had decreased from a treatment period value of 3.2. All placebo subjects tested in the posttreatment period had $PD_{>30}$ indices of ≤ 2 except the subject who responded in the treatment period. This subject continued to dis-

Table I $PD_{>30}$ Doses and $PD_{>30}$ Indices

Treatment Group	Subject	Pretreatment $PD_{>30}$	Treatment $PD_{>30}$	Last Tyramine Dose at Treatment	$PD_{>30}$ Index	Posttreatment $PD_{>30}$	Last Tyramine Dose at Posttreatment
Linezolid	2	300	100	100	3.00	NR	200
	5	500	100	100	5.00	200	200
	8	500	100	100	5.00	NR	300
	10	600	125	125	4.80	NR	250
	15	400	100	100	4.00	200	200
	18	400	200	200	2.00	NR	250
	20	400	150	150	2.67	NR	250
	22	300	200	200	1.50	NR	200
	27	500	150	150	3.33	NR	300
	29	500	NR	300	*	NT	NT
Moclobemide	1	500	100	100	5.00	NR	300
	4	800	200	200	4.00	NR	350
	9	800	250	250	3.20	350	350
	12	700	100	100	7.00	NR	300
	14	600	125	125	4.80	NR	350
	17	600	75	75	8.00	NR	250
	19	700	100	100	7.00	NR	300
	24	400	125	125	3.20	NR	250
	25	600	125	125	4.80	NR	350
	28	300	100	100	3.00	NR	200
	32	700	150	150	4.67	NR	150
Placebo	3	700	NR	400	*	NT	NT
	6	400	NR	250	*	NT	NT
	7	500	NR	300	*	NT	NT
	11	600	350	350	1.71	350	350
	13	500	NR	300	*	NT	NT
	16	400	250	250	1.60	NR	250
	21	500	NR	300	*	NT	NT
	23	600	NR	350	*	NT	NT
	26	700	NR	450	*	NT	NT
	30	600	75	75	8.00	NR	150

NR = subject did not attain $PD_{>30}$; NT = pressure testing not conducted since $PD_{>30}$ was not attained during treatment; * = $PD_{>30}$ index cannot be calculated because $PD_{>30}$ was not attained during treatment. $PD_{>30}$ index would be < 2 based on next testing dose.

play an elevated response to the tyramine test, but his index value decreased from 8 in the treatment period to 4 in the posttreatment period.

The doses for $PD_{>30}$ of all treatment periods and $PD_{>30}$ indices during treatment are presented in Table I.

Urinary Catecholamines and Metabolites

Treatment with linezolid was not associated with any statistically significant changes from pretreatment in the excretion of epinephrine, VMA, and HVA. The excretion of norepinephrine (pretreatment mean ± *SD* = 9.40 ± 3.16 μg; treatment mean ± *SD* = 8.00 ± 2.97 μg) and MHPG (pretreatment mean ± *SD* = 0.83 ± 0.23 μg; treatment mean ± *SD* = 0.40 ± 0.29 μg) were significantly reduced during treatment, while the excretion of dopamine (pretreatment mean ± *SD* = 116.70 ± 39.97 μg; treatment mean ± *SD* = 143.70 ± 51.20 μg) and metanephrine (pretreatment mean ± *SD* = 0.21 ± 0.11 μg; treatment mean ± *SD* = 0.41 ± 0.13 μg) were significantly increased.

Treatment with moclobemide was associated with a statistically significant decrease in the excretion of VMA (pretreatment mean ± *SD* = 3.69 ± 1.43 μg; treatment mean ± *SD* = 1.83 ± 0.96 μg) and MHPG (pretreatment mean ± *SD* = 1.00 ± 0.21 μg; treatment mean ± *SD* =

0.53 ± 0.34 μg), while no significant changes were found with the excretion of other catecholamines and metabolites.

For both the linezolid and moclobemide treatments, the MHPG/norepinephrine ratio (linezolid pretreatment mean ± *SD* = 0.092 ± 0.037; linezolid treatment mean ± *SD* = 0.055 ± 0.045; moclobemide pretreatment mean ± *SD* = 0.110 ± 0.026; moclobemide treatment mean ± *SD* = 0.058 ± 0.042) was significantly decreased, while the HVA/dopamine ratio did not show any significant changes.

Greatest Systolic Blood Pressure/ Heart Rate Ratio

There was a linear relationship between GSBP/HR and tyramine dose, with mean GSBP/HR increasing with tyramine dose during pretreatment for all three treatment groups (Figure 1). During treatment, the mean GSBP/HR generally increased with tyramine dose for the linezolid and moclobemide groups but not for the placebo group (Figure 2). The two outlying points at the 250 and 300 mg tyramine dose level, where the mean GSBP/HR ratio of the linezolid group is exceptionally low, represent the GSBP/HR of a single subject who had flat vital signs during treatment.

An ANOVA using the GSBP/HR ratio as the response variable and treatment group as the independent variable showed that there was no significant difference among the three treatment groups during the pre- and posttreatment periods of the study. In contrast, the responses of the linezolid and moclobemide groups during treatment were significantly different from placebo ($p = 0.0001$). The difference between the linezolid and moclobemide groups was not significant ($p = 0.7001$).

An ANCOVA was then carried out with GSBP/HR as the dependent variable and the following factors as independent variables: treatment, tyramine dose, and treatment and tyramine dose interaction. The interaction between treatment and tyramine dose was significant ($p = 0.0007$) during the treatment period, indicating that responses to treatment were different for the treatment groups across different dose levels. This result is consistent with the results shown in Figure 2 (i.e., the three treatment groups have different regression slopes).

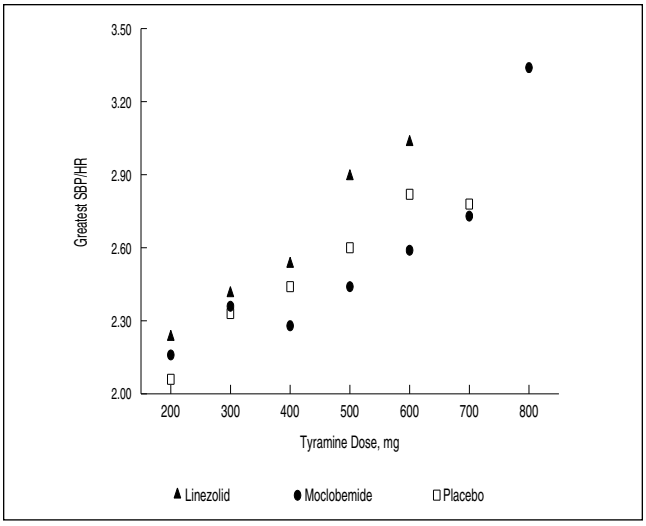

Figure 1. Greatest systolic blood pressure/heart rate ratio (GSBP/HR) treatment group mean by pretreatment tyramine dose (triangles = linezolid; circles = moclobemide; squares = placebo).

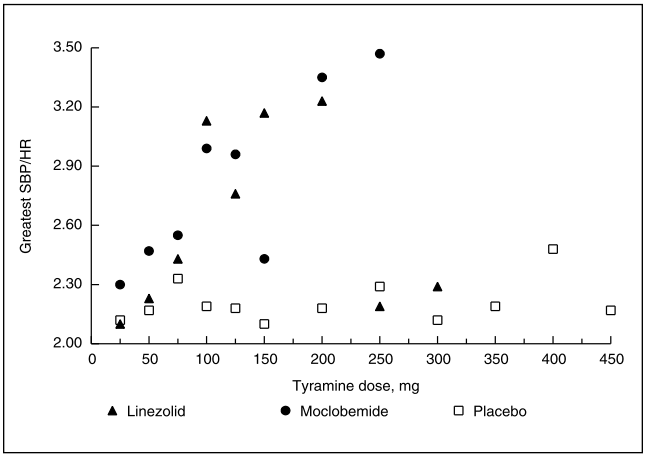

Figure 2. Greatest systolic blood pressure/heart rate ratio (GSBP/HR) treatment group mean by treatment tyramine dose (triangles = linezolid; circles = moclobemide; squares = placebo).

Pharmacokinetics

No statistically significant differences were observed for either linezolid or moclobemide average concentration when compared on Days 4, 5, and 6 of the treatment period. The C_{av} for linezolid for Days 4, 5, and 6 was 15.8, 17.1, and 12.2 μg/mL, respectively; the corresponding C_{av} values for moclobemide were 1308, 1514, and 1507 μg/mL, respectively. Thus, steady state had been achieved during both moclobemide and linezolid treatments, and drug exposure was constant over the tyramine test days.

The mean apparent Cl of tyramine during the placebo treatments was 26,376 ± 9617 L/h, and the mean apparent V_d was 49,249 ± 56,722 L. These values are in agreement with literature accounts of the pharma-

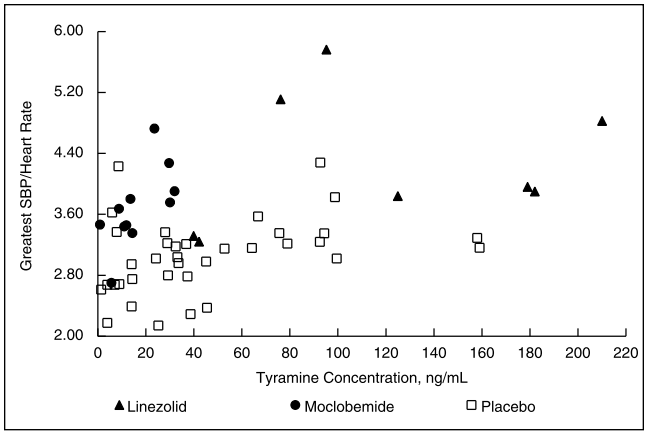

Figure 3. Individual greatest systolic blood pressure/heart rate (GSBP/HR) by corresponding tyramine concentration (triangles = linezolid; circles = moclobemide; squares = placebo).

cokinetic parameters of tyramine.[18] During linezolid treatment, mean apparent tyramine Cl decreased to 2156 ± 692 L/h with a mean apparent V_d of 2215 ± 209 L. During moclobemide treatment, mean apparent Cl decreased to 7570 ± 1323 L/h with a mean apparent V_d of 19,826 ± 21,921 L. Although parameters were not precisely estimated and were found to be highly correlated due to the small data sets involved, a significant decrease in oral tyramine clearance was demonstrated during both linezolid and moclobemide treatment periods. The estimation of posttreatment tyramine parameters was not possible due to the limited data available.

To explore further the relation between response and tyramine exposure, the GSBP/HR ratio was regressed with the tyramine concentration determined at the time of the GSBP of the highest tyramine dose administered in the treatment period. A significant linear relationship was observed ($p = 0.0023$, $R^2 = 0.1626$) (Figure 3). No significant difference was observed among the three treatments in the relationship between concentration and response.

DISCUSSION

The potential interaction between foods containing a high tyramine content and irreversible MAOI drugs is well documented. With the advent of newer drugs with reversible MAOI activity, it is necessary to establish potential interactions with tyramine-containing foods to establish relevant product labeling. Drugs such as moclobemide have been extensively evaluated in tyramine pressor tests to quantify the potential for drug-food interactions. Results of these tests have established that moclobemide causes less potentiation of tyramine than traditional, irreversible MAOIs, and there is therefore no need to restrict dietary tyramine. However, the avoidance of the consumption of excessive amounts of tyramine-rich foods is recommended as a precaution.[19]

The placebo, linezolid, and moclobemide treatments were well tolerated. The tyramine tests produced responses consistent with the literature and the pharmacology of the compound. All subjects recovered from the change in sensitivity to tyramine observed during the treatment period within 2 days of stopping treatment.

Based on an analysis of $PD_{>30}$ indices, the results of this study demonstrated that moclobemide and linezolid potentiate the pressor effect of tyramine in a similar manner. The enhanced effect was moderate, as seen in previous studies reported in the literature for moclobemide, and returned to the baseline effect within 2 days of discontinuation of either drug. The smallest tyramine dose required to cause a 30 mmHg rise in SBP ($PD_{>30}$ dose) was 100 mg with linezolid treatment and 75 mg with moclobemide treatment. Surveys of typical tyramine content of foods have established that it is unlikely that this amount of tyramine would be ingested unless excessive amounts of the most tyramine-rich foods were ingested.[20-22] Foods high in tyramine content include those that have undergone protein changes by aging, fermentation, pickling, or smoking to improve flavor, such as aged cheeses (0-15 mg tyramine/ounce), fermented meats (0.1-8 mg tyramine/ounce), sauerkraut (8 mg/8 ounces), soy sauce (5 mg/teaspoon), tap beers (4 mg/12 ounces), and red wines (0-6 mg/8 ounces). Restrictions to normal dietary intake of tyramine-containing foods are therefore not warranted.

The mechanism of potentiation of the tyramine effect was determined to be the decreased clearance of tyramine by linezolid and moclobemide. This is consistent with their documented MAOI activity. Reduced tyramine clearance will result in higher blood concentrations at any given tyramine dose. Although similar relationships were observed between response (as measured by GSBP/HR) and tyramine concentration for the three treatment groups, the relationship between response and tyramine dose differed between the two drug treatment groups and the placebo group. This was a result of the decreased apparent oral clearance and corresponding increase in blood concentration of tyramine associated with each tyramine dose during drug treatment.

The results indicate that the GSBP/HR ratio was a good measure of the pressor effect. A possible advan-

tage over the traditional $PD_{>30}$ response assessment was demonstrated if the ratio was regressed over both dose and tyramine concentration. This approach allowed further insight into the mechanism of the enhanced response to tyramine during the drug treatment phases of the study. Since the response versus concentration relationship was similar between all three treatments, it would appear that the enhanced response during moclobemide and linezolid treatment was due only to increased tyramine concentrations and not the presence of moclobemide or linezolid. Further research will be necessary to determine its advantages in special subpopulations of subjects or different study conditions.

The monitoring of urinary excretion profiles of endogenous norepinephrine and dopamine and their major metabolites was consistent with the MAOI activity of linezolid and moclobemide. However, the results were highly variable. Although trends were observed in increased parent compound excretion and decreased metabolite excretion, the effects were not consistently reproduced. This monitoring is therefore of questionable value as a tool in assessing relative MAOI activity.

It is concluded that linezolid and moclobemide have similar effects on the pressor response to oral tyramine. The mechanism of potentiation of the tyramine effect was determined to be the decreased clearance of tyramine by linezolid and moclobemide. In common with the product labeling for moclobemide, restrictions to normal dietary intake of tyramine-containing foods are not warranted when taking linezolid. The results also indicate that the GSBP/HR ratio was a good measure of the pressor effect with a possible advantage over the traditional $PD_{>30}$ response assessments. The monitoring of urinary excretion values of endogenous catecholamines demonstrated MAOI activity. However, high variability makes this a limited assessment tool.

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REFERENCES

1. Pawsey SD, Daley-Yates PT, Wajszczuk CP, Stalker DJ: U-100766 safety, toleration and pharmacokinetics after oral and intravenous administration [abstract F151]. Paper presented at the European Congress of Antimicrobial Chemotherapy, Glasgow, Scotland, May 1996.

2. Martin JP, Herberg JT, Slatter JG, Dupuis MJ: Although a novel microtiter-plate assay demonstrates that linezolid (PNU-100766) is a weak, competitive (reversible) inhibitor of human monoamine oxidase (MAO A), no clinical evidence of MAO A inhibition in clinical trials has been observed. Paper presented at the 38th Interscience Conference on Antimicrobials and Chemotherapy, San Diego, CA, September 1998.

3. Bieck PR: Hypertensive krisen unter reversiblen hemmstoffen der monoaminoxidase? *Psychiat Prax* 1989;16:25-31.

4. Simpson GM, White K: Tyramine studies and the safety of MAOI drugs. *J Clin Psychiatry* 1984;45(7, Sec. 2):59-61.

5. Hendershot PE, Antal EJ, Welshman IR, Batts DH, Hopkins NK: Linezolid: pharmacokinetic and pharmacodynamic evaluation of co-administration with pseudoephedrine HCl, phenylpropanolamine HCl and dextromethorphan HBr. *J Clin Pharmacol* 2001; 41:563-572.

6. Korn A, Da Prada M, Raffesberg W, Gasic S, Eichler HG: Effect of moclobemide, a new reversible monoamine oxidase inhibitor, on absorption and pressor effect of tyramine. *J Cardiovasc Pharmacol* 1988;11:17-23.

7. Berlin I, Zimmer R, Cournot A, Payan C, Pedarriosse, Puech AJ: Determination and comparison of the pressor effect of tyramine during long-term moclobemide and tranylcypromine treatment in healthy volunteers. *Clin Pharmacol Ther* 1989;46:344-351.

8. Bieck PR, Firkusny L, Schick C, Antonin K-H, Nilsson E, Schulz R, et al: Monoamine oxidase inhibition by phenelzine and brofaromine in healthy volunteers. *Clin Pharmacol Ther* 1989;45:260-269.

9. Korn A, Wagner B, Mortiz E, Dingemanse J: Tyramine pressor sensitivity in healthy subjects during combined treatment with moclobemide and selegiline. *Eur J Clin Pharmacol* 1996;49:273-278.

10. Dingenase J: An update of recent moclobemide interaction data. *International Clinical Psychopharmacology* 1993;7:167-180.

11. Dollery CT, Davies DS, Strolin-Benedetti M: Clinical pharmacology of MD780515, a selective and reversible MAO-A inhibitor, in: Kamijo K, Usdin E, Nagatsu T (eds.), *Monoamine Oxidase: Basic and Clinical Frontiers.* Amsterdam: Excerpta Medica, 1982;221-229.

12. ABPI Data Sheet Compendium. London, Datapharm Publications Limited, 1995;334:1195-1196, 1447-1448.

13. Beal SL, Sheiner LB: The NONMEM system. *Am Statistician* 1980;34:118-119.

14. Johnson RA, Haan DE, James CA, Hopkins NK: Determination of linezolid, PNU-100766, in human plasma and urine using high-performance liquid chromatography with ultraviolet detection. *Pharm Research* 1997;14(Suppl.):S-374.

15. Cazers AR, Gammill JC, Johnson RA, Hopkins NK: Determination of tyramine in human plasma by LC/MS/MS. Paper presented at the 47th ASMS Conference on Mass Spectrometry and Allied Topics, Dallas, TX, June 13-17, 1999.

16. *SAS/STAT User's Guide*, version 6. Cary, NC: SAS Institute, Inc., 1990.

17. Klee GG: Maximizing efficacy of endocrine tests: importance of decision-focused testing strategies and appropriate patient preparation. *Clin Chem* 1999;45:1323-1330.

18. Provost JC, Funck-Brentano C, Rovei V, D'Estanque J, Ego D, Jaillon P: Pharmacokinetic and pharmacodynamic interaction between toloxatone, a new reversible monoamine oxidase-A inhibitor, and oral tyramine in healthy subjects. *Clin Pharmacol Ther* 1992; 52:384-393.

19. Livingston MG, Livingston MH: Monoamine oxidase inhibitors: an update on drug interactions. *Drug Safety* 1996;14:219-227.

20. McCabe BJ: Dietary tyramine and other pressor amines in MAOI regimens: a review. *J Amer Dietetic Assn* 1986;86:1059-1064.

21. DaPrada M, Zürcher G, Wüthrich I, Haefely WE: On tyramine, food, beverages and the reversible MAO inhibitor moclobemide. *J Neural Transm* 1988;26:31-56.

22. Walker SE, Schulman KI, Tailor SAN, Gardner D: Tyramine content of previously restricted foods in monoamine oxidase inhibitor diets. *Journal of Clinical Psychopharmacology* 1996;16(5): 383-388.