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Effect of oral linezolid on the pressor response to intravenous tyramine

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Aims
To investigate the effect of monoamine oxidase A inhibition from a single oral dose of linezolid on the pressor response to intravenous (i.v.) tyramine, using positive and negative controls to validate the methodology.

Methods
This placebo-controlled, three-period crossover study was conducted in 12 healthy male volunteers. Each volunteer received either one oral dose of moclobemide (300 mg), linezolid (600 mg), or placebo tablet followed by an i.v. tyramine pressor test until an increase in systolic blood pressure of at least 30 mmHg above baseline occurred. Each study day was separated by a 7-day washout period. The dose of tyramine required to raise the blood pressure by 30 mmHg (TYR30) was calculated for each oral treatment by linear interpolation between log-transformed doses of i.v. tyramine. The influence of body mass index (BMI) on TYR30 was also investigated.

Results
The tyramine sensitivity factor (ratio of the geometric least square mean TYR30 for placebo and active oral treatment) was 1.8 [90% confidence interval (CI) 1.6, 2.0, $P < 0.0001$] for linezolid and 2.1 (90% CI 1.8, 2.4, $P < 0.0001$) for the positive control moclobemide. BMI had a statistically significant effect on TYR30.

Conclusions
There was a significant difference in the pressor response to i.v. tyramine between linezolid and placebo. Moclobemide (positive control) and linezolid have a similar pressor response to i.v. tyramine. The statistically significant effect of BMI on TYR30 underlines the advantage of within-individual comparisons of treatments in order to reduce variability and provide more accurate treatment estimates.

Introduction

Tyramine is an indirectly acting sympathomimetic drug, displacing neurotransmitter from adrenergic axonal terminals [1], resulting in an increase in systolic blood pressure (SBP) when administered intravenously and/or in the presence of monoamine oxidase inhibitors (MAOIs) [2]. Coadministration of intravenous (i.v.) tyramine with MAOIs has been used to determine the potential for clinically significant drug interactions [3]. In preclinical studies, the oxazolidinone antibiotic linezolid has been shown to possess weak reversible MAOI activity (MAO-A; $K_i = 55$ μM) [4]. In an oral tyramine interaction study, the MAOI activity of linezolid was similar to that of moclobemide: the tyramine sensitivity factor [ratio between the mean dose of tyramine required to raise the blood pressure by 30 mmHg (TYR30) for placebo and active oral treatment] was 3.48 for linezolid and 4.97 for moclobemide [5]. However, there were no within-individual comparisons made since each volunteer was randomized to receive only

one of the study medications (i.e. moclobemide, linezolid, or placebo alone). The aim of this study was to determine the pharmacodynamic effect of MAO-A inhibition by linezolid on peripheral neurones by the intravenous tyramine pressor test using within-volunteer comparisons. Moclobemide is a known selective, competitive, and reversible MAO-A inhibitor ($K_i = 0.2$–0.4 mM) [6], and was used in this study as a positive control [2, 7], with placebo tablets acting as a negative control.

The target rise of 30 mmHg in SBP was considered to be of sufficient size to be attributable to tyramine rather than natural variability, whilst being clinically inconsequential (increases in SBP of 30 mmHg occur during mild to moderate exercise) [8].

A supplementary examination of the influence of body mass index (BMI) on TYR30 was performed as a consequence of observations made during a pilot study conducted at the same site that suggested a potential effect of BMI on the tyramine pressor response.

Methods

Volunteers

The protocol was approved by the AstraZeneca Ethics Committee, Manchester, UK, and was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. Twelve healthy males (aged 27–46 years) from the volunteer panel at AstraZeneca's Clinical Pharmacology Unit, Alderley Park, Cheshire, UK, participated in the study after giving written informed consent. Inclusion criteria were: BMI 18–31, no history of migraine or serotonin syndrome and resting blood pressure <130/90 mmHg at screening.

Sample size

The sample size calculations were based on the primary endpoint of TYR30. A tyramine sensitivity factor (TSF) of 2 was considered the minimum clinically relevant difference to detect, with the TSF for moclobemide being typically between 2 and 4.2 [2, 9]. Based on the pilot study, a sample size of 10 would have >99% power to detect a doubling of TYR30 between moclobemide and placebo at the 10% two-sided significance level. The sample size was inflated to 12 to allow for withdrawals.

Study design and treatments

The study was a single-centre, randomized, placebo-controlled, three-period crossover design. On each study period the volunteers received a single oral tablet of (i) 300 mg moclobemide (Manerix®; Roche, Welwyn Garden City, UK), (ii) 600 mg linezolid (Zyvox®; Pharmacia & Upjohn, Kalamazoo, USA), or (iii) placebo with 200 ml of purified water 1 h prior to the intravenous tyramine pressor test. The predicted t_{max} values for moclobemide and linezolid were 1–1.5 h (60–75 min) [10] and 1.28 h (76 min) [11] after dosing, respectively. The isotonic tyramine solution was aseptically manufactured by the Pharmaceutical Analytical Research & Development Department of AstraZeneca, Macclesfield, UK, as tyramine 1 mg ml^{-1} in 5-ml vials. It was diluted according to tyramine dose required with 0.9% sodium chloride to make up a final volume of 10 ml 1 h prior to administration in a Class 2 laminar airflow cabinet. Each study period was separated after a minimum washout period of 7 days. The Biostatistics Group, AstraZeneca generated the randomized scheme for the sequence of oral treatments received during the three study periods. Volunteers were allocated to sequences in balanced blocks.

The tyramine challenge tests were performed at the AstraZeneca Alderley Park Clinical Pharmacology Unit, in order to maximize control of the external environment in terms of temperature, noise and physical activity. The volunteers were fasted from midnight. On the morning of each study period volunteers received the oral treatment in a semirecumbent position 1 h prior to the start of the intravenous tyramine challenge. Individuals not involved in data collection or assessment dispensed the tablets.

Volunteers were placed in a fully supine position 10 min prior to the start of the intravenous tyramine challenge. After resting vital signs had been recorded the volunteers received intravenous bolus injections of tyramine of 1, 2, 4, 6, 8 and 10 mg in an ascending dose sequence. The bolus injection was administered over a 10-sec period, followed by a 10-ml saline flush. Each dose administration was separated by a minimum duration of 15 min during which time SBP readings were taken as described below. In addition, one matching injection of 0.9% saline solution was administered randomly in the sequence to maintain the blinding of the intravenous tyramine dose. Once a ≥30 mmHg increase of SBP above predose was achieved, no further intravenous injections were administered. Thus, not all volunteers received all intended doses of intravenous tyramine or saline.

Administration of the oral treatments was not fully blinded as none of the tablets were matching in appearance. Volunteers were asked to close their eyes when taking the tablet, maintaining the subject blinding, and individuals not involved in data collection or blood pressure assessment, maintaining the observer blinding, administered the tablets. The tyramine injections and matching saline injection were fully blinded to volun-

teers, administrator and assessor. The primary endpoint of SBP was measured using an electric sphygmomanometer. Readings from a machine are objective and not influenced by preceding measurements.

On completion of the tyramine challenge the volunteers were allowed to return to the semirecumbent position until a standard low-tyramine meal was provided 3 h after oral dosing, after which time they were fully ambulant within the Unit and were discharged home 4 h later. No additional dietary restrictions were present between visits. Alcohol and strenuous physical activity were prohibited from 24 h predose to 24 h post dose on each visit.

Phentolamine (Rogitine®; Alliance, Bath, UK) for intravenous injection was available to reverse any excessive pressor response to i.v. tyramine [12, 13].

Blood pressure measurements

Supine SBP measurements were recorded at 1-min intervals, using a Critikon Dinamap 1846SX semiautomatic blood pressure recording device, until three consecutive readings were all within ±15 mmHg. The mean of these three consecutive readings was calculated to provide the predose SBP. After administration of the injection, SBP was recorded at 1-min intervals until three consecutive readings were all within ±12 mmHg of the predose SBP or for five consecutive minutes, whichever was longer. The maximum increase in SBP after each tyramine injection was used for the TYR30 calculation.

As tyramine injections were administered at 15-min intervals the predose SBP measurement procedure recommenced 12 min after the preceding tyramine injection.

Statistical analysis

The primary endpoint of TYR30, defined as the dose of tyramine required to cause an increase in SBP of 30 mmHg, was derived by linear interpolation between the two log-transformed doses of tyramine either side of a 30-mmHg increase in SBP from predose to postdose. Analysis of the relationship between tyramine concentration and SBP response was performed using an analysis of covariance (ANCOVA) model with log transformed TYR30 as the response, allowing for the effects of oral treatment sequence, volunteer nested within treatment sequence, period and oral treatment. This was done within the SAS™ procedure PROC MIXED (SAS Institute Inc., Cary, NC, USA), fitting all effects as fixed. All statistical tests were two-tailed, and P-values ≤0.10 were considered significant. The results of the analyses were presented as adjusted means (geometric least square means [glsmeans]), and the treatment effect (TSF, the ratio of glsmeans between placebo and active treatments) and its 90% confidence interval (CI).

The effect of BMI as a covariate was explored using type I sum of squares, fitting BMI before volunteer nested within oral treatment sequence (because BMI only varies between volunteers).

Results

Safety

Twelve volunteers entered the study and 10 completed the study. No serious adverse events were reported. Two volunteers were withdrawn due to adverse events: one due to development of third-degree heart block and one due to renal colic. The episode of third-degree heart block and related paraesthesia occurred 92 min after receiving 600 mg oral linezolid and approximately 90 s after the third tyramine injection (4 mg). After the injection a sinus bradycardia initially developed (heart rate 38–39 beats min^{-1}), progressing after 90 sec to third-degree heart block, with a systolic blood pressure of 154 mmHg. The episode resolved rapidly following administration of 2 mg phentolamine intravenously, with normal sinus rhythm restored and blood pressure returning to baseline within 5 min.

There were no clinically relevant changes in laboratory parameters.

TYR30 and tyramine sensitivity factor

As a consequence of withdrawals due to adverse events, 11 volunteers participated in each of the moclobemide and linezolid study days, and 10 completed the oral placebo study day. All volunteers achieved an increase of at least 30 mmHg in SBP during each study period in which they participated. The mean increase in SBP after each dose of tyramine is presented for each oral treatment in Table 1.

Once an increase of >30 mmHg was achieved a volunteer received no further tyramine injections, hence not all doses of tyramine were administered to all volunteers, and fewer volunteers received the higher doses of tyramine. As the saline injection was administered randomly in the sequence, and if an increase in SBP >30 mmHg had been achieved prior to this injection, it would not be administered on that day. Hence fewer volunteers received the saline injection.

The glsmeans of TYR30 and TSF for each oral treatment are presented in Table 2. The TSF for moclobemide was calculated to be 2.1 (90% CI 1.8, 2.4, $P < 0.0001$). This means that the dose of i.v. tyramine required to cause a 30-mmHg rise in SBP was over two

Table 1
Mean increase in systolic blood pressure (SBP, in mmHg) above pre-tyramine baseline

Intravenous tyramine dose (mg)	Oral treatment Linezolid	Moclobemide	Placebo
0	4.7 ± 3.1 (3)	4.8 ± 6.9 (4)	4.8 ± 2.2 (8)
1	5.9 ± 2.7 (11)	4.1 ± 3.3 (11)	4.0 ± 4.6 (10)
2	12.9 ± 6.5 (11)	18.0 ± 9.8 (11)	8.0 ± 5.2 (10)
4	42.9 ± 13.9 (11)	47.8 ± 13.3 (9)	15.9 ± 11.6 (10)
6	40.0 ± 4.2 (2)	–	31.6 ± 12.7 (9)
8	–	–	40.0 ± 10.3 (6)

Results are expressed as mean ± standard deviation (n).

Table 2
Analysis of TYR30 calculated using linear interpolation between two log-transformed doses of i.v. tyramine

Contrast (placebo to active)	n placebo	glsmean placebo TYR30 (mg)	n active	glsmean active TYR30 (mg)	TSF	90% CI	P-value
Placebo to moclobemide	10	5.4	11	2.6	2.1	1.8–2.4	< 0.0001
Placebo to linezolid	10	5.4	11	3.0	1.8	1.6–2.0	< 0.0001

CI, Confidence interval; glsmean, geometric least square mean; TSF, tyramine sensitivity factor (TYR30 on placebo/TYR30 on active); TYR30, the dose of i.v. tyramine required to raise the SBP by 30 mmHg from preinjection for each dose of oral study medication.

times greater with placebo than with moclobemide. The result confirms a statistically significant difference between the TYR30 of moclobemide and placebo, and is in keeping with previous literature citations [3, 14]. The TSF for linezolid was calculated to be 1.8 (90% CI 1.6, 2.0, $P < 0.0001$), so the dose of tyramine required to cause a 30-mmHg rise in SBP was 1.8 times greater with placebo than with linezolid. This demonstrates a statistically significant difference between the TYR30 of linezolid and placebo.

BMI

The effect of BMI on TYR30 was consistent with all oral treatments with BMI having a statistically significant effect on TYR30. When the TYR30 was calculated by linear interpolation between log-transformed tyramine doses, a unit increase in BMI resulted in a 0.045 increase in log (TYR30), which was equivalent to a 1.046-fold increase in TYR30 ($P = 0.002$). The effect of BMI can be more easily understood by deriving the TYR30 from linear interpolation between tyramine doses which results in a unit increase in BMI producing a 0.84 mg increase in TYR30; however, log-transformed data have been used for all other statistical calculations in this study.

Discussion

Safety

The placebo, moclobemide, and linezolid treatments were well tolerated in this study. The responses to i.v. tyramine were consistent with the monoamine oxidase inhibition-related pharmacology of the compounds.

The volunteer that developed third-degree heart block 92 min after the oral dose of linezolid (600 mg) and 90 sec after the i.v. dose of tyramine (4 mg) was withdrawn from the study after the first study day and was not rechallenged. Thus it is not possible to determine whether this response was due to an excessive sensitivity to tyramine or a synergistic interaction between linezolid and tyramine. To our knowledge, there have been no reports in the literature of such cardiac arrhythmias associated with linezolid at this low oral dose. An episode of complete atrioventricular block in a healthy volunteer after oral administration of tyramine (800 mg) and daily dosing with toloxatone (1200 mg) has previously been reported [15]; as in this study, the volunteer

recovered after administration of i.v. phentolamine. Coadministration of linezolid with some oral sympathomimetic agents has been associated with elevated blood pressure in normotensive patients [16], and a recent review of the safety of linezolid has recommended that administration of adrenergic agents such as dopamine or epinephrine should be performed with careful monitoring [17]. As a consequence of the adverse event in this study vigilance is recommended for any reported episodes consistent with a cardiac arrhythmia.

TYR30 and tyramine sensitivity factor

The glsmean i.v. tyramine dose required to achieve the TYR30 with oral placebo was 5.4 mg. This value is within 0.5 mg of values quoted in the literature (for studies that employed similar methodologies) [18–21].

The tyramine pressor test methodology applied in this study demonstrated a TSF for moclobemide of 2.1 (90% CI 1.8, 2.4, $P < 0.0001$) confirming the sensitivity of the test to detect statistically significant differences between a known MAO-A inhibitor (the positive control) and placebo (the negative control). The TSF for linezolid was calculated to be 1.8 (90% CI 1.6, 2.0, $P < 0.0001$), demonstrating a statistically significant difference between the TYR30 of linezolid and placebo. The results of this study indicate that moclobemide and linezolid have similar pressor responses to i.v. tyramine. These findings are consistent with the oral tyramine challenge study [5]. Therefore caution should be advised when considering the coadministration of linezolid with intravenous sympathomimetic agents with respect to potential interactions due to monoamine oxidase inhibition by linezolid.

Completion of the tyramine challenge tests (SBP increase >30 mmHg) occurred after the anticipated t_{max} for both moclobemide and linezolid. In future studies a tyramine challenge test should be scheduled with the anticipated target tyramine dose being administered at the t_{max} of the test compound, based on a pilot study.

BMI

The results confirm that BMI had a statistically significant effect on the TYR30, a finding that had not been recognized previously in the literature, with the underlying mechanism for this effect being unknown at present. This finding emphasizes the benefit of performing within-individual comparisons of active oral compounds and placebo to reduce variability and provide more accurate treatment estimates.

Previous studies of tyramine pressor responses have reported the finding that male volunteers needed more tyramine than female volunteers to increase the SBP by at least 30 mmHg [18, 22]. The relationship between tyramine pressor response and sex differences may be more complex than BMI alone. It would be advisable to restrict the performance of such comparative studies to one sex, or perform separate analyses for the sexes, until further pharmacokinetic and/or pharmacodynamic factors affecting tyramine pressor responses between the sexes have been quantified.

In conclusion, this study demonstrated that moclobemide and linezolid have a similar effect on the pressor response to i.v. tyramine. The findings are consistent with previous literature on oral tyramine challenge tests with these two compounds. The study also demonstrated a statistically significant effect of BMI on the tyramine pressor response, which underlined the necessity of performing within-individual comparisons of treatments in order to reduce variability and provide more accurate treatment estimates.

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