An investigation of components of variance and tachyphylaxis in a placebo-controlled intravenous tyramine study

M. V. Cantarini, C. L. Watkins, J. Growcott & A. M. Hughes

Experimental Medicine Department, AstraZeneca, Macclesfield, UK

Correspondence

Dr M. V. Cantarini, Experimental Medicine, AstraZeneca, 1G50 Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK.

Tel: +44 (0)1625 518172

Fax: +44 (0)1625 585626

E-mail:

mireille.cantarini@astrazeneca.com

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Aims

To explore inter- and intra-volunteer variability for the dose of intravenous tyramine eliciting a 20 mmHg increase in systolic blood pressure from baseline (TYR20) and to evaluate potential tachyphylaxis.

Methods

Twelve healthy volunteers received blinded placebo-controlled ascending and descending sequences of intravenous tyramine injections on two separate occasions. The TYR20 was derived by linear interpolation, using three interventions to deal with missing data.

Results

Analysis of covariance (ANCOVA) demonstrated no significant difference in TYR20 between sequences, regardless of the missing data methodology applied. Inter-volunteer variability was 2.4–3.4 times larger than within-volunteer variability. No evidence of tachyphylaxis was seen using either the sign test or generalized additive models.

Conclusions

Since inter-volunteer variability was greater than intra-volunteer variability, a crossover study design would be a more efficient study design, and the descending sequence of injections could be omitted since tachyphylaxis was not demonstrated.

Introduction

Tyramine is an indirectly acting sympathomimetic drug, displacing neurotransmitter from adrenergic axonal terminals [1], which can result in an increase in systolic blood pressure when administered intravenously and/or in the presence of monoamine oxidase inhibitors [2]. Previous tyramine pressor studies have reported either large intra- or inter-volunteer variations (3–47% and 30%, respectively) [3], or good reproducibility (intra-volunteer coefficient of variation between 7.4 and 11.2%) [4].

The latter study also demonstrated the potential for tachyphylaxis with repeated administration of tyramine.

The current study was performed to define the components of variance within the Alderley Park volunteer panel, to enable appropriate powering of future drug interaction studies. A descending dose sequence was incorporated into the study design to investigate possible tachyphylaxis following a rapid sequence of ascending intravenous tyramine injections.

Methods

Subjects

The protocol was approved by the AstraZeneca Ethics Committee, Manchester, UK, and was carried out in accordance with the Declaration of Helsinki. Twelve healthy males from the volunteer panel at AstraZeneca's Clinical Pharmacology Unit, Alderley Park, Cheshire, participated in the study after giving written informed consent. Inclusion criteria were: age 18–65 years, body mass index 18–31, no history of migraine, and resting blood pressure <130/90 mmHg at screening.

Design

The study was a single-centre, double-blind, placebo-controlled, two-period design. On each study day, after resting vital signs had been recorded, the volunteers received intravenous bolus injections of tyramine of 0.5–8.0 mg in an ascending then descending dose sequence. Each dose administration was separated by a minimum duration of 15 mins during which time systolic blood pressure readings were taken as described below. In addition, matching placebo injections of saline solution were administered randomly in the sequence for blinding purposes: once randomly in the first three injections, once randomly in the last three injections and once randomly in between the first and last three injections. Once a ≥ 30 mmHg increase of systolic blood pressure above predose was achieved, no further ascending doses were administered, the same dose was repeated, then descending doses were administered from that dose. Thus, not all volunteers received all intended doses of intravenous tyramine or placebo.

The treatment sequence was repeated after a minimum washout period of 7 days.

Blood pressure measurements

Supine systolic blood pressure (SBP) measurements were recorded at 1-min intervals, using a Critikon Dinamap 1846SX semi-automatic blood pressure recording device, until three readings were within ± 15 mmHg to provide the predose SBP. After administration of the intravenous treatment, 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.

Statistical analysis

The TYR20, defined as the dose of tyramine required to cause an increase in SBP of 20 mmHg, was derived by linear interpolation between the two doses of tyramine either side of a 20 mmHg increase in SBP from pre-dose

to post-dose. Estimation of the components of variance for TYR20 (inter-volunteer, intra-volunteer between days, and intra-volunteer within days) was done using an analysis of covariance (ANCOVA) model within the SAS[®] procedure PROC MIXED (SAS Institute Inc., Cary, NC, USA). Tachyphylaxis was explored using (i) the sign test, where the null hypothesis being tested was H_0 : there is no difference in the area under the curve between ascending and descending dose sequences, i.e. no tachyphylaxis, and (ii) Generalized Additive Models (GAMs) [5], where the null hypothesis was H_0 : there is no difference between the fit of the model including the sequence term and the model excluding it, i.e. no tachyphylaxis.

It should be noted that the primary endpoint of this study was TYR30; however, due to the large amount of missing data for this endpoint, the results for TYR20 are presented in this paper.

Results

Intravenous tyramine injections were well tolerated by all 12 volunteers, with no serious adverse events being reported. The mean maximum changes in SBP, diastolic blood pressure (DBP) and heart rate achieved after each dose of intravenous tyramine for each study day are presented in Figure 1 (dosing occurring in volunteers only if an increase in SBP >30 mmHg not yet achieved in the sequence). Placebo injections administered randomly throughout the sequences gave consistent responses on both study days.

TYR20

Two volunteers did not achieve a ≥ 20 mmHg increase in SBP at one visit (both ascending and descending sequences) during the study. The TYR20 and components of variance for TYR20 for these missing data, four out of 48 observations, were calculated using three methods: (i) replacing the missing TYR20 value with the highest dose received by the volunteer in the sequence, (ii) replacing the missing TYR20 value by a linearly extrapolated value and subsequent implausible extrapolated values replaced with the highest dose received, (iii) missing data excluded. The results of these analyses are presented in Table 1.

Results from the ANCOVA analysis indicated that there was no systematic difference in TYR20 between sequences when calculated by any of the three methods listed above (highest dose received $P = 0.2704$, extrapolated value $P = 0.1607$ and data excluded $P = 0.2699$).

Tachyphylaxis

The sign test analysis of difference in area under the curves (AUCs) for systolic blood pressure change for

Figure 1

Vital sign responses in healthy male volunteers to intravenous tyramine injections on each study day. Systolic blood pressure (SBP) (± 0.5 SD) ($\bullet$, ascending, $\circ$, descending); diastolic blood pressure (DBP) ($\blacksquare$, ascending, $\square$, descending); heart rate ($\blacktriangle$, ascending, $\triangle$, descending). Group mean shown, n = number of volunteers receiving each dose of tyramine. Placebo (saline) injections represented as 0 mg tyramine

the ascending and descending arms revealed there were seven positive and five negative differences (12 volunteers, average over the two study days). This gives a two-sided P -value of $P = 0.77$. Therefore there is insufficient evidence to reject the null hypothesis of no tachyphylaxis using the sign test. The generalized additive models analysis gave a P -value of $P = 0.65$ for study day 1, giving insufficient evidence to reject the null hypothesis of no tachyphylaxis for study day 1. The analysis for study day 2 gave a P -value of $P = 0.004$. However, on closer data inspection there was a large outlier value for one volunteer, and when these data were removed the P -value became $P = 0.66$, giving insufficient evidence to reject the null hypothesis of no tachyphylaxis for study day 2.

Discussion

The statistical interpretation of the results was complicated by volunteers failing to achieve the target of ≥ 30 mmHg increase of SBP during the sequence of tyramine injections, however all but two achieved a ≥ 20 mmHg increase in SBP. Missing data were managed by using three different statistical approaches. The dosing schedule had been developed based on previously published reports anticipating a 30 mmHg rise in systolic blood pressure after 3–4 mg intravenous tyramine [3, 6]. However, both of these studies administered the tyramine into an intravenous line with an infusion running, whereas the current study employed a bolus injection followed by a flush of saline. This may not have been sufficient to distribute the tyramine into the circulation as effectively as an infusion.

Table 1

Comparison of TYR20 for each study day and components of variance calculated using alternative methods

Analysis method	Mean TYR20 (SD) mg		Components of variance for TYR20		
	Study day 1 ascending, descending	Study day 2 ascending, descending	Between subject	Within subject between days	Within subject within days
Missing data replaced by highest dose received	5.15 (1.55), 5.47 (1.46)	5.24 (1.81), 5.36 (1.61)	1.6229	0.000	0.4804
Missing data replaced by extrapolated data	5.06 (1.40), 5.75 (2.13)	5.07 (1.61), 5.12 (1.45)*	1.8191†	0.000†	0.7647†
Missing data excluded	4.89 (1.33)*, 5.24 (1.28)*	4.99 (1.66)*, 5.12 (1.45)*	1.3465	0.000	0.5206

* $N = 11$, all other TYR20 are $n = 12$. †Missing data replaced by extrapolated value and implausible extrapolated value replaced by highest dose received.

The finding of no significant rise in diastolic blood pressure or heart rate associated with the increase in systolic blood pressure is consistent with baroreflex buffering [7].

The variability estimates were calculated using three methods to deal with the missing four observations. Replacing TYR20 values with extrapolated values led to some implausible TYR20 estimates of less than the maximum dose received, requiring further data manipulation to obtain realistic TYR20 estimates. However, all analyses found a similar pattern in the relative size of the three types of variability estimate. Inter-volunteer variation was 2.4–3.4 times larger than the intra-volunteer variation, suggesting benefit in performing within-volunteer comparisons to reduce variability. In other words, a crossover study would be more efficient than a parallel group design. In all analyses, there was little or no intra-volunteer variation between days over and above that already accounted for by the intra-volunteer variation within a day. Hence, a crossover study would not lose any efficiency if the same volunteer received two tyramine sequences on two different days instead of on the same day.

There was no statistical evidence of tachyphylaxis in this study. It is acknowledged that this study was not specifically powered to detect tachyphylaxis and therefore a lack of evidence does not necessarily indicate that there was no tachyphylaxis. However, an examination of the individual subject profile graphs of SBP does not reveal any obvious tachyphylaxis pattern and appears to

support the findings of the statistical analyses. Therefore it is recommended that a descending dose sequence would be unnecessary in future studies, permitting the tyramine pressor test to be administered over a shorter period identified around $T_{\max}$ of the interacting agent.

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