Retrospective analysis of ECG data derived from a four-way cross over study involving a broad spectrum anti-infective agent nitazoxanide

J Täubel1, U Lorch1, J Singh1, JF Rossignol2, G Ferber1 and AJ Camm3
1Richmond Pharmacology Ltd, St George's University of London, Cranmer Terrace, London, United Kingdom
2Romark Laboratories, L.C., 3000 Bayport Drive, Suite 200, Tampa, Florida 33607 USA
3Department of Cardiological Sciences, St George's University of London, Cranmer Terrace, London, United Kingdom

Introduction
There is a pressing global clinical need for accelerated treatment strategies for viral infections as the drug-resistance is constantly evolving in viruses thereby limiting treatment options. An example of a wide spreading virus of high variability is influenza. The treatment against it consists of two major classes of drugs: adamantanes and neuraminidase inhibitors. However, resistance to both classes of drugs has been observed, thus raising a concern about efficacious treatments against influenza.

Nitazoxanide is the approved generic name for 2-ethyl-5-(5-nitro-2-thiazolyl)-benzoxazole, also known as PF-5779, NTZ or Alfradin (Romark Laboratories, L.C.). Originally, it has been FDA-approved for the treatment of Cryptosporidium parvum and Giardia lamblia infections. However, it has been surmised that nitazoxanide might be useful for the treatment of drug-resistant influenza due to its ability to block viral replication by a novel mechanism. It is presently being studied for a potential role for licenced use as a treatment of acute uncomplicated influenza.

Previous studies have shown no clinically significant modifications of electrocardiogram (ECG) results and no differences in the dosing of nitazoxanide in the fed condition. However, in mind development of new treatments, it was required to conduct thorough QT/QTc study to rule out the possibility of QTc interval lengthening in respect to its use. Hence, a thorough QTc study was necessary to determine the QT interval lengthening in respect to its use.

Methods
This study was designed as a double-blind, randomised, placebo-controlled, single-blind, cross-over study with healthy male and female subjects to evaluate the cardiovascular safety profile, including rhythm and conduction abnormalities, categorical QT/QTc interval data, and qualitative and quantitative ECG variations from baseline of a therapeutic (675 mg) and a supra-therapeutic (2700 mg) single dose of nitazoxanide compared to placebo, on the mean QTc interval from baseline to under treatment values. A single oral dose of 400 mg moxifloxacin was used as a positive control to confirm assay sensitivity.

Aims
The aim of this study was to characterise the effects of a therapeutic (675 mg) and a supra-therapeutic (2700 mg) single dose of nitazoxanide compared to placebo, on the mean QTc interval from baseline to under treatment values. A single oral dose of 400 mg moxifloxacin was used as a positive control to confirm assay sensitivity.

Statistical Analysis
A linear mixed model with sequence, period, sex and race as fixed effects, and baseline as covariate was adjusted (where nested in sequence) and subject by period interactions as random effects. Two-sided 90% confidence intervals (CIs) for the difference between each dose of nitazoxanide and placebo (safety), and moxifloxacin and placebo (assay sensitivity) were derived. Subjects who had valid ECG data for at least one post-dose measurement during Periods 1-4 were included in the primary analysis set. In accordance with ICH E14, all CIs were required to be completely below 10 ms to show safety. Statistical analysis. CIs were required to be blinded to the values. A time-matched baseline was used in a secondary analysis. The baselines were period specific in order to provide information on period effects and in particular on any possible carryover effects. With respect to correction to heart rate (HR), the method to be used as the primary analysis was determined under blinded conditions based on the "root mean squared squared (RMSSD)" criterion applied to the baseline data. Analyses with other heart rate corrections (TCQ/QT, QT/PR interval) and QTcB were estimated, and the most appropriate QT and QTC as well as those with a time-matched baseline were considered secondary.

Results

QT/QTc Analysis
Choice of primary correction
The smallest RMSSD criterion was obtained for QTcB (19.0 ms), while with QTQc a slightly higher value (20.4 ms) was reached. Therefore QTcB was used as the primary correction method. This decision also took into account that, since this correction method does not rely on an estimate derived from the data, QTcQ is likely to provide a more stable result.

Assay Sensitivity
In order to assess the ability of the study to detect clinical significance, moxifloxacin was used as a positive control since it is expected to have an effect on the mean QT/QTc interval of at least 5 ms compared to placebo. The assessment was made up to 24 hours post-dose. The largest change in QTc between 400 mg moxifloxacin and placebo was observed at 3 hours post-dose with a peak value of 11.2 ms (two-sided 90% CI: 8.8, 13.3 ms). All changes observed in the pre-defined window of maximal effect of moxifloxacin were above 6 ms (Panel A, Table 2) and estimates of the lower limit of the CI were well above 5 ms in all cases, confirming assay sensitivity of the study in this trial-case of the Holm procedure.

Table 2: QTc (best correction) Assay sensitivity

<table>
<thead>
<tr>
<th>Time-point</th>
<th>Difference to baseline (ms)</th>
<th>Estimate</th>
<th>90% CI</th>
<th>Lower limit</th>
<th>Upper limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>1.7</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>2.00</td>
<td>0.1</td>
<td>0.050</td>
<td>0.000</td>
<td>0.000</td>
<td>0.100</td>
</tr>
<tr>
<td>3.00</td>
<td>1.9</td>
<td>0.029</td>
<td>0.000</td>
<td>0.000</td>
<td>0.058</td>
</tr>
<tr>
<td>4.00</td>
<td>0.9</td>
<td>0.029</td>
<td>0.000</td>
<td>0.000</td>
<td>0.058</td>
</tr>
<tr>
<td>6.00</td>
<td>1.1</td>
<td>0.029</td>
<td>0.000</td>
<td>0.000</td>
<td>0.058</td>
</tr>
<tr>
<td>12.00</td>
<td>1.0</td>
<td>0.029</td>
<td>0.000</td>
<td>0.000</td>
<td>0.058</td>
</tr>
</tbody>
</table>

Figure 1: Heart rate effect per treatment and time-point

Relationship between HR and increase in diastole
In order to further investigate the modest increase in HR and its possible relationship with gastrointestinal disturbances (diarrhea), summary statistics for HR were repeated by classifying the subjects in the supra-therapeutic dose treatment group by whether they had diarrhea reported in the period corresponding to the respective treatment or not. The results suggest that the supra-therapeutic dose increase in HR may be correlated to diarrhea. There were also a few (albeit significant) differences, but those subjects with diarrhea showed a slightly greater increase from pre-dose values (Panel C, Figure 2).

Discussion
The results of this thorough QTc study show that nitazoxanide and its active metabolite (tizoxanide) have no effect on cardiac repolarisation. The largest change from average baseline in QTc between 675 mg nitazoxanide and placebo was 1.6 ms (two-sided 90% CI: -0.3, 3.5) and for 2700 mg nitazoxanide and placebo was 3.4 ms (two-sided CI: 1.4, 5.4 ms). The PNOQTc analysis showed that with increasing plasma concentration of tizoxanide and tizoxanide glucuronide there was a slight decrease in the QTc. In order to assess the ability of the study to detect clinical significance, 400 mg moxifloxacin was used as a positive control. The estimated difference for QTcB was 11.6 ms with 90% CI of between 8.8 and 13.3 ms, which is in agreement with published literature and confirms the assay sensitivity of the study in detecting clinically significant QTc changes. The study was powered to show a statistically significant result based on a formula, sample size calculation in which to achieve a power of 80% (α= 0.05) it required the inclusion of 56 subjects.

A slight increase in baseline corrected average heart rate (3Hz) was observed for all active treatments but not placebo. The change was dose dependent and therefore most pronounced (up to 3 beats per min) for subjects in the supra-therapeutic 2700 mg nitazoxanide treatment group between 2% and 6 hours post dose. This may well be associated with nausea and/or diarrhoea frequently observed with high doses. Those in the therapeutic 675 mg nitazoxanide treatment group showed only a minimal change from pre-dose baseline which clearly is not clinically relevant.

References