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

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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-acetyloxy-N-(5-nitro-2-thiazolyl) benzamide, also known as PH-5776, NTZ or Alinia® (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[1]. It is presently undergoing global Phase III clinical studies required for licensure as a treatment of acute uncomplicated influenza.

Previous studies have shown no clinically significant modifications of electrocardiogram (ECG) results including QTc measurements after dosing of nitazoxanide in the fed condition. However, having in mind development of new treatments, it was required to conduct thorough QT (TQT) study to make complete the cardiac safety information about the drug in accordance with the ICH E14 guidelines. The presented double-blind, placebo-controlled TQT study, beside the therapeutic dose, included administration of a supra-therapeutic dose - for this dose to be several times the anticipated therapeutic dose without exposing the subjects to any undue risk whilst exploring the exposure and QT interval relationship.

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.

Methods

This study was designed as a double-blind, randomised, placebo-controlled, single dose, cross-over study in 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. Fifty six (56) subjects participated in the study and attended for screening, four treatment periods (Periods 1-4) and a follow-up visit scheduled 7-14 days after Day 1 of Period 4 (Table 1).

Table 1: Summary of Study Design

Period 1			Period 2			Period 3			Period 4	
Day −1	Day 1	Washout* ≥10 days	Day −1	Day 1	Washout* ≥10 days	Day −1	Day 1	Washout* ≥10 days	Day −1	Day 1
P	P		P	M		P	TD		P	SD
	M			TD			SD			P
	TD			SD			P			M
	SD			P			M			TD

P=Placebo, M=moxifloxacin, TD=therapeutic dose, SD =supra-therapeutic dose
* At least a 10 day wash-out interval between study drug administrations

Statistical Analysis

A linear mixed model with sequence, period, sex gender and race, time and time by treatment interaction as fixed effects, and baseline as covariate was adapted, with subject (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. All subjects who had valid ECG data for at least one post-dosing time-point 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. To show assay sensitivity, CIs were required to be above 5 ms for the results of the 2, 2.5, 3 and 4 hours time-points of the difference between placebo and positive control (moxifloxacin). To account for multiplicity, a Hommel procedure was applied to these 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 slope (RMSS)" criterion applied to the baseline data[2]. Analyses with other heart rate corrections (QTcIL/QTcIP/QTcF/QTcB - excluding the most appropriate) and QT as well as those with a time-matched baseline were considered secondary.

Results

QT/QTc Analysis

Choice of primary correction

The smallest RMSS criterion was obtained for QTcF (19.0 ms/s), while with QTcIP a slightly higher value (20.4 ms/s) was reached. Therefore QTcF 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, QTcF 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 QTcF between 400 mg moxifloxacin and placebo was observed at 3 hours post-dose with a peak value of 11.0 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 8 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 trivial case of the Hommel procedure.

A Table 2: QTcF (best correction) Assay sensitivity

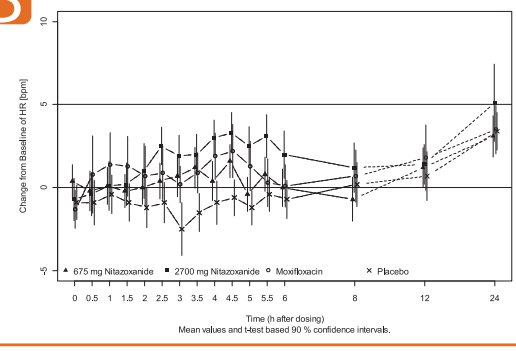
Time-point	Difference to time-matched Placebo			p-value	
	Estimate	90 % CI		Raw	Corrected
		Lower	Upper		
02:00	8.7	6.6	10.8	0.002	0.002
02:30	9.7	7.7	11.7	0	0
03:00	11	8.8	13.3	0	0
*04:00	11	9.1	12.9	0	0
*03:30	10.9	9.1	12.7	0	0

* Time-points are ordered by raw p-values

Heart Rate Effect

The HR analysis revealed that the supra-therapeutic dose (2700 mg) had an effect on HR (Panel B, Figure 1). HR was observed to have increased by about 3 bpm 4 hours after dose with the 2700 mg nitazoxanide treatment group when compared to the 675 mg nitazoxanide and moxifloxacin treatment groups where only marginal changes could be observed. The placebo treatment led to no change or a slight reduction. All groups showed the greatest change in heart rate at the 24 hour time point.

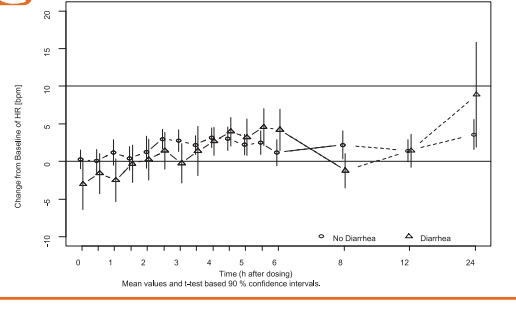
B Figure 1: Heart rate effect per treatment and time-point



Relationship between HR and increase in diarrhea

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 at the supra-therapeutic dose the increase in HR may be correlated to diarrhea. There were no statistically significant differences, but those subjects with diarrhoea showed a slightly greater increase from pre-dose values (Panel C, Figure 2).

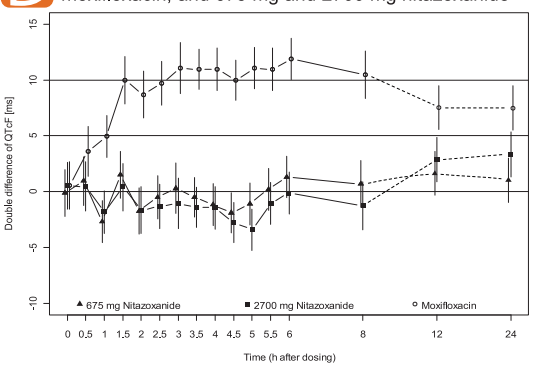
C Figure 2: Supra-therapeutic Dose – Grouped by occurrence of diarrhoea



Mean QTcF difference to placebo of change from average baseline

The effect of 675 mg and 2700 mg nitazoxanide on QTcF was demonstrated by first subtracting the average baseline QTc of the baseline day preceding each of the study days for all four treatments and then by subtracting placebo from the other three treatments. The average baseline was defined as the mean of time-points on the baseline (Day -1). The effect of 675 mg, 2700 mg nitazoxanide (test) and moxifloxacin (control) on QTcF is shown in Panel D (Figure 3). Of note, the greatest effect with moxifloxacin was seen at the 6 hour time-point. This is an expected finding as previously we have shown this occurs when taking moxifloxacin with food[3,4].

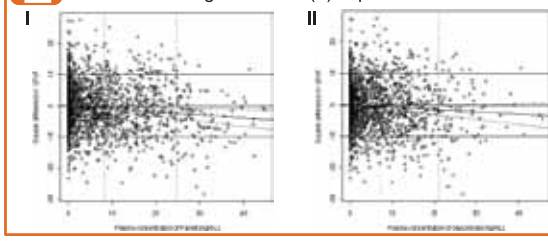
D Figure 3: Changes in QTcF after dosing with 400 mg moxifloxacin, and 675 mg and 2700 mg nitazoxanide



QTc versus PK Analysis

An analysis of the relationship between QTcF and plasma concentration of tizoxanide (Panel E, Figure 4**I**) and tizoxanide glucuronide (Figure 4**II**) shows that with increasing plasma concentration, there was a slight corresponding decrease in QTcF.

E Figure 4: Relationship between QTcF and tizoxanide (**I**) and tizoxanide glucuronide (**II**) in plasma



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 QTcF between 675 mg nitazoxanide and placebo was 1.6 ms (two-sided 90% CI: -0.3, 3.6) and for 2700 mg nitazoxanide and placebo was 3.4 ms (two-sided CI: 1.4, 5.4 ms). The PK/QTc analysis showed that with increasing plasma concentration of tizoxanide and tizoxanide glucuronide there was a slight decrease in the QTcF. 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 QTcF was 11.0 ms with 90% CI of between 8.8 and 13.3 ms, which is in agreement with published literature[2] and confirms the assay sensitivity of the study in detecting clinically significant QT/QTc changes. The study was powered to show a statistically significant result based on a formal 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 day corrected average heart rate (ΔHR) was observed for all active treatments but not placebo. The change was dose dependent and therefore most pronounced (up to 3 beats per minute) for subjects in the supra-therapeutic 2700 mg nitazoxanide treatment group between 2½ 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

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