Reflux disease and achalasia: Failure of the gatekeeper
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Chapter 5

Randomized clinical trial: effect of a novel metabotropic glutamate receptor 5 antagonist (AZD2066) on transient lower esophageal sphincter relaxations and reflux episodes in healthy volunteers

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SUMMARY

Background:
Selective metabotropic glutamate receptor 5 (mGluR5) antagonists inhibit transient lower esophageal sphincter relaxations (TLESRs) in animals and acid reflux in humans.

Aim:
To assess the effect of single doses of the mGluR5 antagonist AZD2066 on TLESRs and reflux in humans.

Methods:
Healthy male volunteers received AZD2066 13 mg and placebo (part A), or AZD2066 2 mg and AZD2066 6 mg and placebo (part B), in a randomized crossover study. Postprandial manometry/pH-impedance measurements were taken after each dose.

Results:
13 individuals completed part A of the study and 19 individuals completed part B. There was a significant reduction in the geometric mean number of TLESRs (27%; \( P = 0.02 \)) and the geometric mean number of reflux episodes (51%; \( P = 0.01 \)) in subjects receiving AZD2066 13 mg compared with placebo. Adverse events in participants receiving AZD2066 13 mg were mostly related to the nervous system (dizziness [3/13]; disturbance in attention [3/13]). Adverse events were reversible and of mild intensity. There were no serious adverse events. The effects of AZD2066 appeared dose-dependent, with smaller reductions in TLESRs and reflux episodes (relative to placebo) and fewer adverse events observed for AZD2066 2 mg and AZD2066 6 mg compared with AZD2066 13 mg.

Conclusions:
mGluR5-mediated inhibition of TLESRs may be a useful approach for inhibiting gastroesophageal reflux.
INTRODUCTION

Gastroesophageal reflux disease (GERD) is a chronic disease caused by reflux of gastric contents into the esophagus.\textsuperscript{1-3} In Western countries, approximately 10–20% of adults are thought to be affected by GERD symptoms, with lower rates of around 5% reported in Asian populations.\textsuperscript{4, 5} The mainstay of GERD treatment is acid suppression, most commonly achieved with the use of proton pump inhibitors (PPIs).\textsuperscript{5-8} However, there is mounting evidence that reflux symptoms persist in a high proportion (20–30%) of patients despite PPI therapy,\textsuperscript{9-10} and that they are associated with decreased psychological and physical well-being.\textsuperscript{11}

Transient lower esophageal sphincter relaxations (TLESRs) are considered to be a major mechanism for generating reflux in healthy individuals and in patients with GERD.\textsuperscript{12, 13} Pharmacological inhibition of TLESRs is a promising new therapeutic approach because it has the potential to reduce most types of reflux. This is particularly relevant for patients who have troublesome reflux symptoms despite acid suppression from PPI use, because weakly acidic and weakly alkaline reflux are thought to play a role in symptom generation in this group.\textsuperscript{14} TLESRs are triggered in response to the activation of stretch receptors in the stomach,\textsuperscript{15} and are thought to be mediated by a vago-vagal reflex pathway.\textsuperscript{16, 17} The gamma-aminobutyric acid type B (GABA\textsubscript{B}) receptor acts at several points along this pathway, and recent clinical studies demonstrate that inhibition of TLESRs using the GABA\textsubscript{B}-receptor agonist lesogaberan reduces the number of reflux episodes and symptoms.\textsuperscript{18, 19} Inhibition of TLESRs may also be achieved via other pathways. In particular, metabotropic glutamate receptor 5 (mGluR5) expressed in the soma of gastric vagal afferents appears to play an important role in triggering TLESRs.\textsuperscript{20} Selective antagonists of mGluR5 have been shown to inhibit TLESRs in animal studies\textsuperscript{21, 22} and to reduce acid reflux and improve clinical symptoms in patients with GERD.\textsuperscript{23} However, the effect of mGluR5 inhibition on the number of reflux episodes has not been assessed in humans in relation to TLESR frequency.

AZD2066 is a novel chemical substance that has been pharmacologically characterized as a selective, non-competitive antagonist of mGluR5, and is being evaluated for the treatment of neuropathic pain, depression and GERD. The primary objective of this pharmacodynamic study was to measure the effect of AZD2066 on the frequency of TLESRs in healthy volunteers. Secondary objectives included assessing the effect of AZD2066 on the frequency of TLESRs, number and type of reflux episodes, esophageal acid exposure, lower esophageal sphincter (LES) pressure and number of swallows, and to determine its pharmacokinetic characteristics and safety and tolerability profile.
METHODS

Study participants
To be enrolled, study participants were required to be healthy men, aged 18–45 years, with a body mass index (BMI = weight/height²) of 19–30 kg/m². Full details of the inclusion/exclusion criteria implemented during the study are presented in supplementary Table 1. At the 21-day pre-entry visit, each subject had a medical examination which included a full laboratory screen, a physical examination (general appearance, cardiovascular, lungs, abdomen, skin, head and neck, lymph nodes, thyroid, musculoskeletal/extremities and reflexes), a 12-lead resting ECG recording, blood pressure measurement and a pregnancy test for females. Tests for Hepatitis B and C, HIV and drug abuse were also performed at the pre-entry visit, and participants also underwent a structured psychiatric interview using the Mini International Neuropsychiatric Interview. Participants had to have clinically normal electrocardiogram, pulse and blood pressure measurements and laboratory values at the pre-entry visit, and normal laboratory values before administering the first dose at the second visit.

This study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Guidelines for Good Clinical Practice, and was approved by the Institutional Review Board associated with the study centre (Department of Gastroenterology, Academic Medical Centre, Amsterdam, Netherlands). Participants provided written informed consent prior to the study. This study is registered on a publicly accessible website (ClinicalTrials.gov Identifier: NCT00813306).

Study design and study drugs
This was a double-blind, randomized, single-centre, phase 1, crossover study (2008–2009) conducted in two parts, each involving a different set of participants. Part A assessed the impact of AZD2066 on gastroesophageal sphincter function and reflux variables using a single dose (13 mg) considered to have an acceptable safety and tolerability profile, based on an interim analysis of a multiple ascending dose study (study code: DO475C00002). Each participant received 130 mL of 0.1 mg/mL oral AZD2066 (13 mg) solution and 130 mL of oral placebo solution, each on different days separated by a 7–28-day washout period (Figure 1, study part A).

Part B of the study explored the dose–response relationship for AZD2066 using two lower single doses (2 mg and 6 mg). Each participant received 60 mL of 0.1 mg/mL oral AZD2066 (6 mg) solution, 20 mL of 0.1 mg/mL oral AZD2066 (2 mg) solution made up to 60 mL with oral placebo solution and 60 mL oral placebo solution each on different days. A washout period of 7–28 days was initially planned for both parts of the study. However, in part A of the study, AZD2066 (5–6 nmol at Cmax) was detected in the plasma of 2 subjects in the placebo treated group after treatment.
with AZD2066 and a washout period of 8 days. The washout period between each treatment in part B of the study was thus increased to 10–28-days (Figure 1, study part B).

In both parts of the study, the first dose (AZD2066 or placebo) was administered ≤21 days after the pre-entry visit. Food and fluids were not allowed after midnight on the night before dose administration. Participants received a standardized breakfast (700 kcal, of which 219 kcal [31.3%] were from fat) 30 min after administration of AZD2066 or placebo, which was to be consumed within 15 min. Esophageal manometric and pH-impedance measurements were collected from participants (while in a semi-recumbent position) for 3 h 45 min, so that 3 h of data collection occurred in the postprandial period. Participants also had a follow-up visit 5–7 days after the last study period.

**Assignment and blinding**

The principal investigator (GB) assigned individuals to unique enrolment numbers, determined subject eligibility, and assigned eligible participants to unique randomization numbers. A randomized dosing regimen allocation list was generated by AstraZeneca R&D Mölndal using a validated computer program, and the randomization was performed within blocks of consecutive patient numbers. The study was double-blinded so that both investigators and participants were unaware of who received which dosing regimen. Treatment codes were not broken until the evaluability of the data from each participant had been established and documented.

**Pharmacodynamic assessments**

Manometric recordings of the pharynx, esophagus, LES and stomach were obtained using a perfused 12-channel silicone rubber assembly (DentSleeve International Ltd, Mississauga, Ontario,
Canada). The manometric catheter was positioned with the proximal border of the sleeve 1 cm above the LES. Manometric readings from dosing until the end of the 3-h postprandial period were used to assess the following.

i) Number of TLESRs, defined according to previously described criteria, and their time of onset (primary outcome in relation to AZD2066 13 mg; secondary outcome in relation to AZD2066 2mg and AZD2066 6 mg).

ii) LES pressure (recorded every 15 min and expressed as the mean difference between the end expiration LES pressure and the end-expiration intragastric pressure over 1 min). Mean LES pressure during the 3-hour postprandial period was calculated from measurements taken 15 min after dose administration and then every 15 min until 3 h after the meal (3 h 45 min in total) (secondary outcome).

iii) Number of swallows (defined as a fast increase in pressure in the pharyngeal channel, clearly distinguishable from baseline activity) (secondary outcome).

For the impedance-pH readings, a catheter, comprising several electrode pairs measuring intraluminal impedance, and an antimony pH sensor were used. The catheter was positioned so that the pH sensor was located 5 cm above the LES. Impedance-pH readings from dosing until the end of the 3-h postprandial period were used to assess the following:

i) Number of reflux episodes, defined as a period when impedance decreased to below 50% of baseline (liquid episode) or increased to above 150% of baseline (gas episode), propagating aborally from the most distal channel (secondary outcome).

ii) Number of reflux episodes that were acidic (intraesophageal pH <4 [or a drop of ≥1 pH unit if pH is already <4] lasting more than 5 s), weakly acidic (intraesophageal pH 4.0–6.5 lasting more than 5 s) and weakly alkaline (intraesophageal pH ≥6.5 lasting more than 5 s) (secondary outcome).

iii) Percentage of time with an esophageal pH <4 (secondary outcome).

Pharmacokinetic, safety and tolerability assessments
Blood samples (2 mL) were collected at frequent intervals for 12 h after each dose of AZD2066 to determine the pharmacokinetic profile (secondary outcome). Plasma AZD2066 concentrations were determined by liquid chromatography and mass spectrometry (quantification limit: 1.00 nmol/L). Adverse events occurring in the first 24 h after dose administration were considered to be during active dosing.
Statistical analysis

For part A of the study, it was determined that complete data from a minimum of 10 subjects would be required to detect a true effect of a 50% reduction in the number of TLESRs (primary variable). This was based on using a one-sided t-test at a significance threshold of 5%, with 90% power and a within subject standard deviation of 0.5 for the difference (placebo versus AZD2066) in the log number of TLESRs. The null hypothesis \( H_0 \) was that the difference in the log number of TLESRs is ≤ 0. This was tested against the alternative hypothesis \( H_1 \) that the difference is > 0. It was determined that at least 14 subjects would need to be enrolled to ensure that a minimum of 10 subjects would complete the study. The results of part A of the study revealed the true effect of AZD2066 on TLESR frequency to be lower than anticipated (i.e. there was a 27% reduction in the number of TLESRs). Based on detecting a true effect of a 27% reduction in the number of TLESRs, it was calculated that at least 18 participants would need to complete part B of the study to provide adequate statistical power based on the same parameters used in part A. It was determined that at least 22 subjects would need to be enrolled to ensure this level of completion.

Pharmacodynamic and pharmacokinetic variables were analysed for all participants not affected by major protocol violations (per protocol data). Major protocol violations were defined as those that would have lead to concerns over the validity of the analysis and included: entering the study despite not meeting study entry criteria; continuing the study despite meeting criteria for being withdrawn; receiving the incorrect treatment or dose; taking prohibited concomitant medications during the study and severe non-compliance with the study protocol. All participants who received at least one dose of AZD2066 or placebo and for whom post-dose information was available were included in the safety analysis.

Geometric means were used to compare treatment effects where possible because this method of calculating the mean is less biased by extreme values than the arithmetic mean. Mean total differences in the effect of AZD2066 and placebo could only be estimated as geometric mean ratios if data were able to be log-transformed i.e. if the value of the variable being assessed was ≥ 1 for every patient. Values for LES pressure and the number of TLESRs, reflux episodes and swallows that occurred during the 0–3 hour postprandial period were able to be log-transformed in the analysis. Analyses for log-transformable data were performed using analysis of variance (ANOVA) with treatment, period and sequence as fixed effects, and participant’s ID as a random effect. Carry-over effects were not specifically analysed because they cannot be distinguished from treatment, sequence and period effects in 2 x 2 crossover studies, and only 3 of these factors can be assessed at the same time in the ANOVA model (i.e. to estimate treatment, sequence and period effects it must be assumed that the carry-over effect is zero). However, in line with recommendations, the washout period was based on the pharmacokinetics profile of AZD2066.
and sequence and period effects were accounted for as a proxy for carry-over effects (i.e. carry-over effects are unlikely if sequence and period effects are not detected). 25

Confidence intervals (CIs) for the true mean were calculated in the logarithmic scale based on the mean square error obtained in the ANOVA. The limits were transformed back to the original scale to give a CI for the ratio of geometric means between dose regimens. The number of acidic, weakly acidic, pure liquid and mixed gas/liquid reflux episodes that occurred in the 0–3 hour postprandial period, and values for the % time with esophageal pH <4, were not able to be log-transformed (i.e. not all values in each dataset were ≥ 1). These variables were thus compared between dose regimens in terms of arithmetic mean differences for which 95% CIs were calculated using Student’s t-distribution.

RESULTS

Participant flow and follow-up
A total of 16 participants enrolled in part A of the study, of whom three did not meet the inclusion, exclusion and/or screening criteria (described above) at the pre-entry visit. The remaining 13 participants were randomized to the dose regimens and all 13 completed part A. Of 24 participants enrolled in part B, two did not meet the inclusion, exclusion and/or screening criteria the pre-entry visit. The remaining 22 participants were randomized to the dose regimens. Two discontinued after the first treatment visit in part B (included in the safety analysis only): one because of severe non-compliance and one because of involvement in the conduct of the study. Another participant discontinued before administration of the first dose because of a fainting episode.

Impedance data for one patient (required to calculate the number of TLESRs and reflux episodes) were excluded from the AZD2066 6 mg analysis owing to poor quality tracings.

Participants’ characteristics at baseline
All 13 participants that completed part A of the study were white men. The mean age of the group was 22.1 years (range, 19–27 years). The mean BMI was 22.4 (Standard deviation [SD]: 2.1) and the median BMI was 22.6 (range, 19.2–26.6). Of the 19 participants who completed part B of the study, 18 were white men and one was a black man. The mean age of the group was 25.5 years (range, 18–32 years). The mean BMI was 24.2 (SD: 2.1) and the median BMI was 24.1 (range, 20.7–28.7). All participants were judged as being healthy at study enrolment based on normal results from the physical examination and investigations.
Analysis

Pharmacodynamic results: effect of AZD2066 on TLESRs

There was substantial variation between participants in terms of the number of TLESRs that occurred in both Part A (Figure 2A) and part B (Figure 3A) of the study. A significant 27% reduction was observed in the geometric mean number of postprandial TLESRs that occurred when participants received AZD2066 13 mg compared with when they received placebo (Table 1). At the individual time points, the greatest reductions in the number of TLESRs were observed 0–1 h and 1–2 h after food intake for AZD2066 13 mg relative to placebo (Figure 2B). In part B of the study, no significant reduction in the mean number of TLESRs was observed during the 0–3-h postprandial period when participants received AZD2066 2 mg (Table 2) or AZD2066 6 mg (Table 3) compared with when they received placebo, and this was reflected across all of the individual time points (Figure 3B).

Figure 2

Figure 2 | Number of TLESRs with 13 mg AZD2066 or placebo (A) presented as individual patient data for the 0–3 hours after the standardized meal consumed 45 min after dose administration (B) presented as geometric means for individual time periods before and after the standardized meal consumed 45 min after dose administration. Error bars show 95% confidence intervals.
### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>AZD2066 13 mg</th>
<th>Geometric mean ratio (95% CI)</th>
<th>( P ) value</th>
</tr>
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<tbody>
<tr>
<td>Number of TLESRs (n = 13)</td>
<td>19.0</td>
<td>14.0</td>
<td>0.73 (0.53, 1.00)</td>
<td>0.02</td>
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<tr>
<td>Number of reflux episodes (n = 13)</td>
<td>13.0</td>
<td>6.2</td>
<td>0.49 (0.29, 0.85)</td>
<td>0.01</td>
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<tr>
<td>LES pressure (n = 13)</td>
<td>9.8</td>
<td>10.0</td>
<td>1.05 (0.83, 1.32)</td>
<td>0.33</td>
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<tr>
<td>Number of swallows (n = 13)</td>
<td>233.0</td>
<td>242.0</td>
<td>0.92 (0.77, 1.10)</td>
<td>0.34</td>
</tr>
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</table>

### Arithmetic mean difference (95% CI) \( P \) value

<table>
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<th></th>
<th>Arithmetic mean difference (95% CI)</th>
<th>( P ) value</th>
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<tr>
<td>Number of acid reflux episodes (n = 13)</td>
<td>8.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Number of weakly acidic reflux episodes (n = 13)</td>
<td>4.1</td>
<td>3.5</td>
</tr>
<tr>
<td>Pure liquid reflux episodes (n = 13)</td>
<td>8.7</td>
<td>6.6</td>
</tr>
<tr>
<td>Mixed gas/liquid reflux episodes (n = 13)</td>
<td>4.4</td>
<td>2.4</td>
</tr>
<tr>
<td>% time with esophageal pH &lt;4 (n = 13)</td>
<td>2.5</td>
<td>3.7</td>
</tr>
</tbody>
</table>

TLESR, transient lower esophageal sphincter; LES, lower esophageal sphincter; CI, confidence interval

**Figure 3** | Number of reflux episodes with 13 mg AZD2066 or placebo (A) presented as individual patient data for the 0–3 hours after the standardized meal consumed 45 min after dose administration (B) presented as geometric means for individual time periods before and after the standardized meal consumed 45 min after dose administration. Error bars show 95% confidence intervals. Pharmacodynamic results: effect of AZD2066 on reflux episodes
Effect of AZD2066 on TLESRs and reflux | Chapter 5

<table>
<thead>
<tr>
<th>Geometric mean</th>
<th>Placebo</th>
<th>AZD2066 6 mg</th>
<th>Geometric mean ratio (95% CI)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Number of TLESRs (n = 18)</td>
<td>16.0</td>
<td>16.0</td>
<td>0.98 (0.82, 1.17)</td>
<td>0.41</td>
</tr>
<tr>
<td>Number of reflux episodes (n = 16)</td>
<td>13.0</td>
<td>7.9</td>
<td>0.81 (0.60, 1.10)</td>
<td>0.08</td>
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<tr>
<td>LES pressure (n = 19)</td>
<td>12.0</td>
<td>12.0</td>
<td>0.96 (0.79, 1.18)</td>
<td>0.65</td>
</tr>
<tr>
<td>Number of swallows (n = 19)</td>
<td>212.0</td>
<td>206.0</td>
<td>0.97 (0.82, 1.16)</td>
<td>0.74</td>
</tr>
<tr>
<td>Arithmetic mean</td>
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<td></td>
</tr>
<tr>
<td>Number of acid reflux episodes (n = 16)</td>
<td>7.8</td>
<td>6.1</td>
<td>-1.74 (-4.45, 0.96)</td>
<td>0.10</td>
</tr>
<tr>
<td>Number of weakly acidic reflux episodes (n = 16)</td>
<td>3.3</td>
<td>3.5</td>
<td>0.18 (-1.94, 2.30)</td>
<td>0.57</td>
</tr>
<tr>
<td>Pure liquid reflux episodes (n = 16)</td>
<td>5.2</td>
<td>6.3</td>
<td>1.09 (-1.38, 3.55)</td>
<td>0.81</td>
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<td>Mixed gas/liquid reflux episodes (n = 16)</td>
<td>6.1</td>
<td>3.4</td>
<td>-2.63 (-5.03, -0.23)</td>
<td>0.02</td>
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<tr>
<td>% time with esophageal pH &lt;4 (n = 18)</td>
<td>3.8</td>
<td>6.3</td>
<td>2.54 (-0.84, 5.92)</td>
<td>0.14</td>
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</table>

TLESR, transient lower esophageal sphincter; LES, lower esophageal sphincter; CI, confidence interval

Table 2 | Pharmacodynamic effects of AZD2066 6 mg compared with placebo 0–3 h after food intake (study part B).

<table>
<thead>
<tr>
<th>Geometric mean</th>
<th>Placebo</th>
<th>AZD2066 2 mg</th>
<th>Geometric mean ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of TLESRs (n = 19)</td>
<td>16.0</td>
<td>16.0</td>
<td>0.99 (0.83, 1.18)</td>
<td>0.45</td>
</tr>
<tr>
<td>Number of reflux episodes (n = 19)</td>
<td>13.0</td>
<td>11.0</td>
<td>0.87 (0.61, 1.26)</td>
<td>0.23</td>
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<tr>
<td>LES pressure (n = 19)</td>
<td>12.0</td>
<td>14.0</td>
<td>1.15 (0.94, 1.40)</td>
<td>0.08</td>
</tr>
<tr>
<td>Number of swallows (n = 19)</td>
<td>212.0</td>
<td>225.0</td>
<td>1.06 (0.89, 1.25)</td>
<td>0.50</td>
</tr>
<tr>
<td>Arithmetic mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of acid reflux episodes (n = 19)</td>
<td>7.8</td>
<td>7.7</td>
<td>-0.12 (-2.64, 2.41)</td>
<td>0.46</td>
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<tr>
<td>Number of weakly acidic reflux episodes (n = 19)</td>
<td>3.3</td>
<td>3.7</td>
<td>0.37 (-1.61, 2.36)</td>
<td>0.65</td>
</tr>
<tr>
<td>Pure liquid reflux episodes (n = 19)</td>
<td>5.2</td>
<td>7.4</td>
<td>2.26 (-0.04, 4.57)</td>
<td>0.97</td>
</tr>
<tr>
<td>Mixed gas/liquid reflux episodes (n = 19)</td>
<td>6.1</td>
<td>4.0</td>
<td>-2.06 (-4.32, 0.19)</td>
<td>0.04</td>
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<tr>
<td>% time with esophageal pH &lt;4 (n = 18)</td>
<td>3.8</td>
<td>5.1</td>
<td>1.30 (-1.94, 4.55)</td>
<td>0.42</td>
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</table>

TLESR, transient lower esophageal sphincter; LES, lower esophageal sphincter; CI, confidence interval

Table 3 | Pharmacodynamic effects of AZD2066 2 mg compared with placebo 0–3 h after food intake (study part B).

There was also substantial variation in the total number of reflux episodes that occurred during both parts of the study (Figure 4A and Figure 5A). There was a significant 51% reduction in the geometric mean number of reflux episodes that occurred during the 0–3-h postprandial period when participants received AZD2066 13 mg compared with when they received placebo (Table 1). The 13 mg dose of AZD2066 appeared to reduce the number of reflux episodes relative to placebo both before and after food intake, with the greatest effects observed at the 1–2- and 2–3-h postprandial time points (Figure 4B).
The initial analysis of data from part B of the study showed a significant 39\% reduction in the geometric mean number of reflux episodes during the 0–3-h postprandial period for AZD2066 6 mg compared with placebo (geometric mean ratio, 0.61; 95\% CI, 0.41–0.90; \( P = 0.01 \)). However, closer inspection revealed that this effect was mainly driven by two high responders. Exclusion of these outliers changed the estimated mean reduction in reflux episodes for AZD2066 6 mg to a non-significant decrease of 19\% compared with placebo (Table 2). Compared with when they received placebo, participants did not have a significant reduction in the geometric mean number of postprandial reflux episodes when they received AZD2066 2 mg (Table 3). The mean number of reflux episodes that occurred at the individual time points for AZD2066 6 mg, AZD2066 2 mg and placebo are presented in Figure 5B.
Pharmacodynamic results: effect of AZD2066 on different types of reflux

In part A of the study, the significantly lower geometric mean number of reflux episodes that occurred when participants received AZD2066 13 mg compared with when they received placebo was largely accounted for by a statistically significant reduction in the mean number of acid reflux episodes (Table 1). The mean number of acid reflux episodes was not significantly reduced when participants received AZD2066 6 mg (Table 2) or AZD2066 2 mg (Table 3) compared with when they received placebo, although AZD2066 6 mg reduced the mean number of acid reflux episodes to a larger extent relative to placebo than did AZD2066 2 mg. None of the AZD2066 doses in part A or B of the study significantly reduced the mean number of weakly acidic reflux episodes (Table 2 and Table 3). Analyses were not performed for weakly alkaline reflux episodes owing to their infrequent nature.
In part A of the study, the mean numbers of pure liquid and mixed gas/liquid reflux episodes occurring during the postprandial period were lower when participants received AZD2066 13 mg than when they received placebo (Table 1), although these effects were not statistically significant.

In part B of the study, the mean number of mixed gas/liquid reflux episodes that occurred in participants receiving AZD2066 6 mg (Table 2) and AZD2066 2 mg (Table 3) was significantly lower than in those receiving placebo. Pure gas reflux episodes were not analysed owing to the infrequent nature of these events.

Pharmacodynamic results: relationship between TLESRs and reflux episodes
When patients received AZD2066 13 mg in part A of the study, 43% of TLESRs were temporally related to reflux episodes, compared with 56% of TLESRs when they received placebo, although this difference was not statistically significant. In part B, the proportion of TLESRs temporally related to reflux episodes was 56% for AZD2066 2 mg and 48% for AZD2066 6 mg, compared with 55% for placebo.

In part A of the study, the proportion of reflux episodes temporally related to TLESRs was 65% when participants received AZD2066 13 mg compared with 68% when they received placebo. In part B, 73% and 72% of reflux episodes were temporally related to TLESRs when participants received AZD2066 2 mg and AZD2066 6 mg, respectively, compared with 80% when they received placebo. The proportion of acid reflux episodes temporally related to TLESRs was 82.4% when patients received AZD2066 13 mg compared with 86.4% when they received placebo, while the proportion of weakly acidic reflux episodes related to TLESRs was 61.1% for AZD2066 13 mg and 83.7% for placebo.

Pharmacodynamic results: effect of AZD2066 on esophageal pH
In part A of the study, time with esophageal pH <4 was reduced when participants received AZD2066 13 mg compared with when they received placebo, although this effect was not significant (Table 1). In part B of the study, no effect relative to placebo was observed for AZD2066 6 mg (Table 2) and AZD2066 2 mg (Table 3) on time with esophageal pH <4.

Pharmacodynamic results: effect of AZD2066 on LES pressure and swallowing
There were no significant differences relative to placebo in the mean LES pressure or in the mean number of swallows for any of the dose regimens investigated (Tables 1–3).

Sequence, period and carry-over effects
For the reflux variables assessed, no significant sequence or period effects were detected, thus indicating an absence of carry-over effects. A significant sequence effect was detected for AZD2066 13 mg in relation to TLESRs in part A of the study (P = 0.02), indicating that sequence,
period and/or carry-over effects may have occurred. However, the sequence effect was accounted for in the ANOVA model used to calculate the statistical significance of the treatment effect. Furthermore, when results of only the first treatment period were analysed, thus reducing the sample size (AZD2066 13 mg: n = 7; placebo: n = 6) but eliminating potential sequence, period and/or carryover effects, the impact of AZD2066 13 mg on TLESRs was still significant (GMR, 0.49; 95% CI, 0.28–0.88); \( P = 0.02 \).

**Figure 6** | Mean plasma concentration of AZD2066 during the 12 h after taking 2 mg, 6 mg or 13 mg doses of the drug.

<table>
<thead>
<tr>
<th>Part A</th>
<th>Placebo (n = 13)</th>
<th>AZD2066 13 mg (n = 13)</th>
<th>Placebo (n = 20)</th>
<th>AZD2066 6 mg (n = 19)</th>
<th>AZD2066 2 mg (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any adverse event</td>
<td>3</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Serious adverse event</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Discontinuation due to an adverse event</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Dizziness</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Disturbance in attention</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Somnolence</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fatigue</td>
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<td>1</td>
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<td>0</td>
<td>1</td>
</tr>
<tr>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Increase in blood bilirubin</td>
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</tr>
<tr>
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<tr>
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<td>0</td>
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<tr>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Haematuria</td>
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<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Erection increased</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 4** | Number of participants with one or more adverse events during active dosing.
Safety and tolerability assessments

The adverse events experienced during the study are reported in Table 2. All adverse events were mild in intensity and reversible. No serious adverse events were reported during the active dosing periods, and there were no discontinuations due to adverse events.

In part A of the study, eight of the 13 participants reported an adverse event when they received AZD2066 13 mg compared with three when they received placebo. Most of the adverse events reported when participants were receiving AZD2066 13 mg were related to the nervous system, the most common being dizziness (3/13) and disturbance in attention (3/13). Only one nervous system disorder (somnolence) was reported when participants were receiving placebo. In part B of the study, the number of participants reporting adverse events was similar for AZD2066 2 mg (5/20) and AZD2066 6 mg (4/19) compared with placebo (5/20). Headache was the most common adverse event reported during dosing with AZD2066 2 mg and AZD2066 6 mg (3/20 and 3/19, respectively), and the same number of headaches was reported when these participants were taking placebo (3/20).

DISCUSSION

In this randomized, double-blind, crossover study conducted in healthy volunteers, a single oral 13 mg dose of AZD2066 was found to reduce the numbers of TLESRs, reflux episodes and acid reflux episodes significantly compared with placebo.

The overall frequency of TLESRs was greater during the 3-h postprandial period than in the preprandial period, and differences in the number of TLESRs and reflux episodes between AZD2066 and placebo were generally more pronounced during the postprandial period. These observations are consistent with previous findings that TLESRs can be activated by food intake via stretch receptors in the stomach.15

A dose dependent effect was seen with AZD2066: only the AZD2066 13 mg dose significantly reduced the geometric mean number of TLESRs (27%) and geometric mean number of reflux episodes (51%) compared with placebo, and there was a trend towards a greater effect for AZD2066 6 mg than for AZD2066 2 mg. A possible exception to the dose dependent effects of AZD2066 was the counterintuitive observation of a significant reduction relative to placebo in mixed gas/liquid reflux when participants received AZD2066 2 mg and AZD2066 6 mg, but not when they received the higher AZD2066 13 mg dose. However, a similar reduction in the absolute number of mixed gas/liquid reflux episodes relative to placebo was observed for AZD2066 13 mg and AZD2066 6
mg, both of which reduced mixed gas/liquid reflux to a greater extent that AZD2066 2 mg. The lack of a statistically significant effect for AZD2066 13 mg in relation to mixed gas/liquid reflux may have been because of the smaller sample size used in part A of the study (AZD2066 13 mg n = 13) versus part B (AZD2066 2 mg: n = 19; AZD2066 6 mg: n = 16).

This is not the first study to show that pharmacological inhibition of TLESRs reduces the number of reflux episodes in humans. Activation of inhibitory GABA B receptors using baclofen, a drug indicated for spasticity, has been shown to reduce the number of TLESRs and reflux episodes in healthy volunteers and in patients with GERD. 26-28 In more recent studies, inhibition of TLESRs using the novel GABA B agonist lesogaberan reduced the number of reflux episodes in healthy men and in patients with symptomatic GERD despite PPI treatment. 18, 19 The current study is, however, the first to demonstrate that the reduced frequency of reflux episodes previously shown to be associated with inhibition of mGluR5 in humans is related to the ability to reduce the number of TLESRs.

Interestingly, AZD2066 13 mg reduced the geometric mean number of reflux episodes in this study by 51%, despite a reduction in the geometric mean number of TLESRs of only 27%. Indeed, the number of TLESRs temporally related to reflux episodes was reduced (although not significantly) in participants receiving AZD2066 13 mg compared with those receiving placebo (43% vs 56%). Lesogaberan has also been shown to reduce the number of reflux episodes to a greater extent than the number of TLESRs. In the case of lesogaberan, this difference may be partially explained by a concomitant increase in mean LES pressure, which has been shown to reduce reflux via a mechanism that is unrelated to TLESRs. 15 However, AZD2066 was not found to increase LES pressure in the current study, and the mechanism by which AZD2066 reduces the number of TLESRs generating reflux remains unclear. One possibility is that some TLESRs were not detected in the current study. While this is consistent with the observation that only 65–80% of reflux episodes were temporally related to TLESRs, it is difficult to envisage why a smaller proportion of TLESRs would be detected in participants receiving AZD2066 13 mg than in those receiving placebo. It is also possible that inhibition of mGluR5 may influence both the quantity and quality of TLESRs. Thus, in the presence of AZD2066, LES relaxations may occur that meet the criteria for a TLESR but do not generate reflux with sufficient properties to meet the definition of a reflux episode (i.e. a drop in impedance to <50% of baseline). Other risk factors for acid reflux, such as the position of the acid pocket, 29 may also be affected by blocking mGluR5, although to our knowledge no studies have investigated this.

Another interesting observation from this study was that acid reflux, and not weakly acidic or weakly alkaline reflux, was significantly reduced in participants receiving AZD2066 compared with those receiving placebo. A similarly disproportionate effect on acid reflux has been seen for both
lesogaberan and baclofen. It has been postulated that this may be due to the greater effect of these drugs on liquid and mixed liquid/gas reflux compared with pure gas reflux, the former being more often associated with acid reflux. In this study, AZD2066 13 mg reduced liquid and mixed liquid/gas reflux more than pure gas reflux. However, pure gas reflux made up a very small proportion (~6%) of the total number of reflux episodes observed in this study. It therefore seems unlikely that the inability of AZD2066 to inhibit pure gas reflux could account for its lack of effect on weakly acidic reflux, which accounted for approximately one-third of the total number of reflux episodes. There is some evidence to suggest that GABA<sub>B</sub> agonists may also alter the amount and distribution of acid in the stomach. Given that GABA<sub>B</sub> and mGluR5 receptors are both involved in regulating TLESRs, it is conceivable that mGluRS may also help regulate digestive components in the stomach, such as acid. It should also be noted that the proportion of weakly acidic reflux episodes that were unrelated to TLESRs increased from 13.6% when patients received placebo to 38.9% when patients received AZD2066 13 mg. The lack of effect of AZD2066 13 mg on weakly acidic reflux may thus be attributed to an increase in the proportion of these events that occur independently of TLESRs, although the mechanism behind this is unclear.

In conclusion, the novel mGluR5 antagonist AZD2066 reduces the number of postprandial reflux episodes that occur in healthy individuals, and this effect is correlated with a reduction in the frequency of TLESRs. In addition, AZD2066 appeared to have an acceptable safety and tolerability profile in this study. However, it should be noted that the GABA<sub>B</sub>-receptor agonist lesogaberan was previously shown to reduce the geometric mean number of TLESRs in healthy volunteers to a greater extent (36%) than AZD2066 did in the current study. Lesogaberan has since been discontinued owing to a lack of evidence for a clinically significant effect as an add-on to PPI therapy in patients with reflux symptoms despite PPI treatment. Nevertheless, the results of the current study reinforce the importance of TLESRs in generating reflux and provide another potential avenue for the development of novel therapeutic agents for GERD (ClinicalTrials.gov Identifier: NCT00813306).
REFERENCES


