Statistical analysis plan

POLO study: Combined effect of Pregabalin and Oxycodone, and Lacosamide and Oxycodone on breathing: an exploratory study in healthy volunteers

17-01-2024

Trial registration number: P22.044

ClinicalTrials.gov Identifier: NCT05598905

Baseline data

Baseline characteristics such as age, sex, height and weight will be documented and will be summarized using the mean (standard deviation), while non-normally distributed data will be stated by the median (interquartile ranges).

Primary endpoint

The primary endpoint of this study is the extrapolated end-tidal pCO2 at 55 mmHg (7.3 kPa) or VE55 during re-breathing of carbon dioxide. To determine the VE55, subjects inhale a mixture of 7% CO2 and 93% O2 from a 4-6L rebreathing bag to obtain the hypercapnic ventilatory response (HCVR). In this response the end-tidal pCO2 is plotted against ventilation (measured using a pneumotachograph system). Linear regression is then applied to the data using R to derive the slope (Si) and the x-axis intersection (Bi), the latter representing the point at zero ventilation (apnea). The level of breathing is determined using Formula 1, which is centred on an extrapolated end-tidal pCO2 of 55mmHg, abbreviated as VE55 (see also figure 1).

$$VE55 = S \times (55 - B) \quad (1)$$

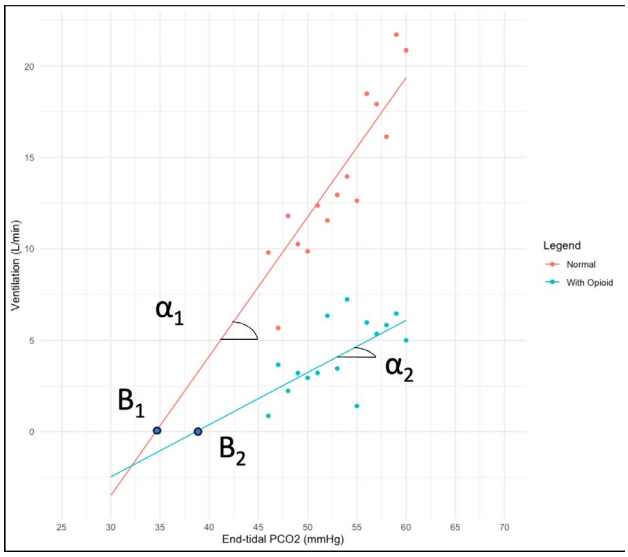

Figure 1: Example of two simulated breathing tests. Slope Si is obtained by taking tan(αi). Lower VE55 values may result from less steep increases (i.e. the blue line) in HCVR interpolations or from higher apnea points (i.e. point B_2) and suggest greater respiratory depression.

Secondary endpoints

The first secondary endpoint is the decrease of nociception, which will be quantified using 3 different pain tests (electrical threshold, electrical tolerance, pressure pain). The electrical pain threshold and tolerance are measured using a locally designed and manufactured transcutaneous electrical stimulation device (CICS, Leiden University Medical Center, Leiden, The Netherlands), able to create a constant current between 0 and 128 mA. Participants will be instructed to press a button when the stimulus becomes painful (electrical pain

threshold) and when the stimulus is perceived as too painful (electrical pain tolerance), also ending the stimulus. The electrical tests will be performed three times per timepoint, and a mean score will be calculated. The pressure pain will be measured using the Wagner Instruments DFN 200 Algometer. A pressure stimulus will be applied on the skin area (1cm2) between the thumb and index finger. Participants will indicate when the pressure stimulus becomes painful (pain threshold). The pressure tests will be carried out four times, twice on each hand, and the mean score will be calculated.

Other secondary endpoints include the level of sedation and the occurrence of nausea which will be queried using an NRS scale from 0 - 10 (0 = no effect, 10 = most severe effect). The occurrence of vomiting will be counted. The pupil diameter will be measured using a handheld pupillometer (Neuroptics PLR-3000 pupillometer).

Statistical principles

Data with a normal distribution will be summarized using the mean (standard deviation), while non-normally distributed data will be conveyed through the presentation of the median (interquartile ranges). The primary and secondary endpoints are repeatedly measured for the same patients across multiple visits, with each visit involving a different intervention. This constitutes a repeated measurement design. Given that measurements from the same patient tend to be related, it is important to account for the “participant effect”. To address this, a Linear Mixed Model (LMM) will be used, which extends the traditional linear model, when the assumption of observation independence is not met. Furthermore, the LMM approach ensures that the estimates derived remain unbiased despite the presence of data said to be missing (not) at random and it allows for the inclusion of random effects, which are essential in accounting for individual differences among participants. The data analysis will be performed using the statistical software R [1], specifically employing the ’nlme’ package [2] for model fitting. A significance level of 5% will be used for all tests. No adjustment of p-values will be made for multiple testing for secondary endpoint analyses.

Data handling

Potential outliers will be investigated. They will be excluded only if there is reasonable justification to assume discrepancies in the data. Upon completion of the study, the data will be cleaned and validated. Subsequently, the data set will be locked to prevent any alterations.

Missing data

Every participant will visit the research facility multiple times. At every visit, participants will be under strict supervision for the entire procedure, so all possible missing data will be accounted for. The LMM approach ensures that the estimates derived remain unbiased despite the presence of missing data.

Statistical analysis

Sequence randomization

The study uses a three-arm structure design, but the patients are randomized only for the first two visits. To check that the third visit can still be considered valid, regardless of the subjects being not randomized, a LMM, specified in Formula 2, can be used to check if there is any significant difference between the first two visits and the third one.

$$VE55_{i,j} = \beta 0 + \beta 1 sequence1_{,j} + bi + \varepsilon i \quad (2)$$

with:
i = 1, ..., 12: denotes the patient of sequence 1 (12 patients will be included in sequence 1, the remaining 12 ones will be included in sequence 2);

j = yes, no: binary variable that denotes the visit (’yes’ for the visits where randomization took place (visit 1 or visit 2, considered as baseline values) or ’no’ for the visit where randomization did not happen, so visit 3;

$VE55_{i,j}$: represents the observed response or dependent variable for the i-th subject of a j-th level of randomization at baseline (measurement = 1);

β0, β1: represent the fixed-effect coefficients associated with the intercept (in this case it captures the effect of the visits with randomization) and the effect of the third visit with no randomization, respectively;

$bi \sim N(0, \sigma^2 b)$: denotes the deviation of the intercept of subject i from the population intercept β0

$\varepsilon i,j \sim N(0, \sigma^2)$: denotes the variability not explained by the fixed and random effects. $bi \perp \varepsilon i,j$: the random effects and the errors are independent. This means that the individual-specific variations captured by the random effects are not correlated with the unexplained variability in the observations.

4.2 Primary and secondary endpoint analysis

A LMM will be used to describe the VE55 trajectory by incorporating time to examine the progression of ventilatory depression, and treatment to assess its potential influence on VE55, both serving as predictors in the analysis. However, taking into account the possible steep decrease in ventilation after oxycodone intake, seen by Van der Schrier et al. [3] and the trajectory of the respiratory depression over time, including the possible effect of lacosamide and pregabalin, adjustments will have to be made to the model.

We will compose a preliminary LMM formula in which the time component is partitioned into a categorical variable for the initial three measurements to capture the steep decrease more precisely. Additionally, a continuous linear trend is introduced, augmenting the categorical effect of measurement 4 for all subsequent time points. This continuous trend represents the minutes elapsed after the lacosamide/pregabalin intake (if provided, e.g., 30 minutes for measurement 4, 60 minutes for measurement 5, and so forth). The treatment is considered in conjunction with its interaction with continuous time following the actual administration of the treatment (lacosamide or pregabalin). This interaction is added to investigate whether the responses change differently over time given a certain treatment. The main effect of treatment is explicitly excluded from the model's mean structure to ensure that it does not impact the prediction of responses during time points when no treatment is given to the subjects. Specifically, when a second treatment (lacosamide or pregabalin) is administered, omitting the main effect prevents it from being added for measurements before lacosamide/pregabalin intake. In reality, during these measurements, respiratory depression is influenced only by oxycodone, and including the main effect of the additional treatment could lead to incorrect predictions.

For the analysis of the relief of nociception, sedation and pupil diameter (secondary endpoints), the same statistical procedure will be applied. Three different LMM models will be used for each of the pain tests. For the other secondary endpoints (e.g. nausea/vomiting) an exploratory descriptive approach will be applied.

4.3 Assumption validations

When dealing with mixed models, it is crucial to validate model assumptions to ensure the reliability and accuracy of the findings. All assumptions regarding a linear mixed model will be checked appropriately, according to the usual practises.

References

1. R Core Team. R: A Language and Environment for Statistical Computing R Foundation for Statistical Computing (Vienna, Austria, 2021). https://www.R-project.org/.

2. Pinheiro, J., Bates, D. & R Core Team. nlme: Linear and Nonlinear Mixed Effects Models

3. Van Der Schrier R.and Jonkman, K. et al. An experimental study comparing the respiratory effects of tapentadol and oxycodone in healthy volunteers. British Journal of Anaesthesia 119, 1169–1177. issn: 0007-0912 (2017)