**SUPPLEMENT 1**



**Figure S1.** Cortexolone 17α propionate



**Figure S2.** Cortexolone 21 propionate



**Figure S3.** Cortexolone

**SUPPLEMENT 2**

**High-performance liquid chromatography gradient conditions**

The following conditions were used to detect cortexolone 17α-propionate and its metabolites/degradation products using chromatographic separation on a Kinetex XB-C18 column.

**Table S1.** High-performance liquid chromatography gradient conditions

|  |  |  |  |
| --- | --- | --- | --- |
| Pump Time (min) | % Mobile Phase A | % Mobile Phase B | Flow Rate (mL/min) |
| Initial | 55.0 | 45.0 | 0.450 |
| 0.50 | 55.0 | 45.0 | 0.450 |
| 4.00 | 42.0 | 58.0 | 0.450 |
| 6.50 | 38.0 | 62.0 | 0.450 |
| 6.60 | 15.0 | 85.0 | 0.600 |
| 9.00 | 15.0 | 85.0 | 0.600 |
| 9.10 | 55.0 | 45.0 | 0.450 |
| 10.0 | 55.0 | 45.0 | 0.450 |

**Table S2.** High-performance liquid chromatography mass transitions

|  |  |
| --- | --- |
| **Function 1 (0.00-3.20 min):** |  |
| **Cortexolone** |  |
| Mass Transition | 347.20 > 109.00 |
| Cone (V) | 30 |
| Collision (eV) | 26 |
| Dwell time (s) | 0.150 |
|  |  |
| **Cortexolone-d7 (I.S.)** |  |
| Mass Transition | 354.20 > 113.00 |
| Cone (V) | 30 |
| Collision (eV) | 26 |
| Dwell time (s) | 0.150 |
|  |  |
| **Function 2 (3.20-4.60 min):** |  |
| **Cortexolone-17-alpha-propionate** |  |
| Mass Transition | 403.20 > 329.00 |
| Cone (V) | 20 |
| Collision (eV) | 13 |
| Dwell time (s) | 0.150 |
|  |  |
| **Cortexolone-17-alpha-propionate-d6 (I.S.)** |  |
| Mass Transition | 409.20 > 335.00 |
| Cone (V) | 20 |
| Collision (eV) | 13 |
| Dwell time (s) | 0.150 |
|  |  |
| **Function 3 (4.60-10.0 min):** |  |
| **Cortexolone-21-propionate** |  |
| Mass Transition | 403.20 > 109.00 |
| Cone (V) | 30 |
| Collision (eV) | 30 |
| Dwell time (s) | 0.200 |
|  |  |
| **Cortexolone-21-propionate-d6 (I.S.)** |  |
| Mass Transition | 409.20 > 113.00 |
| Cone (V) | 30 |
| Collision (eV) | 30 |
| Dwell time (s) | 0.200 |

**SUPPLEMENT 3**

**Introduction**

An increase of heart rate (HR) compared to vehicle placebo was observed in the first few hours post-dose on Day 1, with a similar, but less pronounced increase also seen post-dose on Day 4. Exploratory post-hoc analysis of this increase in HR was conducted because the results of the vehicle placebo volunteers’ response suggested a possible effect on HR. The analysis and results of the tachycardic effect are summarized below and are for exploratory purposes only.

**By-timepoint-analysis of HR**

Throughout this study, there was an increase of HR under active treatment, which became significant within 5 hours post-dose on Day 1 and for a number of time points on Day 4, as seen in Figure S1. Figure S2 shows there is little indication that this difference was due to differences at baseline. Figure S3 shows the uncorrected HR data.



**Figure S4.** Placebo-controlled change from baseline of HR: Time course (arithmetic mean) by treatment with 90% confidence intervals



**Figure S5.** Change from baseline of HR: Time course (arithmetic mean) by treatment with 90% confidence intervals. Red = treatment; black = vehicle placebo.

**Figure S6.** Absolute values of HR: Time course (arithmetic mean) by treatment with 90% confidence intervals. Red = treatment; black = vehicle placebo.

**Comparison of time courses of HR and concentration**

The time courses as seen in Figure S7 do not indicate a causal relationship of the changes in HR with drug concentration.

**Figure S7.** Joint presentation of time course of ΔΔHR (top panel), mean concentration of Cortexolone 17α-propionate (middle panel) and the two metabolites (lower panel)

**Concentration-HR modeling**

A linear mixed effects model was applied to the HR data. The model included fixed effects and was of the form:

ΔHR ~ C + treat + T + BL

where ΔHR is the change from baseline of HR; C is the plasma concentration of Cortexolone 17α-propionate; “treat” is a discrete factor with levels "Active" and "Placebo"; and T is a discrete time effect with one level for each time point. Note that including a treatment effect will allow some judgement on the appropriateness of the model, as a well-fitting linear model should have a non-significant treatment effect. BL is the baseline value of HR for each volunteer, with the mean across volunteers subtracted.

BLi = HRi,0 – meanj (HRj, 0)

for volunteer i where HRj,0 is the baseline value for volunteer j.

As a consequence, the mean of BL across volunteers is zero. No fixed intercept was allowed.

The model has random effects per volunteer for the intercept and the concentration. An unstructured covariance matrix was assumed. As this model converged – although with a singular fit – no modifications of this model were necessary.

The Kenward-Roger approximation was used to calculate degrees of freedom and any t-test-based quantities, in particular two-sided 90% confidence intervals for the model parameters.

**Table S3.** Base model – Slope parameters

| **Effect** | **Estimate** | **SE** | **df** | **t-value** | **90% Confidence interval** |
| --- | --- | --- | --- | --- | --- |
| Cortexolone 17-α propionate [bpm per ng/mL] | -0.24 | 0.142 |  7.5 | -1.68 | -0.505 | 0.028 |
| Baseline HR [ms/bpm] | -0.03 | 0.110 | 28.5 | -0.25 | -0.214 | 0.159 |

**Table S4.** Base model – Treatment effect

| **Treatment effect [bpm]** |
| --- |
| **Estimate** | **SE** | **df** | **t-value** | **90% Confidence interval** |
| 4.2 | 2.01 | 27.7 | 2.11 | 0.8 | 7.7 |

As shown in Table S3 and Table S4, the base model had a significant treatment effect with negative slope which indicated that the model did not describe the observed increase in HR. Figure S5 and Figure S6 indicate a predicted difference between the active and the vehicle placebo group according to this model, in the absence of Cortexolone 17α propionate.

The line gives the regression derived from the base model by adjusting for the time effect, the shaded area is the 90% confidence range.

**Figure S8.** Unchanged values of ΔHR plotted versus concentration of Cortexolone 17α-propionate

The line gives the predicted effect, the shaded area the 90% confidence range. The intervals in red give the deciles of concentration and the bars give the means and 90% confidence intervals for the ΔHR values in the respective decile, adjusted for the placebo effect.

**Figure S9.** Predicted effect on HR using the base model

**Exploratory models**

A series of models similar to the base model were fitted.

In summary, there was no indication that any of these models provided a substantially better fit than the base model. However, as one model (ΔHR ~ C1 + treat + T + BL) had the lowest t-value for the intercept and had a slightly lower AIC than that of the basic model, this model was investigated further.

As a consequence, the results of this model were not fundamentally different from the base model. Both models presented a negative slope which might be an indication of a decrease in HR with increasing concentration, conflicting with observed data.