Appendix S1.

MATERIALS AND METHODS

Study design and participants

A crossover design was used in this single-centre randomized controlled trial, resulting in each participant being their own control.

Patients with genetically and biochemically confirmed Sjögren-Larsson syndrome (SLS), aged 7–40 years were included in the study. All patients continued current dermatological treatment during the study period. Study approval was obtained from the Regional Committee on Research involving Human Subjects.

Treatment

Zileuton is approved by the US Food and Drug Administration (FDA) for treatment of asthma in patients ≥ 12 years. Inhibition of LTB4 biosynthesis in blood is directly related to zileuton plasma concentration. Since we expected the same mechanism of action to be responsible for reduction of pruritus in SLS, our aim was to achieve similar exposure to zileuton as in asthmatic patients.

For patients ≥ 12 years of age, dosages as currently approved for the treatment of asthma were used. To determine dosages for younger patients (≥ 5 and < 12 years of age), the guidance for paediatric dosing was used.

Randomization and allocation to treatment

Based on findings from pharmacokinetics and pharmacodynamics, all patients received treatment with either zileuton or placebo over 2 periods of 8 weeks, separated by a wash-out period of 4 weeks. Treatment order was randomly assigned and randomization was performed by the hospital pharmacy. Assessors and patients were blinded to assignment of treatment.

Data collection

Visits were scheduled for all patients at t=0 (start first treatment period), t=8 weeks (stop first treatment period), t=12 weeks (stop wash-out period and start second treatment period), t=20 weeks (stop second treatment period) and t=24 weeks (stop second wash-out period). Baseline measurements (t=0) were performed by 2 assessors (MS and JF) producing one mutual assessment for all patients. All subsequent measurements were performed by one assessor (JF).

During all visits, structured dermatological assessment was performed using the Sjögren-Larsson Severity Index (SLaSI) specially designed for this study using item from the Psoriasis Area and Severity Index (PASI) scoring of psoriasis (8). For each SLaSI outcome, the mean of all body areas scored was calculated for each patient at each visit leading to "SLaSI mean scores". To measure global changes, the Physician Global Assessment (PGA) tool (range 0–5; 5 being most severe) was performed during all visits (9).

Urine samples were collected at each visit and stored at –80°C within maximally 6 h after collection. Unused capsules were returned to the study staff at each visit to measure treatment compliance.

Primary caregivers scored pruritus and excoriations in all patients weekly during the entire study period using a visual analogue scale (VAS) instrument consisting of 100-mm horizontal lines.

Primary outcome measures (POM)

POM were the differences in SLaSI mean scores and PGA scores before and after treatment with zileuton or placebo.

Secondary outcome measures (SOM) were individual changes in VAS scores.

Statistical analysis

Primary analysis involved studying changes in SLaSI mean scores and PGA scores during treatment. Data were analysed for the 2 treatment periods combined and separately using paired samples *t*-tests, comparing: (*i*) scores at the start of the treatment period with those at the end of the treatment period (treatment); (*ii*) scores at the end of the treatment period with those at the end of the wash-out period (wash-out); and (*iii*) scores at the start of the treatment period with those at the end of the wash-out period (treatment + wash-out).

SOM were studied by calculating all individual mean VAS scores during treatment periods with zileuton and periods with placebo treatment for the outcomes pruritus and excoriations separately.

Previous experience led to the assumption that only a minority of patients would respond to zileuton. Therefore, POM were also analysed in each individual patient to detect responders. Regarding SLaSI and PGA scores, we defined a positive response to zileuton as a decrease of ≥ 0.5 after treatment. Based upon use in psoriasis, a positive VAS response was defined as a decrease of ≥ 20 during treatment (10). All separate outcomes scoring positive were given 1 point (maximum score 7 points). Patients scoring ≥ 5 points were considered responders.

Leukotriene analysis

For LTB4 and 20-OH-LTB4 analysis samples were purified using anion exchange solid phase extraction with subsequent high-performance liquid chromatography (HPLC) fractionation on a Acquity HSS T3 column (100×1 mm/ dp=1.8 µm; Waters Chromatography, The Netherlands). Relevant fractions were measured using an enzyme immunoassay (Leukotriene B4 Express EIA Kit, Cayman Chemical Company nr 10009292, USA and standard for 20-OH-LTB4 from the same company number 20190). Reference values for age-matched healthy controls were determined in-house. Total procedure was validated by performing recovery experiments for LTB4 and 20-OH-LTB4, which gave satisfactory results.