Differential Effects of Digoxin on Imiquimod-Induced Psoriasis-Like Skin Inflammation on the Ear and Back

Supplementary File 1

Detailed materials and methods

Female C57Bl/6j mice were purchased from Taconic (Ry, Denmark). They were housed in single-ventilated cages in a temperature- and humidity-controlled 12 hour light/dark room and given free access to water and chow diet. At 8 weeks of age they received topical Aldara (5% imiquimod, Meda AB, Solna, Sweden) or vehicle cream (Skanderborg Apotek, Denmark) daily for 5 days, with 45 mg on the shaved back (1x2 cm area) and 5 mg on the right ear. During applications the mice were anaesthetized with isofluran (IsoFlo vet 100%, Abbott, Berkshire, UK). All mice were treated with either i.p. digoxin SAD (Amgros I/S, Copenhagen, Denmark; 20 $\mu$g/mouse on day 1, 10 $\mu$g/mouse on day 3, and 20 $\mu$g/mouse on days 4 and 5) or saline (control group). The digoxin dose was somewhat lower than dosages (20 $\sim$ 40 $\mu$g/d) previously used in other Th17-dependent disease models ameliorated by digoxin, but we found that the mice were in marked acute distress after the first dose, wherefore treatment was avoided on day 2. During skin applications mice were given neutral paraffin oil/vaseline ointment on the eyes (Ophtha, Tubilux Pharma, Rome, Italy) to avoid cream getting into the eyes. The mice (n=32; 8 mice/study group) were sacrificed at day 6, 24 hours after the last cream application and i.p. treatment. All animal experiments were performed according to the principles stated in the Danish law on animal experiments and were approved by the Animal Experiment’s Inspectorate, Ministry of Justice, Denmark (license number 2012-15-2934-00119). The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the European Parliament [EU directive 2010/63/EU]. The ethical policy of the University of Copenhagen complies with that of the NIH (A5846-01). Back skin was scored visually for erythema (0 $\sim$ 4) and scaling (0 $\sim$ 4) daily prior to each skin application. Ear and back fold thickness was measured using a digimatic thickness gauge (Mitutoyo, Illinois, US) from treatment days 1 and 3, respectively, and until study termination. Prior to retro-orbital bleed out and cervical dislocation, mice were anaesthetized subcutaneously with a 0.1 ml/10g mouse dose of a mixture of tiletamine (1.63 mg/mL), zolazepam (1.63 mg/mL), xylazin (2.61 mg/mL), and butorphanol tartrate (0.065 mg/mL). For histological analyses, skin samples from the right ear and back were fixed in 10% neutral buffered formalin (“Lillie” formaldehyde solution 4%, Hounisen, Skanderborg, Denmark). Four mm biopsies from the ear and the back were paraffin embedded and sectioned at 4 $\mu$m. The sections were stained biochemically with hematoxylin and eosin. Digital photos of histological sections were acquired using a slide scanner (Axio Scan.Z1, Zeiss, Birkerød, Denmark). Plasma interleukin-17A and serum amyloid A levels were measured by commercial ELISA according to the manufacturer’s instructions (R&D Systems, Minneapolis, US and Tridelita, Kildare, Ireland, respectively). Statistical analyses were performed using GraphPad Prism 7 (GraphPad Software Inc., La Jolla, USA). A $p$-value below 0.05 was considered statistically significant. Data was analysed using two-way ANOVA with Tukey’s multiple comparisons test. The minimal detection limit for the IL-17A assay was 5 pg/ml, but although the measured IL-17A levels were below 5 pg/ml in 6 out of 16 mice receiving vehicle cream, these data were included in the two-way ANOVA. Values are represented as means $\pm$ SDs. Missing data due to technical difficulties are reported in the figure and table legends.

REFERENCES