Appendix S1

SUPPLEMENTAL MATERIALS AND METHODS

Tissue samples

Four-mm punch biopsies from buccal mucosa were collected after obtaining informed consent from 8 patients clinically and histologically diagnosed with oral lichen planus (OLP; 4 females, 4 males; age range 43–79 years, median 61 years). All biopsies were taken from reticular areas. Buccal mucosal biopsies were also obtained from 8 volunteers without OLP (6 females, 2 males; age range 43–67 years, median 50 years; *p*-value vs OLP age 0.49, Mann–Whitney *U* test). Neither controls nor patients were medicated with immunosuppressors or non-steroidal anti-inflammatory drugs (NSAIDs) at the time of biopsy. All patients with OLP had more or less symptomatic lesions at the time. OLP was diagnosed according to the modified World Health Organization (WHO) diagnostic criteria, as described in (12). The samples were embedded in Tissue-Tek®, snap-frozen in liquid nitrogen and thereafter stored at –80°C. The study was approved by the ethical review board at Umeå University (Dnr 09-083M). Four of the OLP biopsies and 7 of the control samples have previously been described with respect to an Illumina Gene expression study (12).

Analysis of linoleic acid derivatives

The linoleic acid derivatives were quantitated using an ultra-performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS) method described in detail in (S1). In brief, samples were spiked with oxylipin and N-acylethanolamine internal standard solutions (9(S)-HODE-d_4, 12(13)-DiHOME-d_4, 12(13)-EpOME-d_4, (Cayman Chemical Co., Ann Arbor, MI, USA) for the data reported here), diluted in 1 ml 5% methanol in MilliQ distilled water and grounded using a bead mill (Retsch MM400; 30 oscillations/s for 2 min). After centrifugation the supernatant was applied into solid-phase extraction Waters Oasis HLB cartridges (60 mg sorbent, 30 µm particle size) followed by elution with 2 ml methanol and 2 ml ethyl acetate into polypropylene tubes containing 6 µl glycerol solution (30% in methanol). The eluates were evaporated under vacuum and reconstituted in methanol containing 12-[[(cyclohexylamino)carbonyl]amino]-dodecanoic acid as a recovery standard. UPLC-MS/MS analysis was performed immediately using a Waters BEH C18 column (2.1 × 150 mm, 2.5 µm particle size) and the mass analysis was performed on an Agilent 6490 Triple Quadrupole system equipped with the iFunnel Technology source (Agilent Technologies, Santa Clara, CA, USA) in negative multiple reaction monitoring mode. The internal standard recoveries were 62%, 78% and 60% for 9(S)-HODE-d_4, 12(13)-DiHOME-d_4 and 12(13)-EpOME-d_4, respectively. In addition to the linoleic acid derivatives reported here, arachidonic acid derivatives were also detected. The samples were also run in positive multiple reaction monitoring mode to identify *N*-acylethanolamines and related compounds (S1). The data for the arachidonic acid-derived oxylipins and the N-acylethanolamines will be reported elsewhere.

Lipid abbreviations: 9-HODE, 9-hydroxy-10*E*,12*Z*-octadecadienoic acid; 13-HODE, 13-hydroxy-9*Z*,11*E*-octadecadienoic acid; 9,10-DiHOME, 9,10-dihydroxy-12*Z*-octadecenoic acid; 12,13-DiHOME, 12,13-dihydroxy-9*Z*-octadecenoic acid); 9,10,13-Tri-HOME, 9,10,13-trihydroxy-11*E*-octadecenoic acid; 9,12,13-TriHOME, 9,12,13-trihydroxy-10*E*-octadecenoic acid; 9(10)-EpOME, 9(10)-epoxy-9*Z*-octadecenoic acid.

Statistical analysis

Two tailed *t*-tests not assuming equal variances and 2-tailed Mann–Whitney tests were undertaken using the GraphPad Prism statistical software for the Macintosh (v8, GraphPad Software Inc., San Diego, CA, USA). A 5% false discovery rate (S2) was used when multiple testing was employed. MANOVA was conducted using the stat package built into the R statistical software package (v. 3.5.1).

SUPPLEMENTARY REFERENCES

S1. Gouveia-Figueira S, Nording ML. Validation of a tandem mass spectrometry method using combined extraction of 37 oxylipins and 14 endocannabinoid-related compounds including prostamides from biological matrices. Prostaglandins Other Lipid Mediat 2015; 121: 110–121.

S2. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. JR Statist Soc B 1995; 57: 289–300.