**Dermal exposure to the immunomodulatory antimicrobial chemical triclosan alters the skin barrier integrity and microbiome in mice**

**Dataset Number:** RD-1034-2022-0

**Introductory Information**

Triclosan is an antimicrobial chemical used in healthcare settings that can be absorbed through the skin. Exposure to triclosan has been positively associated with food and aeroallergy and asthma exacerbation in humans and, although not directly sensitizing, has been demonstrated to augment the allergic response in a mouse model of asthma. The skin barrier and microbiome are thought to play important roles in mediating inflammation and allergy and disruptions may contribute to development of allergic disease. To investigate potential connections of the skin barrier and microbiome with immune responses to triclosan, SKH1 mice were dermally exposed to triclosan (0.5-2%) or vehicle for up to 7 consecutive days.

**Methods Collection**

1. Triclosan Exposures
   1. Female SKH1 mice (6-7 weeks old)
   2. Triclosan (0.5%, 2%) or acetone (vehicle) on dorsal back skin for 7 days
2. Trans-Epidermal Water Loss Measurements
   1. Trans-Epidermal Water Loss was measured daily with VapoMeter (Delfin)
3. Histology
   1. Back skin was formalin-fixed, embedded in paraffin, and H&E stained
   2. Slides were brightfield imaged at 40X
   3. Epidermal thickness was measured with 3 measurements/sample and averaged
4. Immunofluorescence
   1. Back skin was fixed in 4% paraformaldehyde, frozen, sectioned, and stained with primary antibody (filaggrin, filaggrin 2, keratin 10, or keratin 14) and secondary antibody (Alexa Fluor 594)
   2. Images were acquired at 20X and distribution of protein was measured with 3 measurements/sample and averaged
5. Protein Analysis
   1. Back skin was collected into tubes containing a steel bead and T-PER with Halt Protease and Phosphatase Inhibitor cocktail and 0.5 M EDTA
   2. Processed on a Tissue Lyser II and supernatant was collected
   3. Total protein was quantified using the BCA protein assay
   4. Proteins (filaggrin, filaggrin 2, keratin 10, keratin 14) were quantified with ProteinSimple Anti-Rabbit and Total Protein Detection Modules on Wes machine
   5. Area under the curve was calculated and normalized to total protein
6. Gene Expression Analysis
   1. Back skin was collected into tubes containing 500 µL RNAlater
   2. Total RNA was isolated using RNeasy kit and reverse transcribed
   3. Real-time quantitative PCR was performed with TaqMan Fast Universal PCR master mix, cDNA, and genes-specific primers
   4. Genes evaluated include filaggrin, filaggrin 2, involucrin, loricrin, keratin 10, keratin 14, tight junction protein 1, occludin/ELL domain containing 1, integrin subunit beta like 1, S100a8, thymic stromal lymphopoietin, e-cadherin, Toll-like receptor 4, interleukin 4, interleukin 22
7. Bacterial Collection, Isolation, Sequencing, Analysis
   1. Skin bacteria was collected with a sterile foam tipped applicator prior to exposure (day 0), throughout exposure (day 1 and 3), one-day after the last exposure (day 7), and one-week after the last exposure (day 13)
   2. Gut bacteria were collected following euthanasia on day 7
   3. Microbial DNA was isolated and sequenced on an Illumina MiSeq
   4. Paired-end sequencing reads were analyzed by QIIME2

**Citations**

Rachel Baur, Jasleen Gandhi , Nikki B Marshall , Ewa Lukomska , Lisa M Weatherly, Hillary L Shane, Gangqing Hu, Stacey E Anderson. Dermal Exposure to the immunomodulatory Antimicrobial Chemical Triclosan Alters the Skin Barrier Integrity and Microbiome in Mice. Toxicol Sci. 2021 Nov 24;184(2):223-235. doi: 10.1093/toxsci/kfab111.

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