Systemic and immunotoxicity induced by topical application of perfluoroheptane sulfonic acid (PFHpS) or perfluorooctane sulfonic acid (PFOS) in a murine model

Introductory Information

Per- and polyfluoroalkyl substances (PFAS) are a large group of synthetic man-made surfactants of over 12,000 compounds that are incorporated into numerous products for their chemical and physical properties. Numerous PFAS have been associated with adverse health effects. Although there is a high potential for dermal exposure, these studies are lacking. The present study evaluated the systemic and immunotoxicity of sub-chronic 28- or 10-day dermal exposure, respectively, to of PFHpS (0.3125-2.5% or 7.82-62.5 mg/kg/dose) or PFOS (0.5% or 12.5 mg/kg/dose) in a murine model.

Methods Collection

1. Animal Exposures
    - Female $B_6C_3F_1$ mice (7-8 weeks at start of study)
    - Perfluoroheptane sulfonic acid (PFHpS) (25 µl/ear; 0.3125-1.25%), perfluorooctane sulfonic acid (PFOS) (25 µl/ear; 0.5%) or acetone on dorsal surface of both ears for 10 or 28 days
2. Tissue Collection
    - Thymus were collected, weighed, and then discarded.
    - Kidneys were weighed and then one was discarded and one (right) was collected in 10% formalin for histopathology analysis.
    - Liver was weighed, caudate lobe was collected in RNA later and then homogenized on a TissueLyser II in Buffer RLT for RNA extraction. The remainder of the liver was collected in 10% formalin for histopathology analysis.
    - Spleen was weighed, 1/2 was collected in 4 mL RPMI and processed into single cell suspensions by mechanical disruption of tissues between frosted microscope slides. The remainder of the spleen was collected in 10% formalin for histopathology analysis.
    - Ear pinnas (1 per mouse; split into ventral and dorsal halves) was collected in RPMI and processed into single cell suspensions prepared by incubating with a 0.25 mg/ml Liberase-TL Research grade (Roche) enzymatic digestion for 90 min at 37°C in RMPI with 100 µg/ml DNase I.

The second ear pinna (1/2) collected in 10% formalin for histopathology analysis and 1/2 was collected in RNA later and then homogenized on a TissueLyser II in Buffer RLT for RNA extraction.
    - Right and left auricular draining lymph nodes (dLNs) collected in 2 mL sterile phosphate-buffer saline (PBS) (pH 7.4) and single cell suspensions (2 nodes/animal) were prepared by mechanical disruption of tissues between frosted microscope slides
3. Serum chemistries
    - Blood samples were collected via cardiac puncture and separated by centrifugation. Selected serum chemistries were evaluated using Catalyst DX Chemistry Analyzer.
4. Analytical PFAS detection
    - Serum collected from each animal and urine pooled for each group of mice were analyzed for PFHpS or PFOS by Vista Analytical Laboratory.
5. Gene expression
    - Real-time PCR (Applied Biosystems 7500 RT-PCR System).
6. Immune Cell Subsets
    - Flow cytometry using BD LSRII Flow Cytometer and data was analyzed using FlowJo software.
7. Histology
    - Tissue samples were stained via hematoxylin and eosin (H&E) and evaluated by a veterinary pathologist.
8. Spleen IgM response to SRBC
    - The primary IgM response to SRBC was enumerated using a modified hemolytic plaque assay
9. Serum IgM response to SRBC
    - Serum samples were analyzed for anti-SRBC IgM using a commercially available ELISA kit

Publications

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