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Effect of dietary pristane and other saturated mineral oils (MOSH) on autoimmune arthritis in rats

Monica Andreassen[a,*,1], Hege Hjertholm[a,1], Jean-Pierre Cravedi[b], Koni Grob[c], Jan Alexander[a], Unni C. Nygaard[a]

[a] Norwegian Institute of Public Health, PO Box 4404, NO-0403 Oslo, Norway
[b] Toxalim, INRA, ENVT, INP-EIPurpan, Université de Toulouse, F-31027 Toulouse, France
[c] Official Food Control Authority of the Canton of Zurich, P.O. Box 1471, CH-8032 Zurich, Switzerland

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ABSTRACT

Pristane and other adjuvants based on mineral oil saturated hydrocarbons (MOSH) may induce autoimmunity in rodents after intradermal injection; however there is a lack of information on immune effects after oral MOSH exposure. The aim of our study was to determine the impact of dietary exposure to pristane and other MOSH on the development of autoimmune arthritis.

Dark Agouti (DA) rats were given feed containing 4000 mg/kg pristane or a broad MOSH mixture in various concentrations (0–4000 mg/kg) for 90 days, or a single intradermal injection of 200 μl pristane (positive control). Arthritis scores, and serum and splenocyte markers previously associated with arthritis development, were determined.

All rats injected with pristane displayed arthritis symptoms and higher levels of certain serum markers. None of the rats fed pristane or MOSH developed arthritis symptoms or demonstrated clear changes in any measured arthritis-associated biological markers in serum or splenocytes.

The absence of clinical arthritis symptoms or any increase in common arthritis-associated biological markers in sera and spleen following dietary exposure to pristane or a broad MOSH mixture in a subchronic rat model of arthritis suggest that dietary MOSH have low capacity to promote development of autoimmunity.

1. Introduction

Mineral oils are commonly used as adjuvants to boost the immune system response to an antigen in human and veterinary vaccines [1–3]. Apart from therapeutic use, humans are exposed to mineral oils mainly via the food (see below), While oral administration of MOSH generally has low acute toxicity, MOSH has been reported to accumulate in tissues in both humans [4,5] and animals [6]. In Fischer-344 rats, the formation of liver microgranulomas associated with inflammatory responses was observed after feeding with white mineral oils [7,6,8]. While there is generally little information about the potential of long term dietary MOSH to affect immune functions, single intradermal and intraperitoneal injections of certain mineral oils induce autoimmune responses in rodent models sharing both clinical and pathological features with human rheumatoid arthritis (RA) [9–11]. A few epidemiological studies suggest an association between exposure to high doses of MOH and an increased risk of developing autoimmune diseases. In a case control study in Sweden, self-reported occupational exposures to MOH primarily via skin and inhalation were associated with an increased relative risk of developing rheumatoid arthritis (RA) in men [12]. Furthermore, a significantly higher prevalence of RA and Systemic lupus erythematosus was observed in a population living close to an oil field waste site compared to another community with no known exposures of this type [13]. In the latest scientific opinion of mineral oils in food, The European Food Safety Authority (EFSA) identified a knowledge gap on potential effects on systemic autoimmune diseases or altered immune function after dietary exposure [14].

Mineral oil hydrocarbons (MOH) may unintentionally contaminate the food chain at various stages of food production or migrate

Abbreviations: MOH, mineral oil hydrocarbons; MOSH, mineral oil saturated hydrocarbons; DA, Dark Agouti; i.d., intradermal; RF, rheumatoid factor; TLR, toll like receptor.

* Corresponding author at: Domain for Infection Control and Environmental Health, Department of Toxicology and Risk Assessment, Norwegian Institute of Public Health, Oslo, Norway.
E-mail address: monica.andreassen@fhi.no (M. Andreassen).
[1] These authors contributed equally to this work.

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from food packaging materials [15], while some MOH are intentionally used as food additives and in pesticides [14]. Oral and dermal exposure to MOH may also occur through use of cosmetics and pharmaceuticals and medicinal use [16,17]. Some saturated hydrocarbons occur naturally in marine and terrestrial biota [14]. MOH consist of complex mixtures of mineral oil aromatic hydrocarbons (MOAH) and mineral oil saturated hydrocarbons (MOSH). Due to carcinogenic properties of MOAH, food grade MOH-products are treated in such a way that the MOAH content is minimized, while technical grades of MOH typically contain 15–35% MOAH. MOSH are present at different levels in nearly all foods, with the highest concentrations detected in bread, rolls, grains (mainly rice) confectionary (non-chocolate), vegetable oil, canned fish and oilseeds [14]. The estimated dietary MOSH exposure in Europe ranged from 0.03 to 0.3 mg/kg body weight (b.w.) per day, and higher in younger consumers and children, probably due to a higher intake of food per kg b.w. as well as age-related differences in dietary habits [14].

One model used for studies on arthritis is the arthritis-susceptible Dark agouti (DA) rats. In this model a single intradermal injection of medicinal white oil (commonly used for food, pharmaceutical and cosmetic use) as well as common commercial cosmetic products containing up to 80% mineral oils (like body lotion and baby oil), induced arthritis symptoms (joint synovitis). Percutaneous application of these products on abraded skin resulted in similar, but milder and transient, clinical arthritis symptoms in 5 out of 10 animals being exposed to a certain baby oil [10]. Although short term (five subsequent) per oral doses of medicinal white oil did not have any apparent effect in this arthritis model, the authors speculate that oral administration of adjuvants in conjunction with inflammation of the gut by other agents could parallel the arthritic effects of percutaneous exposure on abraded skin.

As described above, several MOSH have the ability to induce autoimmune responses in rodents after dermal/intradermal exposures, but so far, no studies have investigated whether long term (sub-chronic or chronic) dietary exposure to MOSH can promote autoimmunity [14,18]. A single intradermal pre-administration of Incomplete Freund's adjuvant or hexadecane was able to prevent development of disease induction by the intradermal injection with complete Freund's adjuvant (including bacterial components and mineral oils) [19]. While incomplete Freund's adjuvant has been reported not to demonstrate any significant immune responses after oral administration Silin et al., 2007, neither of the above studies investigated the potency to induce or prevent autoimmunity after oral exposure. Another aspect of concern is that previous safety evaluations regarding MOSH have been based on chemical and physical properties (such as viscosity) instead of sub-classes of mineral oils. The present study provides novel experimental data on the potential effects on autoimmunity of the whole MOSH range to which humans are exposed to via the diet. More precisely, we conducted a sub chronic study to determine whether 90 days with dietary exposures to a broad MOSH mixture can induce clinical signs and/or biological markers for autoimmune arthritis in the arthritis prone DA rat model.

2. Materials and methods

2.1. Animals

Inbred DA male and female rats (DA/OlaHsd), 7–8 weeks of age, was obtained from Harland Laboratories (Indianapolis, USA). In the pilot experiment, the female rats were randomly assigned to two groups (pristane injection and control, n = 6) and in the main experiment 5 female and 5 male rats were randomly assigned to one of six experimental units (see experimental overview in Section 3.2.1; Table 2). The rats were housed two or three animals in each cage, and acclimatized for a minimum of 9 days. The makrolon cages, containing cage enrichments, were randomly placed in ventilated Scantainer filter cabinets, 12 h light/dark cycle, temperature 21 °C ± 2, relative humidity 35–75% ± 10. The cage position in the rack was changed at least twice a week. Feed and tap water were given *ad libitum*. The rats were given a standard diet until start of the experiment (Teklad 2018S, Envigo, Cambridgeshire, UK.) and standard rodent bedding (NESTPAKS, Datesand Ltd). All rats were marked by ear puncture before entering the experiments. The experiments were performed in conformity with Norwegian laws and regulations for live animals, and approval was given by the Norwegian Animal Research Authority under the Ministry of Agriculture (FOTS 7084).

2.2. Chemicals and reagents

Pristane (tetramethylpentadecane, purity ≥97%), Concanavalin A (ConA), Lipopolysaccharide (LPS), and 10% buffered formalin were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). All ELISA kits were purchased from eBioscience (San Diego, CA, USA), with the exception of the RF kit (from MyBioSource, San Diego, CA USA). All CBA kits were purchased from BD Bioscience (San Jose, CA, USA). Antibodies for detection of Toll-like receptor (TLR)2 and TLR 3 were purchased from Santa Cruz Biotechnology Inc. (Dallas, Texas 75220 USA); anti-TLR2 (H-175, rabbit polyclonal IgG), anti-TLR3 (N-14, goat polyclonal IgG), goat anti-rabbit IgG-FITC and donkey anti-goat IgG-APC. Hank's Balanced Salt Solution (HBSS), fetal calf serum (FCS) and the cell growth medium RPMI 1640 were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA USA), and penicillin/streptomycin mix was acquired from PAA The Cell Culture Company (GE Healthcare Little Chalfont, Buckinghamshire, UK).

2.3. Preparation of the MOSH −mixture

A MOSH mixture ranging from about C_{14} to C_{50} was prepared by combining the following products: 295 g/kg paraffin wax Ph Eur, low viscosity, Fluka 76233 (Buchs, Switzerland), 295 g/kg paraffin wax Ph Eur, high viscosity, Fluka 76234, 147 g/kg Catenex Ph 941 FU (Shell) and 263 g/kg distillate from paraffin highly liquid Ph Eur, BP NF, Merck JP K43210074 209 1.17174.1000 (Darmstadt, Germany). This distillate was obtained by discarding the first 25 ml from 700 ml and consecutive fractions of 25 ml. Fractions 2 and 3 were mixed at a ratio of 1:2.

2.4. Preparation of MOSH- and pristane containing feed

A standard pelleted diet for rats (AIN–93 M), as described by Reeves et al. Reeves et al. (1993), was selected. Prior to the preparation of the feed, the major ingredients were analysed by on-line HPLC-GC-FID for MOSH or polyolefin oligomeric saturated hydrocarbons (POSH) to rule out disturbing interferences. For all ingredients, the contamination was below 15 mg/kg and was considered acceptable. Before being incorporated into the diet, the MOSH mixture and pristane was dissolved in soybean oil and stirred for 4 h at 40 °C. MOSH were incorporated into the diet at 40, 400 and 4000 mg/kg and pristane at 4000 mg/kg, and in each case an equivalent mass of soybean oil was replaced by the MOSH solution. Diet concentrations were verified in March 2014 and again in September 2014. The measured doses were within 88–95% of the nominal concentrations.

2.5. Anaesthetics

Animals received anaesthetics prior to and during intradermal injections and at termination (3% Isofluran gas anaesthetics (Isoba