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In vitro and in vivo comparison of dermal irritancy of jet fuel exposure using EpiDerm™ (EPI-200) cultured human skin and hairless rats

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Abstract

The purpose of this study was to evaluate an in vitro EpiDerm™ human skin model (EPI-200) to study the irritation potential of jet fuels (JP-8 and JP-8+100). Parallel in vivo studies on hairless rats on the dermal irritancy of jet fuels were also conducted. Cytokines are an important part of an irritation and inflammatory cascade, which are expressed in upon dermal exposures of irritant chemicals even when there are no obvious visible marks of irritation on the skin. We have chosen two primary cytokines (IL-1α and TNF-1α) as markers of irritation response of jet fuels. Initially, the EPI-200 was treated with different quantities of JP-8 and JP-8+100 to determine quantities which did not cause significant cytotoxicity, as monitored using the MTT assay and paraffin embedded histological cross-sections. Volumes of 2.5–50 μl/tissue (~4.0–78 μl/cm^2) of JP-8 and JP-8+100 showed a dose dependent loss of tissue viability and morphological alterations of the tissue. At a quantity of 1.25 μl/tissue (~2.0 μl/cm^2), no significant change in tissue viability or morphology was observed for exposure time extending to 48 h. Nonetheless, this dose induced significant increase in IL-1α and TNF-α release versus non-treated controls after 24 and 48 h. In addition, IL-1α release for JP-8+100 was significantly higher than that observed for JP-8, but TNF-α release after 48 h exposure to these two jet fuels was the same. These findings parallel in vivo studies on hairless rats, which indicated higher irritation levels due to JP-8+100 versus JP-8. In vivo, transepidermal water loss (TEWL) and IL-1α expression levels followed the order JP-8+100 > JP-8 > control. Further, in vivo TNF-α levels for JP-8 and JP-8+100 were also elevated but not significantly different from one another. In aggregate, these findings indicate that EPI-200 tissue model can be utilized as an alternative to the use of animals in evaluating dermal irritation.

Keywords: Dermal irritancy; Jet fuel; Epiderm; In vitro; In vivo; Hairless rats

1. Introduction

The main function of the skin is to protect the body from infection, dehydration and other environmental insults by creating an impermeable barrier of cornified cell layers, the stratum corneum. When the barrier function is perturbed by application of chemicals, solvents (e.g. acetone), detergents or mechanical forces, the epidermis responds in multiple ways to restore the barrier function. The epidermis response includes an increase in synthesis of cytokines (Wood et al., 1992) that triggers the inflammatory cascade.

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Dermal exposure of jet fuels is well known to cause skin irritation and sensitization (Kinkead et al., 1992; Kanikkannan et al., 2000). JP-8 is a mixture of aliphatic and aromatic hydrocarbons plus three additives, an icing inhibitor (diethylene glycol monomethyl ether), an antistatic compound (Stadis 450), and a corrosion inhibitor (DC1-4A). JP-8+100 contains additional ingredients such as an antioxidant, chelator, or a metal deactivator (McDougal and Rogers, 2004; Allen et al., 2000). Petroleum middle distillates such as kerosene, which have similar components of JP-8, were determined to cause edema, erythema, blisters, and burning sensation following acute exposures (Koschier, 1999).

Skin barrier changes and irritation potential of jet fuels have been thoroughly studied using various animal models (Muhammad et al., 2005; Singh et al., 2003; Kanikkannan et al., 2002). However, the use of animals in research has been a great concern in many countries and therefore development of an in vitro system equivalent to human skin is considered important. In vitro skin irritation testing methods could be helpful not only as pre-clinical safety screens but also for use in ranking chemicals for their skin irritation potential, even at the low end of the irritation spectrum (Hayden et al., 2003). The use of keratinocyte cultures has been gaining acceptance as an alternative to animal studies for skin irritation research (Roguet, 1998; Bernstein and Vaughan, 1999). Keratinocytes are responsible for formation of stratum corneum, the outermost layer of the skin as protective and impermeable barrier. Human epidermal keratinocytes (NHEK) exposed to three jet fuels, Jet A, JP-8 and JP-8+100 in a culture medium demonstrated that these chemicals induce production and release of pro-inflammatory cytokines as TNF-α and IL-8 (Allen et al., 2000). The exposure of the aromatic hydrocarbon components of jet fuels (e.g. cyclohexylbenzene, trimethylbenzene, xylene, dimethylnaphthalene, ethylbenzene, toluene and benzene) to NHEK resulted in a dose-related differential response in IL-8 release (Chou et al., 2003). Both of these studies utilized monolayer keratinocytes growing submerged in a culture medium.

Tissue engineering techniques have been used to create three dimensional tissue substitutes that may be used to restore tissue function, and as biological models for studying disease pathogenesis (Langer and Vacanti, 1993; Berthiaume and Yarmush, 1995). The engineered tissues can also be used as realistic biological models to obtain fundamental understanding of the structure–function relationships following exposure to various exogenous conditions.

In organotypic cultures, keratinocytes are cultivated at the air–liquid interface on various substrates serving as dermal equivalents. The dermal equivalents consist of microporous membranes either cell free, coated with extracellular matrix, or seeded with fibroblasts on the underside of the membranes (Rosdy and Clauss, 1990; Limat et al., 1996; Tammi and Maibach, 1987). Another system used to approximate the in vivo situation is achieved by keratinocyte cultures on de-epidermized and devitalized dermis, a nonliving tissue which is still covered by its basement membrane (Regnier and Darmon, 1991; Gibbs et al., 1997; Ponec et al., 1997). Alternatively, living dermal equivalents consisting of collagen gels populated with fibroblasts provide an appropriate substratum for the development of a polarized and stratified epithelium, displaying many morphological and functional characteristics of native epidermis (Bell et al., 1981; Asselineau et al., 1989; Parenteau et al., 1991).

In contrast to cells in monolayer culture, engineered skin equivalents mimic human epidermis in terms of tissue architecture and barrier function (Nolte et al., 1993; Andreadis et al., 2001). After a week of culture at the air–liquid interface, they form a well-stratified epidermis with basal, spinous, granular and cornified layers. Biochemical and ultrastructural studies have shown that this epidermal culture was similar to human skin (Asbill et al., 2000; Hayden et al., 2003). Therefore three-dimensional engineered tissues may provide realistic models to study the molecular mechanism of skin irritation.

In the present study, we have used the 3-dimentional EpiDerm^{TM} culture (EPI-200) as a human skin equivalent to systematically study the effect of JP-8 and JP-8+100 on tissue viability, structure, and cytokine release. Parallel dermal irritancy studies were performed in hairless rats using these jet fuels. The results lay the initial groundwork for establishing the EPI-200 as an in vitro human skin equivalent for determining skin irritation potential and molecular responses of skin following exposures to various chemicals and fuels.

2. Materials and methods

2.1. Materials

Three-dimensional, organotypic epidermal skin cultures (EPI-200) containing normal human epidermal keratinocytes were obtained from MatTek Corporation (Ashland, MA). A Dulbecco's Modified Eagle's (DME) based medium (EPI-100-NMM, MatTek Corporation) for maintaining the cultures was supplied by the manufacturer. Jet fuels (JP-8 and JP-8+100, Lot no. 3509) were obtained from Wright Patterson AFB, OH. Human IL-1α and TNF-α EIA kits were obtained from Pierce Biotechnology Inc., Rockford, IL. Rat IL-1α and TNF-α EIA