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Dermal microdialysis of inflammatory markers induced by aliphatic hydrocarbons in rats

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ABSTRACT

In the present study we made an attempt to understand the skin irritation cascade of selected aliphatic hydrocarbons using microdialysis technique. Microdialysis probes were inserted into dermis in the dorsal skin of hairless rats. After 2 h of probes insertion, occlusive dermal exposure (2 h) was carried out with 230 μl of nonane, dodecane and tetradecane, using Hill top chambers®. Inflammatory biomarkers such as substance P (SP), α-melanocyte stimulating hormone (α-MSH) Interleukin 6 (IL-6) and prostaglandin E2 (PGE_2) were analyzed in the dialysis samples by enzyme immunoassay (EIA). SP, α-MSH and IL6 were released in significant amounts following the dermal exposure of nonane and dodecane, whereas tetradecane did not induce any of these markers in significant amounts compared to control. Nonane increased the PGE_2 levels in significant amounts within 2 h of chemical exposure compared to dodecane and tetradecane. IL-6 response was found to be slow and 2–3-fold increase in IL-6 levels was observed after 5 h following nonane and dodecane application. The magnitude of skin irritation exerted by all three chemicals was in the order of nonane ≥ dodecane ≥ tetradecane. The results demonstrate that microdialysis can be used to measure the inflammatory biomarkers in the skin irritation studies and irritation response of chemicals was quantifiable by this method. In conclusion, microdialysis was found to be an excellent tool to measure several inflammatory biomarkers as a function of time after dermal exposures with irritant chemicals.

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1. Introduction

For many years assessment of skin response to irritants or noxious stimuli had to be performed from outside the skin surface. Thus, the methods have been limited to observing changes in skin color (erythema), transepidermal water loss (TEWL) and measuring temperature changes to gain information about dermal blood flow. The application of molecular biology to the study of these functional responses in animals or humans in vivo, has been proven difficult because these techniques require direct access to the dermal tissues while causing minimal disruption or damage to the local environment (Clough and Church, 2002). Microdialysis is a widely used technique to determine the endogenous and exogenous solutes in the extra-cellular space of tissues under minimally invasive conditions. The microdialysis principle is based on the sampling of soluble molecules from the interstitial spaces of the tissues where dialysis probe is inserted into the tissue and perfused at an optimal flow rate with a physiological solution (Muller, 2002; Schmidt et al., 2008). Initially microdialysis technique was used for the recovery of brain neuropeptides (Ungerstedt and Pycock, 1974) and later on this technique was adopted to use in various tissues like skin (Kreilgaard, 2002; Fulzele et al., 2007), adipose tissue (Lindberger et al., 2001), muscle (Newman et al., 2001) and gastrointestinal tract (Iversen et al., 1997). Very few studies have been carried out on the dermal microdialysis to assess the skin irritation of irritant chemicals. During the inflammatory cascade, numerous soluble components such as cytokines and chemokines (IL-1α, IL-1β, IL-6, IL-8 and IL-10), and free radicals are released in to the surrounding tissue medium (Angst et al., 2008). Application of dermal microdialysis to the area of skin irritation/inflammation can be a very useful tool to quantify the release of neuropeptides, cytokines or chemokines as a function of time.

Microdialysis technique is minimally invasive and provides biomarkers in relatively pure form and no further purification process is required for their quantification. In an earlier report, we have shown that dermal microdialysis can be used for skin irritation assessment of jet fuel (JP8), by measuring substance P (SP) and prostaglandin E_2 (PGE_2) levels (Fulzele et al., 2007). The current study is focused on the evaluation of skin irritation potential

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Table 1
In vitro recovery of SP, PGE_2, α-MSH and IL6 at two different flow rates.

Chemical	Molecular weight (kDa)	In vitro relative recovery (%)	
		2 μl/min	4 μl/min
SP	1.35	13 ± 0.49	2.76 ± 0.07
PGE_2	0.35	33.85 ± 0.72	34.73 ± 0.44
α-MSH	1.67	27.36 ± 6.7	21 ± 3.8
IL6*	21.7	15 ± 0.3[a]	11 ± 0.9[b]

* IL6 recovery was carried out with 0.1%, w/v BSA in Krebs ringer solution at a flow rate of (a) 0.5 μl/min and (b) 1 μl/min. Values represent mean ± S.D. ($n = 3$).

of selected aliphatic hydrocarbons (nonane, dodecane and tetradecane) by measuring various inflammatory biomarkers such as SP, PGE_2, alpha melanocyte stimulating hormone (α-MSH) and interleukin 6 (IL-6) using dermal microdialysis technique. We selected these biomarkers on the basis of their molecular weight, and easy recovery by microdialysis. Furthermore, these compounds are most commonly expressed in a wide variety of cutaneous irritation and inflammatory conditions in response to noxious chemicals after dermal exposures. Aliphatic hydrocarbons are the primary hydrocarbon components; C8–C14 hydrocarbons constitute about 74% of the jet fuel composition (Chou et al., 2002). Dermal exposures of individual hydrocarbons and quantification of biological markers as a function of time could provide an in depth understanding of events of skin irritation and inflammatory cascade by jet fuels. In the present study we selected nonane, dodecane and tetradecane as representative aliphatic hydrocarbons and measured inflammatory biomarkers by a microdialysis technique following dermal exposures.

Important aspect in the microdialysis studies is the selection of suitable probes for the recovery of various biomarkers; wide range of microdialysis probes of different molecular weight (MW) cutoffs and configurations (concentric and linear) are commercially available. The low MW compounds (2–5 kDa) can easily be recovered from linear probes with a MW cutoff below 30 kDa. However, recovery of large MW compounds (MW above 8 kDa) is challenging and the performance of microdialysis probe will depend on the several factors like flow rate, probe selection, perfusion fluid and nature of the substance to be recovered. With recent introduction of large MW cutoff probes up to 3000 kDa, it is possible to conduct microdialysis studies of large MW compounds including proteins and peptides. Angst et al. (2008) measured several cytokines and nerve growth factors using a larger MW cutoff microdialysis probe (3000 kDa) with an outside diameter of 400 μm. The 3000 kDa probe is not easily available for research purpose and most of the studies have used CMA 20 PES probe (100 kDa) for the recovery of cytokines (Ao and Stenken, 2006; Rosenbloom et al., 2005). In the present study we used both linear low molecular weight LM-10 (30 kDa) and CMA 20 (MW cut off 100 kDa) microdialysis probes for the recovery of four inflammatory markers, SP, PGE_2, α-MSH and IL-6 (Table 1). We selected these biomarkers on the basis of their molecular weight, and easy recovery by microdialysis. Furthermore, these compounds are most commonly expressed in a wide variety of cutaneous irritation and inflammatory conditions in response to noxious chemicals after dermal exposures. The outcome of these studies will help in evaluating the usefulness of microdialysis technique in determining the structure activity relationship of aliphatic hydrocarbons in terms of skin irritation.

2. Materials and methods

2.1. Materials

The nonane, dodecane, tetradecane, urethane, and halothane were obtained from Sigma–Aldrich (St. Louis, MO). α-MSH (Assay > 98%) was obtained from Biopeptide Co. LLC (San Diego CA). Bovine serum albumin (BSA) was obtained from Cell Signaling Technology (Denver, MA). Enzyme immunoassay (EIA) kits for SP and rat IL-6 were purchased from Cayman Chemicals (Ann Arbor, MC) and Pierce Biotechnology Inc (Thermo scientific, Rockford, IL), respectively. EIA kits for α-MSH and PGE_2 were procured from Phoenix Pharmaceuticals (Belmont, CA) and R&D Systems (Minneapolis, MN), respectively. Linear microdialysis probe, 30 kDa MW cut off and 10 mm dialysis membrane (LM-10) was procured from Bio-analytical Systems (West Lafayette, IN) and non-linear CMA20 microdialysis polyethersulfone (PES) probe with a 100-kDa MW cutoff and 10 mm dialysis membrane was obtained from CMA Microdialysis (North Chelmsford, MA). All other chemicals used in this research were of analytical or US pharmacopeial grade.

2.2. Animals

CD®(SD) hrBi hairless rats (250–300 g; Charles River Laboratories) were utilized for the studies. The protocol for in vivo experiments was approved by the Animal Care and Use Committee, Florida A & M University. The animals were acclimatized to laboratory conditions for 1 week prior to experiments and were on standard animal chow and water *ad libitum*. The temperature of the room was maintained at 22 ± 1 °C and the relative humidity of the experimentation room was found in the range of 35–50%. For microdialysis experiments the animals were anesthetized by intraperitoneal (i.p.) injection of Urethane (1.5 g/kg; 300 mg/ml; i.p.) with the anesthesia lasting for the entire period of experiment and after completion of the study animals were sacrificed with an overdose of halothane

2.3. In vitro recovery

To characterize the transfer rate of the probes, in vitro recovery of α-MSH and IL-6 was assessed. In case of SP, PGE_2 and α-MSH recovery studies, a LM-10 microdialysis probe was placed in a 5 ml vial containing 1000 pg/ml stock solution in Krebs–Ringer solution. The inlet end of the probe was connected to a CMA/102 microinjection pump (CMA microdialysis, North Chelmsford, MA) using a tubing connector while the outlet was connected to a CMA/142 micro-fraction collector (CMA microdialysis, North Chelmsford, MA). The probe was perfused with Krebs–Ringer solution at a flow rate of 2.0 and 4.0 μl/min for 60 min in two different experiments. Dialysate samples were collected every 30 min for 2 h. For the recovery of IL-6, CMA 20 microdialysis probe was placed in 2000 pg/ml IL-6 stock solution and perfused with 0.1%, w/v BSA dissolved in Krebs–Ringer solution at a flow rate of 0.5 and 1 μl/min, respectively. To obtain adequate amount of sample for IL-6 analysis, prior to the start of the experiment, 30 μl of 0.1%, w/v BSA solution was added to each vial and samples were collected every 60 min for 2 h. The inflammatory biomarkers concentration was measured in the dialysate (C_{out}) along with the concentration in the surrounding medium (C_m). The relative recovery was calculated by the equation: dialysate concentration (C_{out}) × 100/standard concentration (C_m).

2.4. In vivo studies

For in vivo recovery of SP, α-MSH and PGE_2 the linear microdialysis LM-10 probe was used. The inlet end of the probe was connected to a CMA microinjection pump using a tubing connector. In case of LM-10 catheter, Krebs–Ringer solution (138 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 11 mM $NaHCO_3$, 1 mM NaH_2PO_4) was pumped at a flow rate of 2 μl/min. For in vivo IL-6 recovery, 100 kDa microdialysis CMA-20 probe was used. The probe was perfused at a flow rate of 0.5 μl/min with 0.1%, w/v BSA dissolved in Krebs–Ringer solution. In order to ensure the proper fluid filling, prior to start of the experiment all the probes were perfused for 1 h with perfusion fluid. In case of IL-6 recovery studies the sample vials were filled with 30 μl perfusion fluid as described in in vitro recovery studies. Dermal implantation of the probes was carried out as per manufacturer's instructions. After implantation of microdialysis probes in the rat skin, the outlet end of the probe was connected to a refrigerated micro-fraction collector and throughout the study period samples were maintained at 4 °C. Two baseline dialysate samples were collected at 1 h intervals during initial equilibration period.

Occlusive dermal exposure (2 h) was carried out with 230 μl each of nonane, dodecane and tetradecane using Hill top chambers® (Babu et al., 2004a). Following dermal exposures, dialysate samples were collected for 5 h at 1 h intervals and stored at −80 °C until analyzed.

2.5. Evaluation of probe depth

To measure the probe depth after implantation and to determine the exact location of the probe in the dermis, histological evaluation was performed (Mathy et al., 2005). After biopsy, the tissue was fixed in 4% formalin solution and embedded in paraffin wax. Sections were cut perpendicular to the surface of the skin. Tissues were processed and stained with hematoxylin/eosin following standard procedure. Location of the probe in dermis was performed using optical microscope with graduated lens.

2.6. Analysis

The concentration of SP, PGE_2, α-MSH and IL-6 in the dialysis samples was analyzed by using EIA kits as per manufacturer's instructions.