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PRELIMINARY FINDINGS THAT KEROSENE ALTERS THE DISTRIBUTION OF TOPICALLY APPLIED BENZO[*A*]PYRENE IN MICE

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The dermal route is important in occupational exposure to polycyclic aromatic compounds (PACs), but other organs may be affected. We reported that kerosene-cleaning following treatment with used engine oil increased DNA adducts in the lungs of mice viz. *animals treated with used oil alone. To determine the mechanism we topically applied ^{3}H-BAP(100 nmol in 25 μL acetone) and washed half the mice with 25 μL kerosene 1 h after carcinogen application. Groups of four mice were sacrificed from 1 to 72 h after treatment. Lung, liver, and skin were harvested. The fraction of the radiolabel remaining in the skin of animals treated with benzo[*a*]pyrene (BAP) and washed with kerosene was significantly less than those not washed, beginning at 24 h ($p < .05$). Fractional distribution to the lungs and livers of these animals became significantly elevated. Kerosene increased transdermal water loss. Kerosene treatment*

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compromises dermal barrier function, enhances carcinogen absorption, and alters organ distribution.

Keywords benzo[*a*]pyrene, dermal route, mixtures, organotropism

There are millions of workers with occupational exposure to polycyclic aromatic compounds (PACs). For example, it is estimated that there are about one million automobile mechanics in the United States alone; a significant fraction of these may be exposed to PACs in used gasoline engine oil (UGEO) (1) which coats the engine parts being repaired. While only a fraction of all mechanics are working heavily with these oils, the occupation as a whole has been shown to be at increased risk for lung and urinary bladder tumors (2, 3). While the lung has been known to be a site of PAC carcinogenicity in humans, recently more attention has been paid to the dermal route as means of exposing the lung. Part of the reason for this interest is the relatively recent demonstrations using biological monitoring that the skin is a (if not the) major source of exposure for occupations such as roofers, pavers, coke oven workers, and aluminum workers (4–7). A second reason is that the physiology of blood flow from the skin favors distribution of absorbed materials to the lung. The consequences of genetic disease are far more serious in the lung compared to the skin which highlights the importance of this work.

While the skin has metabolic capacity, its total activity is a small fraction on a per weight basis of more active tissues such as lung and, particularly, liver. That carcinogen-DNA adducts and tumors have been seen in the skin indicates that this organ can metabolize PACs to electrophilic species. However, these lesions are seen only at the site of application and not at distal skin sites (8) which also indicates that the skin is not a selective target for these materials. Thus, there may be only fractional metabolism of a topically applied dose of PAC, with the remainder of the material entering the venous blood and being returned to the heart and then to the lung.

The metabolic activity of the lung is greater than that of the skin and the lung receives a diffuse dose of materials via the dermal route. Several workers have demonstrated the formation of high levels of DNA damage in the lung following topical application of PACs (9, 10). One of the interests of our laboratory is to determine the circumstances that favor increased passage of PACs through the skin to affect the lung. For example, it is not uncommon that workers will use solvents intended for cleaning tools for cleaning their skin following dermal application. While the

use of materials like gasoline and kerosene is not recommended, there has been little empirical data demonstrating deleterious effects. We recently showed that kerosene cleaning following topical application of UGEO significantly increased the levels of carcinogen-DNA adducts in mice as compared to animals that were treated with UGEO but cleaned with a commercially available cleaner (11). The purpose of the research reported here was to determine the mechanism for the increase in the DNA adduct level following "cleaning" with kerosene. We attempt to determine which of three alternative (not necessarily exclusive) mechanisms may be at work: Does kerosene simply solublize the PAC and facilitate passage through the skin? Does kerosene alter the epidermal barrier? Does kerosene circumvent first-pass metabolism in the skin? The following details our preliminary findings.

MATERIALS AND METHODS

C57 black male mice were purchased from Jackson Laboratories at 6–8 weeks old and quarantined for 1 week before use in the study. Animals' backs were shaved with clippers 24 h prior to treatment. A 100 nmol benzo[*a*]pyrene (BAP) (Sigma Chemical Co., St. Louis, Missouri, USA). solution was prepared in acetone (Fisher Scientific Co., Fair Lawn, New Jersey, USA) and 2 μCi of ^{3}H-BAP was added to each sample. The treatment groups included acetone only, kerosene only, and (^{3}H) BAP in acetone. A single topical application of 25 μL was applied to the shaved intrascapular region of the animals. This area was approximately 20 mm by 12 mm. One hour after treatment one-half of the animal's backs were washed with 25 μL of kerosene (Sunny Side, Wheeling, Illinois, USA). Animals were then sacrificed at 1, 4, 12, 24, 48, and 72 h. Each animal was injected i.p. with 0.4 mL of a solution of pentobarbital sodium (200 mg/kg Nembutal sodium solution (Abbott Laboratories, Abbott Park, Illinois, USA) for sacrifice. The lungs, liver, and skin were harvested for tritium radioactivity determinations. Each tissue sample was placed into a labeled cryovial (Nalge Co., Rochester, New York, USA) and immediately placed on dry ice. After harvesting procedures, all samples were stored at −80°C (Revco Freezer, Revco Scientific, Inc., Ashville, North Carolina, USA). Radioactivity levels were determined by mincing the tissue, solubilizing in 600 μL of Scintigest (Fisher Scientific) at 60°C for 1 h before neutralizing with acetic acid (Fisher Scientific). Then, 5 mL of ScintiLene (Fisher Scientific) was added to each vial and the samples were counted on a Packard Model 2200TR liquid scintillation analyzer (Packard Instruments Company, Downers Grove, Illinois, USA). Disintergrations/min (dpm) were automatically

determined from the transformed spectral index coupled with automatic efficiency correction and correction for luminescence and quenching.

The integrity of the skin as a barrier was assessed in two ways: by measuring transepidermal water loss (TEWL) in vivo and by measuring the flux of tritiated water across the skin in vitro. Damage to the skin barrier was assessed in vivo by measuring TEWL over the dorsal skin surface following light CO_2 anesthesia. TEWL data were collected over a 60 s interval using a DermaLab evaporimeter (Cortex Technology, Hadsund, Denmark) connected to a Compaq Armada 4120 laptop computer (Compaq Computer Corporation, Houston, Texas, USA). The device was controlled using cyberDERM software (CyberDERM, Inc., Media, Pennsylvania, USA).

Cutaneous barrier integrity was evaluated in vitro by measuring tritiated water flux across whole skin specimens using Franz diffusion cells. The diffusion cells (Dana Enterprises, West Chester, Ohio, USA) are comprised of two compartments, an upper donor compartment and a lower receptor compartment with a diffusional surface area of 0.79 cm^2. Excised dorsal whole skin was mounted between the two compartments with the dermal surface facing the receptor compartment and the stratum corneum surface facing the donor compartment. The receptor compartment was filled with PBS, pH 7.4 and was thermocontrolled at 37°C in a Reacti-Therm heating/stirring module (Pierce Chemical Company, Rockford, Illinois, USA), resulting in a skin surface temperature between 30°C and 32°C. A small magnetic stirrer was used to mix the contents of the receptor compartment. Following an hour equilibration, the stratum corneum surface was dosed with 150 μL of ^3H_2O, 0.4 μCi/mL (New England Nuclear, Boston, Massachusetts, USA). After 5 min, unabsorbed ^3H_2O was removed by wicking the skin surface with a dry cotton swab. Receptor fluid was collected after 1 h precisely. The amount of ^3H_2O that penetrated through the barrier was determined by adding 12 mL of Ultima-gold scintillation cocktail (Packard Bioscience Company, Meriden, Connecticut, USA) to the receptor solution, mixing well, and counting the radioactivity using a Beckman scintillation counter LS-600 (Beckman Instruments, Inc., Palo Alto, California, USA).

RESULTS AND DISCUSSION

Figure 1 shows the results of studies measuring the passage of tritiated water through the skin. The impact of kerosene cleaning on this parameter is indicated by the significant differences which are seen when kerosene treatments were performed. BAP had a small effect when added to the acetone, but this effect was not significant. A simple kerosene wash

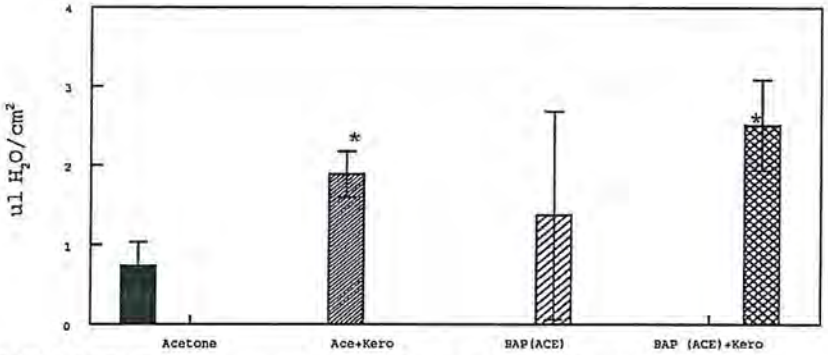

FIGURE 1. Rate of movement of tritiated water into the receptor fluid of Franz cells with mouse skin in vitro with indicated treatments. Asterisks indicate levels which are different significantly ($p > .05$) from the acetone treated group.

significantly increased the permeability of the skin to tritiated water. The addition of BAP with the kerosene cleaning reduced the barrier function of the skin to an even greater degree. Figures 2–4 similarly evidence the kerosene effect in the reverse direction when the loss of water through the treated surface of the skin is measured. In this case there was no clear impact of BAP above the acetone vehicle alone, while kerosene cleaning after acetone or BAP-acetone led to significant increases in water loss.

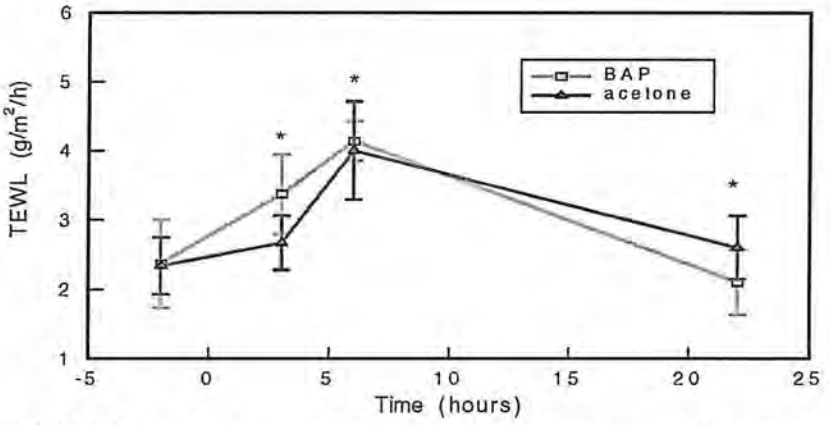

FIGURE 2. Transepidermal water loss in vivo of groups of animals treated with either BAP in acetone or acetone only. The data suggest that there are no differences between these groups of animals. The −2 h data are baseline measurements taken prior to agent administration.

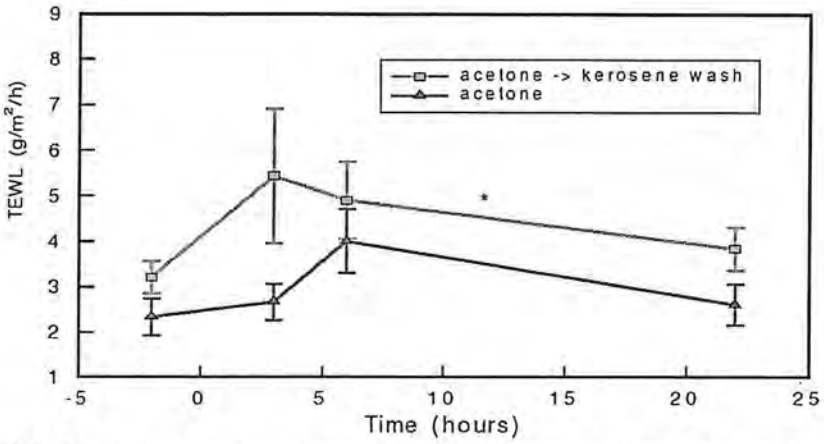

FIGURE 3. Transepidermal water loss in vivo in groups of animals treated with acetone alone or acetone then washed 1 h later with kerosene. The acetone + kerosene animals showed significantly more water loss than the acetone control at 3 and 22 h after washing. The −2 h data are baseline measurements taken prior to agent administration.

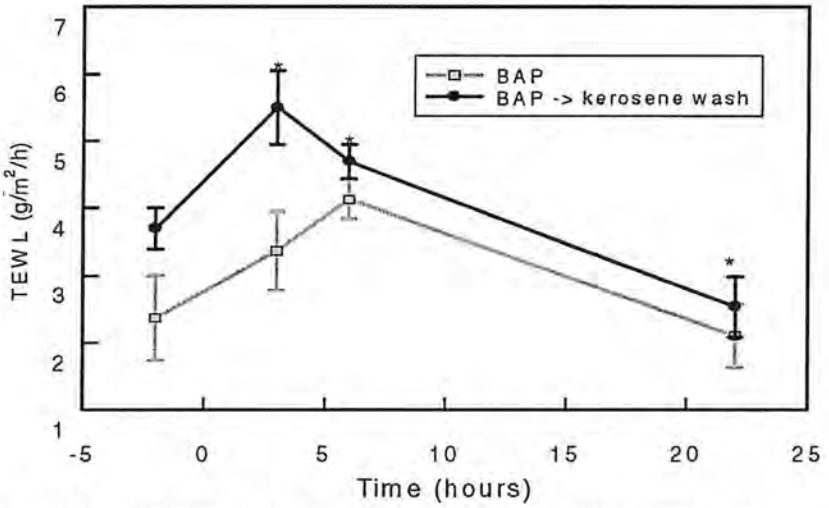

FIGURE 4. Transepidermal water loss in groups of animals treated with BAP in acetone or BAP in acetone and then washed 1 h later with kerosene. The kerosene-treated group was significantly different at 3 h after the treatment. The −2 h data are baseline measurements taken prior to agent administration.

Taken together these data suggest strongly that kerosene has a deleterious effect on the barrier function of the skin; significantly reducing the ability of the skin to protect against loss of internal water, a critical function.

Figure 5 shows the distribution of tritiated BAP in the tissues of mice treated once with either BAP-acetone, or BAP-acetone and washed with kerosene. No difference is seen the first 12 h after application of the BAP. Beginning at 24 h, a significant and consistent decrease in the fraction of the radiolabeled BAP remaining in the skin is noted. At the same time, the fraction of BAP in the liver and lungs of the kerosene treated animals increases significantly as compared to those treated with BAP-acetone (Figures 6 and 7).

These data corroborate our earlier work that kerosene application as a cleaner increases the levels of DNA damage in the lungs of mice treated topically with BAP or UGEO. We show that the mass of BAP transferred to the lung is significantly increased beginning at 24 h following exposure and that the difference remains significant through 72 h following treatment. This follows a single application of the BAP and kerosene. It can readily be seen that an increase in the amount of BAP in the lung

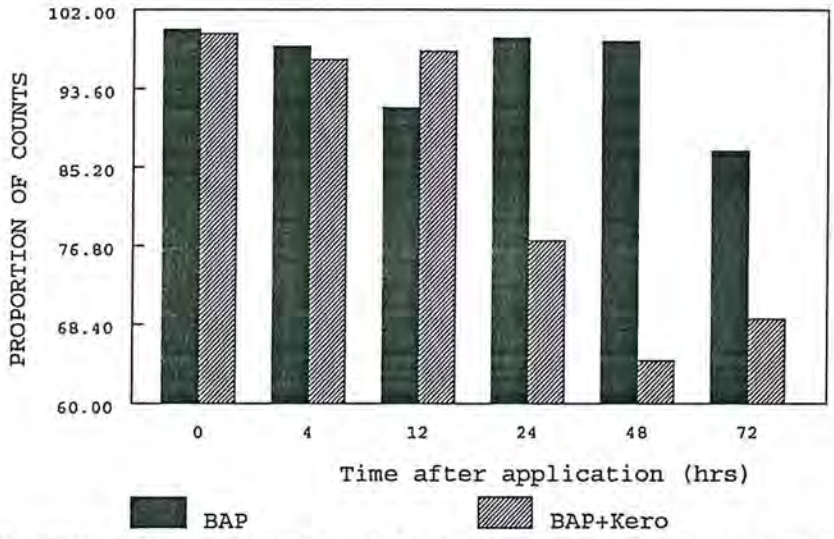

FIGURE 5. Time course of the tritiated BAP residence in the skin for groups of animals treated either with BAP in acetone or BAP in acetone followed 1 h later by a wash of the area with 25 μL of kerosene. Differences between the groups are statistically different ($p > .05$) beginning at 24, 48, and 72 h after treatment.

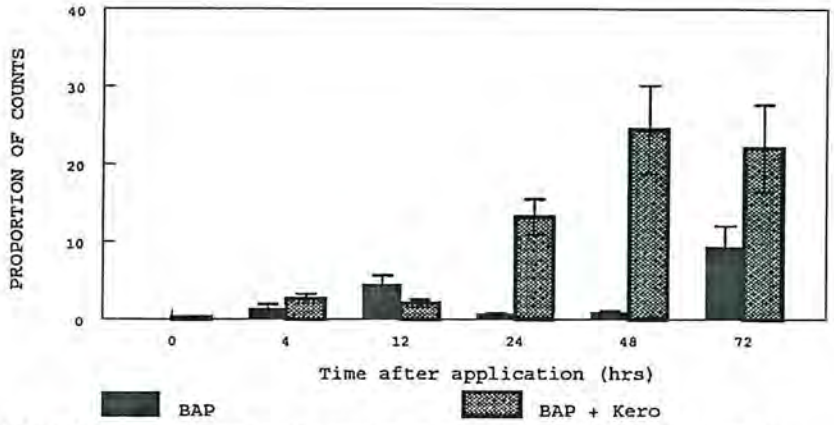

FIGURE 6. Time course of distribution of tritiated BAP into the liver of groups of mice treated either with BAP in acetone of BaP in acetone and then washed 1 h later with 25 μL kerosene. There were significant differences at 24, 48, and 72 h in the fraction of the total counts in the liver of the kerosene-treated animals.

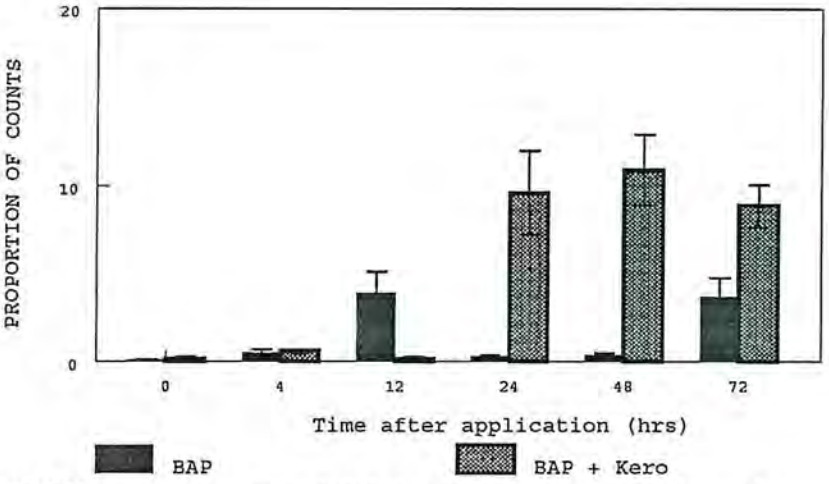

FIGURE 7. The time course distribution tritiated BAP into the lungs of groups of animals treated topically with either BAP in acetone or BAP in acetone followed 1 h later by a wash with kerosene. There were statistically significant differences in the groups at 24, 48, and 72 h after the treatment.

would lead to an increase in DNA adducts. At least one mechanism for the increased internal organ levels appears to be a decrease in the ability of the skin to act as a barrier. The skin of mice treated with kerosene increasingly allows the passage of tritiated water into the body (as measured in vitro) and of water to the atmosphere.

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