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MICROSCOPE IMMERSION OILS: EFFECTS OF SKIN APPLICATION ON CUTANEOUS AND HEPATIC DRUG-METABOLIZING ENZYMES*

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Abstract—Cutaneous application in rats of two widely used microscope immersion oils known to contain the environmental pollutant chemicals, polychlorinated biphenyls, was evaluated. After skin application of 1 or 10 μl of these oils, significant increases in liver weight, microsomal protein, cytochrome P-450 and in the metabolism of ethylmorphine and benzo(a)pyrene resulted. Cutaneously treated sites also showed large increases in benzo(a)pyrene hydroxylation. Immersion oil treatment resulted in the induction of hepatic cytochrome P-448 and caused spectral changes in the hemoprotein analogous to those produced by polycyclic hydrocarbons, such as 3-methylcholanthrene. A single cutaneous application resulted in significant enhancement of the same parameters; this response reached a maximum at 2 days and lasted at least 10 days with each immersion oil tested. The data presented show that extremely small exposures of the skin to microscopic immersion oils lead to marked induction of hepatic and cutaneous drug-metabolizing enzymes.

The polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants that are extremely resistant to normal pathways of biodegradation within the ecosystem. Although these chemicals have been used for over 40 years in a variety of industrial applications, it is only in the last few years that their pharmacological and toxicological effects have come to be appreciated [1]. A major reason for recent interest in environmental PCBs lies in the fact that these compounds have been shown to be potent inducers of the hepatic mixed-function oxidase system. A unique feature of the PCBs is that they possess the inducing properties of both the phenobarbital and the polycyclic hydrocarbon class of inducing substances [2, 3]. For example, they induce ethylmorphine *N*-demethylase, NADPH cytochrome *c* reductase, cytochrome P-448 and benzo(a)pyrene hydroxylase activity in the liver of experimental animals.

PCBs have been detected in the tissues of numerous animals and birds [4] as well as in human adipose tissue [5] and human breast milk [6]. Accidental ingestion of rice oil contaminated with PCBs resulted in several hundred Japanese individuals developing chloracne, loss of vision, jaundice, edema and protracted episodes of abdominal pain [7]. Newborn infants of poisoned mothers had skin discoloration that may have been due to the passage of PCBs through the placenta. Skin application of PCBs to rabbits has resulted in hepatic porphyria [8]. Increased urinary excretion of uro- and coproporphyrins has also been observed in long-term feeding of female rats with commercial mixtures of PCBs [9].

In a previous study [3] we showed that PCBs, after cutaneous application, are rapidly absorbed through rat skin, leading to induction of hepatic cytochrome P-450 and drug-metabolizing enzymes. Furthermore, the directly treated skin sites demonstrated enhanced activity of the carcinogen-metabolizing enzyme, aryl hydrocarbon hydroxylase. This suggested that percutaneous absorption of the PCBs may be a significant portal of entry to the organism for these compounds and, in addition, suggested that perhaps the skin is capable of metabolizing topically applied environmental chemicals.

A newly reported source of exposure to PCBs is in microscope immersion oils and a recent report showed that the majority of such immersion oils currently marketed in the United States contain 30–45 per cent PCBs [10]. Preliminary studies from our laboratory have shown that one microscope immersion oil caused the induction of cytochrome P-448 and enhanced the activity of cytochromes P-450- and P-448-dependent hepatic mixed-function oxidases after intraperitoneal injection [11]. Since immersion oils are widely used by microscopists in many scientific disciplines as well as by school teachers and students in high schools and colleges, we have extended these studies to assess the potential effects of skin exposure to the immersion oils. The present study was designed to assess: (1) the comparative inducing properties of the two most widely used immersion oils in the United States after skin application; (2) the duration of the inducing effects after a single skin exposure to these oils; and (3) to ascertain which specific component of the immersion oils is responsible for their enzyme induction effects.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 80–100 g were used. After administration of light ether anesthesia, a 3 × 3 cm area in the nuchal region was carefully shaved with electric clippers so as not to abrade the skin. Visibly lacerated animals were not used. The animals were kept in stainless steel cages and allowed food and water *ad. lib.*

Among the most widely used immersion oils in the United States are those produced by the Cargille

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Laboratories, Cedar Grove, N.J. Mr. John S. Cargille kindly supplied samples of the two major types designated A and B. In addition, the constituents of these products include mineral oil and polybutene in varying percentages which basically serve to change the viscosity characteristics of the immersion oils. Immersion oils A and B contain the following constituents, per dent by weight respectively: mineral oil, 47 and 29; polybutene, 19 and 43; and Aroclor 1254, 34 and 28. Aroclor 1254 is the trade name of one PCB mixture currently in wide use in the United States. Because of their viscosity, the immersion oils were diluted 1:5 with mineral oil and 50 μl of the diluted sample was applied to the skin so as to deliver the equivalent of 10 μl of undiluted immersion oil A or B. Similarly, the oils were diluted 1:50 with mineral oil and 50 μl was applied to deliver the equivalent 1 μl of immersion oil A or B. The oils were applied to the skin sites with an Eppendorf micropipette and spread over the 3 × 3 cm area with the side of the pipette. No attempt was made to rub the oil into the skin. Animals received either 10 or 1 μl equivalent doses of the immersion oil daily for 6 days and animals were sacrificed on day 7. Control animals received mineral oil only. In other experiments, rats received a single 10-μl application of immersion oil A or B and were sacrificed at various time intervals thereafter.

After sacrifice, the livers and the treated skin sites of the animals were removed and placed in 1·15% KCl solution. Washed hepatic microsomes were prepared and cytochrome P-450 [12], ethylmorphine *N*-demethylase [13], NADPH cytochrome *c* reductase [14] and the ethyl isocyanide difference spectra [15] were determined as described previously. Hepatic benzo(a)pyrene hydroxylase was determined on whole liver homogenate by the method of Nebert and Gelboin [16]. Skin sites to which the oils had been applied were prepared and homogenized and benzo(a)pyrene hydroxylase activity was determined as previously described [3].

RESULTS

Comparative effects of skin application of immersion oils A and B on hepatic drug-metabolizing enzymes and cytochrome P-450. The effects of applications of the immersion oils are shown in Table 1. At a dose of 10 μl/day for 6 days, both immersion oils caused large increases in liver weight and microsomal protein. Cytochrome P-450 content and ethylmorphine *N*-demethylase activity were increased three- to four-fold. NADPH cytochrome *c* reductase was significantly increased and 15- to 20-fold increases in benzo(a)pyrene hydroxylase activities were observed. In addition, both the immersion oils caused a marked increase in the ratio of the 455:430 nm peaks of the ethyl isocyanide difference spectrum of reduced microsomes. No marked differences were observed between the respective enzyme induction effects of immersion oils A or B. As also shown in Table 1, even at doses of 1 μl, there were significant though lesser effects on liver weight, microsomal protein, cytochrome P-450 content, ethylmorphine *N*-demethylase and the change in the ratio of the 455:430 nm peaks. Interestingly, the lower dose of the immersion oils caused essentially the same degree of benzo(a)pyrene hydroxylase induction as did the larger dose. Unlike the larger dose, treatment with 1 μl of immersion oil A did not cause a significant increase in NADPH cytochrome *c* reductase activity.

Effects of immersion oils A and B on the carbon monoxide and ethylisocyanide difference spectra of hepatic microsomes. Ten-μl cutaneous applications of immersion oils A and B for 6 days caused changes in the CO and ethyl isocyanide difference spectra as shown

Table 1. Effect of skin application of microscope immersion oils on hepatic drug-metabolizing enzymes

Measurement	Controls	10 μl		1 μl	
		Oil A	Oil B	Oil A	Oil B
Liver wt (g/g body wt)	0·046† ± 0·001	0·076‡ ± 0·003	0·063‡ ± 0·001	0·055‡ ± 0·001	0·056‡ ± 0·001
Microsomal protein (mg/g liver)	20·65 ± 0·71	26·56‡ ± 0·70	32·20‡ ± 1·71	26·00‡ ± 0·40	25·10‡ ± 0·80
Cytochrome P-450 (nmoles/mg protein)	0·637 ± 0·064	2·658‡ ± 0·063	2·280‡ ± 0·081	2·084‡ ± 0·056	2·026‡ ± 0·094
Ethylmorphine *N*-demethylase (μmoles HCHO/mg protein/hr)	0·309 ± 0·024	0·941‡ ± 0·061	0·996‡ ± 0·022	0·803‡ ± 0·011	0·904‡ ± 0·028
Benzo(a)pyrene hydroxylase (nmoles OHBP/mg protein/hr)	3·04 ± 0·26	69·08‡ ± 1·01	48·73‡ ± 2·44	71·48‡ ± 2·72	44·84‡ ± 3·94
Ratio of 455:430 peaks	0·59 ± 0·01	1·60‡ ± 0·02	1·28‡ ± 0·02	1·29‡ ± 0·03	1·02‡ ± 0·03
455 peak Δ O.D. 455–500	0·060 ± 0·005	0·521‡ ± 0·015	0·515‡ ± 0·022	0·405‡ ± 0·015	0·393‡ ± 0·040
430 peak Δ O.D. 430–500	0·101 ± 0·007	0·327‡ ± 0·015	0·401‡ ± 0·021	0·314‡ ± 0·018	0·386‡ ± 0·042
NADPH-cyt. c reductase (nmoles cyt. c red./mg protein/min)	70·84 ± 3·43	179·91‡ ± 9·33	110·00‡ ± 13·45	77·16 ± 3·42	83·39‡ ± 5·88

* Immersion oil A or B, dissolved in mineral oil, was applied in amounts indicated to the nuchal region daily for 6 days and animals were killed on day 7.

† Control values represent mean ± S.E. for ten rats; treated values represent mean ± S.E. for five rats.

‡ Values significantly different from control values ($P < 0·05$).

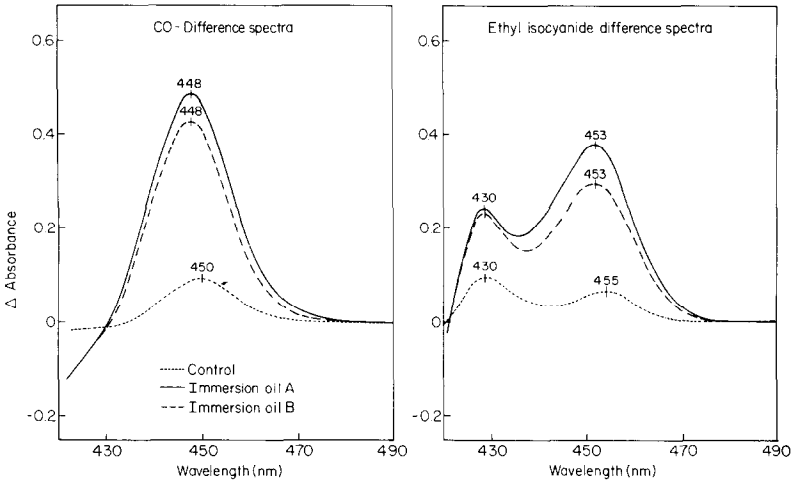

Fig. 1. Carbon monoxide and ethylisocyanide difference spectra of liver microsomes from untreated rats and rats treated with immersion oils A or B. Ten μl of the immersion oil was applied to the nuchal region daily for 6 days.

in Fig. 1. The CO difference spectrum of microsomes from control rats showed the expected absorbance maximum at 450 nm [12]. After treatment with immersion oil A or B, there was a marked induction of hepatic cytochrome P-450 and the absorbance maximum was shifted to 448 nm. The ethyl isocyanide difference spectrum of reduced microsomes from control rats demonstrated two peaks with absorbance maxima at 455 and 430 nm, respectively, as previously reported [17]. However, the difference spectra of microsomes from rats treated with immersion oils A or B showed a shift in the absorption maximum of the 455 nm peak to 453 nm. The 430 nm peak was unaffected. The ratio of the 455:430 nm peak increased from 0·59 observed in control animals to 1·60 and 1·38, respectively, in animals treated with immersion oils A and B. These effects on the spectral properties of cytochrome P-450 are similar to those observed in rats pretreated with polycyclic hydrocarbons such as 3-methylcholanthrene [18, 19].

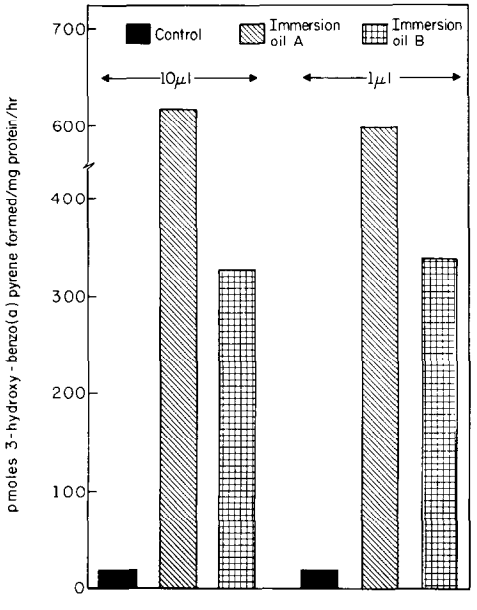

Fig. 2. Induction of skin benzo(a)pyrene hydroxylase by microscope immersion oils A and B. Skin sites to which immersion oils A or B had been applied at a dosage of 1 or 10 μl of oil/day for 6 days were assayed for benzo(a)pyrene hydroxylase. Each value represents the mean of five.

Effects of cutaneous application of immersion oils A and B on skin benzo(a)pyrene hydroxylase. As shown in Fig. 2, cutaneous application of 1 or 10 μl of immersion oils A or B caused 25- to 40-fold enhancement of skin benzo(a)pyrene hydroxylase activity. Immersion oil A appeared to be about twice as potent as immersion oil B in this regard. However, the percutaneous absorption of the two immersion oils appeared to be similar, at least insofar as their effects on hepatic drug-metabolizing enzymes and cytochrome P-450 was concerned (Table 1).

Time course of the comparative effects of a single skin application of the immersion oils on hepatic and skin microsomal enzymes. Ten μl of immersion oils A or B was applied to skin of the nuchal region on day zero and animals were sacrificed at various intervals thereafter. These data are shown in Fig. 3. Both immersion oils had maximum inductive effects on day 2 after treatment and there was a gradual decline thereafter. Immersion oil A had greater effects on all parameters than did immersion oil B. Approximate doubling of cytochrome P-450 and ethylmorphine *N*-demethylase activity persisted 10 days after a single cutaneous exposure. Hepatic benzo(a)pyrene hydroxylase showed 12- and 9-fold enhancement by day 2 after treatment with immersion oils A and B respectively. Although this gradually decreased, at 28 days immersion oil A still showed a 4-fold increase of benzo(a)pyrene hydroxylase, whereas the hydroxylase activity of rats treated with immersion oil B had returned to control values. Skin benzo(a)pyrene hydroxylase activity was markedly enhanced at day 2 by both oils but essentially had returned to normal by day 14.

Effect of polybutenes on hepatic drug-metabolizing enzymes and on skin benzo(a)pyrene hydroxylase. In order to make certain that the enzyme induction effects of the immersion oils were due to their PCBs content, the effects of polybutene dissolved in mineral oil were studied as well. Application of 10 μl of the polybutene to rat skin for 6 days had no significant effect on liver weight, microsomal protein, P-450 or drug-metabolizing enzyme activities in the liver. Similarly, in data not

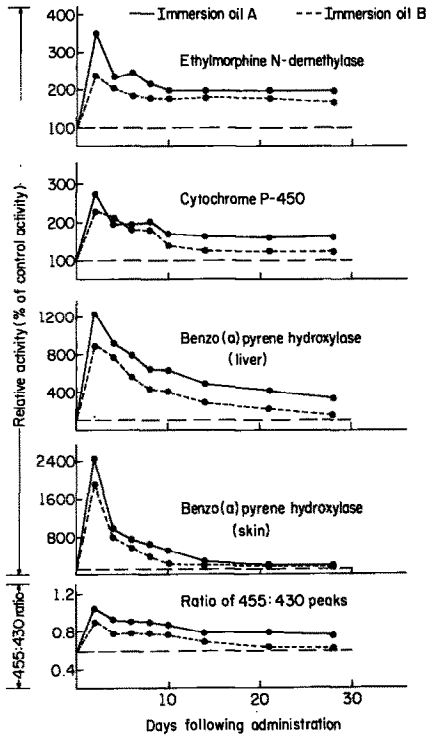

Fig. 3. Comparative effects of immersion oils A and B after a single percutaneous dose of 10 μl. Rats were killed at various time intervals after a single cutaneous dose and enzyme activities assayed as described in Materials and Methods. Each point represents the mean of three pooled livers or skins. Mean hepatic control values were: ethylmorphine *N*-demethylase, 0·323 μmole HCHO/mg protein/hr; cytochrome P-450, 0·712 nmole/mg protein; benzo(a)pyrene hydroxylase, 4·12 nmoles hydroxybenzpyrene/mg protein/hr. Mean control value for skin benzo(a)pyrene hydroxylase was 12·72 pmoles hydroxybenzpyrene/mg protein/hr.

shown here, application of 10 μl of mineral oil for 6 days showed no significant increases in any of the hepatic or skin enzymic activities when compared with those obtained from untreated rats. These data indicate, therefore, that any enzyme induction effects (cutaneous and hepatic) of the whole microscope immersion oils studied here are due to their PCBs content.

DISCUSSION

Immersion oils are widely used substances in microscopy and the skin becomes a point of contact with these immersion oils for many laboratory workers. In view of a recent report showing that microscope immersion oils contain 30–45 per cent PCBs, it seemed of considerable interest to investigate potential hazards to which such workers may be exposed. Immersion oils A and B utilized in this study differ in their viscosity and fluorescence characteristics. Chemically, they differ in composition only in the percentages of mineral oil, polybutene and the PCB mixture, Aroclor 1254. The present studies show that after application to rat skin the immersion oils cause marked increases in hepatic microsomal enzymes which are cytochrome P-450-dependent. Skin application of doses as low as 1 μl/day for 6 days caused significant increases in liver weight, microsomal protein, three-fold increases in cytochrome P-450 content and ethylmorphine *N*-demethylase activity, and 15- to 25-fold increases in benzo(a)pyrene hydroxylase in the liver. The inductive effects on the hepatic enzymes observed with skin application of 1 μl of the oils were similar in magnitude to those observed with 10 μl. Cutaneous application of the immersion oils caused 25- to 40-fold enhancement of skin benzo(a)pyrene hydroxylase activity. However, the inductive effects on skin hydroxylase activity were greater with immersion oil A than with immersion oil B. The higher viscosity of oil B could interfere with its ability to reach skin sites where such inducible enzyme activity is localized.

After a single application of 10 μl of the immersion oils, the inductive effects peaked at 2 days and the increases in hepatic ethylmorphine *N*-demethylase, benzo(a)pyrene hydroxylase, cytochrome P-450 and the changes in spectral properties of the hemoprotein were observable for at least 4 weeks. These increases were more pronounced with immersion oil A than with immersion oil B. Benzo(a)pyrene hydroxylase activity of the skin sites to which the oils had been applied also peaked at 2 days but returned essentially to normal by day 14. This may be related to the fact that the epidermis is constantly being renewed and hence may have completely turned over by 14 days after skin application. The decrease in skin activity of aryl hydrocarbon hydroxylase at 14 days suggests that the rapidly dividing epidermis is the primary site of inducible aryl hydrocarbon hydroxylase activity in the skin, since the slowly turning over dermis would not normally have undergone significant change 14 days after the single treatment. The persistence of the inductive effects after skin exposure to the immersion oils has added significance in light of studies by Kuratsune and Masuda [20]. They showed that in individuals handling carbonless copy paper containing PCBs, only one-third of the PCBs adherent to the fingers could be removed by ordinary hand washing with soap and water. This suggests that skin contact with PCBs may be prolonged and this could lead to significant percutaneous absorption.

Inducers of the hepatic drug-metabolizing enzymes have been generally categorized into the phenobarbital or the polycyclic hydrocarbon classes of inducing substances [21, 22]. Immersion oils A and B share the inducing properties of both these classes of inducers. Specifically, like the barbiturates, both immersion oils induce ethylmorphine *N*-demethylase, liver weight, microsomal protein and NADPH cytochrome *c* reductase activity. Like the polycyclic hydrocarbon class of inducers, immersion oils A and B induce the formation of cytochrome P-448, cause an increase in the ratio of the 455:430 nm peaks of the induced microsomal heme-protein using ethylisocyanide as the ligand and cause marked enhancement of aryl hydrocarbon hydroxylase activity. The latter may be of considerable importance, since enhanced activity of aryl hydrocarbon hydroxylase may have relevance to the induction of tumorigenesis in animal and human systems. Moreover, since immersion oils possess the inducing properties of barbiturates and since barbiturates have been found to stimulate the metabolism in man of drugs such as diphenylhydantoin, bishydroxycoumarin and phenylbutazone [23, 24], it would be of considerable importance to determine if individuals handling immersion oil in the laboratory or in industrial exposures

possess an enhanced capacity to metabolize drugs as compared to the general population.

It must be emphasized that these immersion oil studies were performed in rats after removing protective hair. The epidermis of hairy animals is much thinner than that of relatively hairless man. It is, therefore, possible that the thicker epidermis of man is a much better barrier to the percutaneous absorption of the PCBs found in microscope immersion oils. Nonetheless, the observation that cutaneous benzo(a)pyrene hydroxylase activity is induced by PCBs indicates that further studies to evaluate skin hydroxylase activity in individuals handling these oils should be undertaken. Further studies are also needed to evaluate the significance in man of skin exposure to a class of potentially toxic environmental chemicals which have the capacity to induce hepatic drug-metabolizing enzymes after percutaneous absorption.

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