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Assessment of Ornithine Decarboxylase (ODC) Induction Potential of Cutting Oils over Mouse Skin

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Cutting oils are complex mixtures of hydrocarbons, comprising of certain fractions of mineral oil which are being frequently used as lubricating and heat transferring agents in various machine and tool industries (Cruickshank and Squires 1951). Although skin carcinoma has been reported to develop among the industry workers exposed to the mineral oils (Cruickshank and Squires 1951), not much has been done on the biochemical events related to the carcinogenic/cocarcinogenic potential of the cutting oils. Besides this, no report is available on the role of cutting oils with respect to the multistage process of tumorigenesis. In the present study ornithine decarboxylase (ODC) enzyme levels have been studied in the mouse skin after being exposed to the cutting oils. ODC is considered to be marker enzyme for assessing the activity of tumour promoters in the multistage initiation-promotion tumorigenesis and has been found to increase multifold in neoplastic, proliferating and regenerating tissues (Russel 1973; Bacharch 1976; O'Brien et al. 1975). Although the exact mechanism of ODC induction and its role in tumour promotion has not been yet established, still the ODC induction is regarded by and large to be an essential event in tumorigenesis (O'Brien et al. 1975; O'Brien 1976).

On the basis of ODC induction potential, in the present study we wanted to have an idea whether the cutting oils possessed any tumour promoting activity. Hence, the level of ODC activity after the topical application of fresh (unused) as well as used (after industrial use) cutting oils on the mouse skin was determined. 12-O-tetradecanoyl phorbol-13-acetate (TPA), a potent tumour promoter, was used as a positive control.

MATERIALS AND METHODS

Cutting oil samples, both fresh and used, were obtained from an industry making nuts and bolts at Bombay, India. TPA was purchased from Sigma Chemical Co., USA and DL-(1- C) ornithine hydrochloride from Amersham Corp., Arlington Heights, IL, USA. Rest of the

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chemicals were of analytical grade and purchased form local commercial sources.

Female Swiss albino mice (8-10 wks of age) were kept on synthetic pellet diet and water ad libitum. Two or 3 days prior to the experiment, dorsal hair was shaved in the interscapular region with surgical hair clippers; and mice, only in the resting phase of hair growth, were used for the experiment.

Animals were treated topically with TPA or fresh/used cutting oil at different time intervals with various doses, in the manner as described in the results/figures. After killing the animals by decapitation, skin was separated and epidermal extract for the enzyme assay was prepared according to the method of Verma and Boutwell (1980). The 1.5 ml of reaction mixture contained 0.04 M phosphate buffer (pH 7.2) and pyridoxal phosphate, dithiothreitol, EDTA and DL-ornithine in the optimum concentrations along with the suitable aliquot of epidermal extract equivalent to 0.50 to 0.8 mg of protein and 0.25 μCi of DL ($1-^{14}$ C) ornithine. Reaction was allowed to run for 1 hr at 37°C before the addition of suitable aliquot of 50% W/V citric acid solution. Enzyme activity was determined by measuring the $^{14}CO_2$ released from DL-($1-^{14}$ C) ornithine and trapped in hyamine hydroxide (Verma and Boutwell 1980). Corresponding blanks and standards were run simultaneously. Enzyme activity was calculated in terms of pmoles of ^{14} CO_2 released/mg protein/hr. Protein contents were measured as per Lowry's method (Lowry et al. 1951).

RESULTS & DISCUSSION

Topical application of 50 l of neat, used/fresh cutting oil on the mouse skin led to an increase in the ODC activity measured at 2,4,5,6 and 7 hr. after the treatment. The ODC induction was found to be maximum between 4-5 hr after oil application, when compared with the unexposed control animals. For positive control, TPA (10 μg in 0.2 μl acetone) was applied in a similar manner which induced ODC tremendously after 4 hr of treatment (Fig. 1).

For eliciting the function of dose dependence, 25, 50, 100 and 200 μl of used/fresh oil was painted on the back skin and ODC activity was measured 5 hr after treatment. It was found that the maximum effect of cutting oils over ODC induction was between 4-5 hr (Fig. 1), but no linear dose dependent enzyme induction was noticed in the epidermis after oil treatment (Fig. 2).

Another experiment was planned to see if multiple applications of oil could lead to still higher levels of ODC induction. Varying doses (50, 100 & 200 μl) of neat, fresh/used oil over mouse skin were painted daily for four days. ODC activity was estimated 5 hr after the last application. It was observed that enzyme activity was increased slightly as compared to that after single application of the oils (Fig. 3). However, in this experiment used oil induced more ODC activity as compared to that of fresh oil.

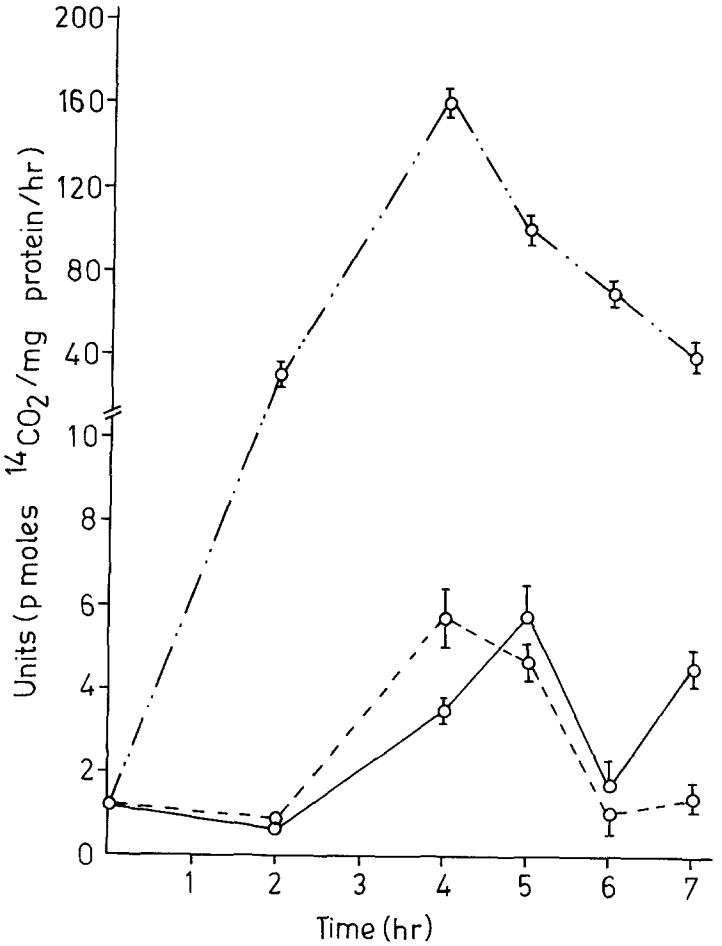

Figure 1. Time dependent effect of cutting oils on mouse skin ODC activity. Groups of mice were treated with 50 μl of fresh o——o or used cutting o- - -o oil or 5 μg TPA o—·—o. Animals were killed for enzyme assay at various times indicated after the application of cutting oil. Each value represents the average of triplicate determinations of enzyme activity from soluble extracts prepared from four animals.

These results demonstrate that the cutting oils possess very low mouse skin ODC induction potential. We can, thus, conclude that these oil varieties may not be the skin tumour promoters of great significance whatsoever.

Here we would further like to mention that the oils used for this study contained different known/unknown compounds, i.e. polycyclic aromatic hydrocarbons (PAHs), nitrosamines, fat, chlorine and

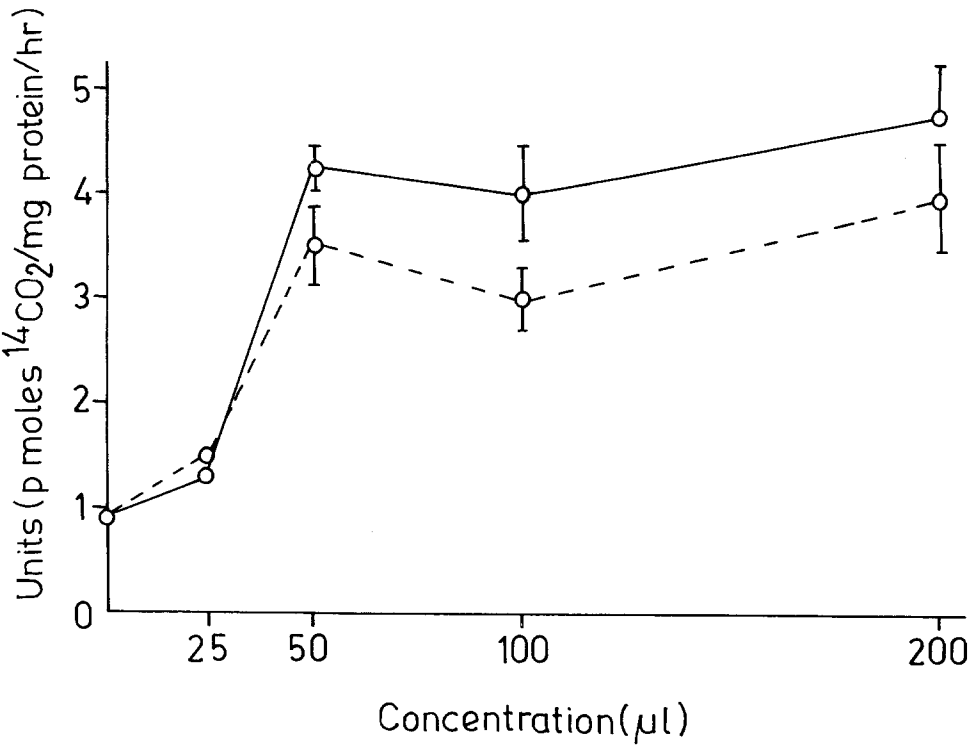

Figure 2. Effect of the dose of cutting oils on mouse skin ODC activity. Mice were treated with different doses of fresh o——o or used o----o cutting oil and enzyme activity was measured 4 hr after oil treatment. Each value represents the mean of triplicate determinations of enzyme activity from the soluble extracts prepared from four animals.

probably metals (after industrial use), etc. At this stage, we cannot say what exact role is being played by these substances in the cutting oils. On the other hand, in contrast to our observations, workers have reported ODC induction in the mouse skin even after a single application of other mineral oils (Rahimtula et al. 1987). It is surmised that the difference in the observations is probably due to the difference in the constituents of the oils.

It has been reported that ODC induction is an essential component of tumour promotion (O'Brien 1976) but not all ODC inducers are necessarily tumour promoters (Marks et al. 1979). We have already shown the aryl hydrocarbon hydroxylase (AHH) induction by these

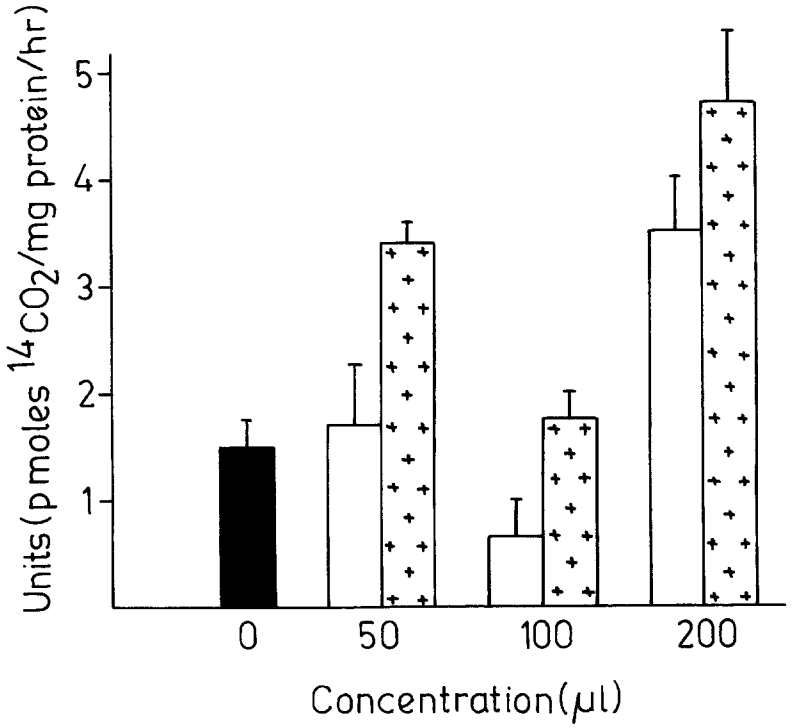

Figure 3. Effect of multiple application of cutting oils on skin ODC activity. Mice were treated with 50, 100 and 200 μl of fresh ▭ or used ⊠ cutting oil daily for four days and enzyme activity was measured 4 hour after the last application of the oils. Each value represents the mean of triplicate determinations of enzyme activity from soluble extracts prepared from four animals. Control ■.

cutting oils (Agarwal et al. 1986) and have found tumour initiating potential of these oils following the initiation-promotion protocol of multistage carcinogenesis (Gupta and Mehrotra 1989).

To make sure that these cutting oils have no tumour promoting potential, the chronic animal bioassay following the 2 stage initiation-promotion protocol will be required to be done. Besides this, fractionation of these oils and study of ODC induction/tumour promoting potential of the individual fractions thus obtained will be needed to assess the tumour promoting activity in these fractions.

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