Toxicity of Aromatic Hydrocarbons on Normal Human Epidermal Cells in Vitro

Michael H. Dietz and B. Allen Flaxman

The Skin and Cancer Hospital of Philadelphia, Department of Dermatology, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140

SUMMARY

Outgrowth cultures of normal human epidermis were continuously exposed for 4 days to the aromatic hydrocarbons 3-methylcholanthrene and benzo[a]pyrene at a concentration of 1 µg/ml. After exposure to the hydrocarbons, the amount of epithelial outgrowth was markedly reduced in 10 of 13 3-methylcholanthrene and 14 of 14 benzo[a]pyrene cultures when compared with that present before treatment. Giant cells and a more disorderly pattern of growth were also observed in the treated cultures weeks earlier than similar occurrences in controls. Fibroblast growth was unaffected. There was no evidence of malignant transformation.

INTRODUCTION

Many studies have demonstrated that carcinogenic aromatic hydrocarbons such as MCA and BP may have both a toxic effect on normal rodent cells in culture and the ability to induce malignant transformation. In contrast, other studies have failed to demonstrate either neoplastic change or toxic response of human and primate cells in vivo and in vitro in the presence of these same hydrocarbons (1, 5, 17). One study, however, has shown epithelial hyperplasia of human tissue in vitro in the presence of BP (14). In general, in nonprimate systems toxicity is shown by normal cells, whereas transformed cells are resistant to this effect (6, 7). However, the reliability of this observation may depend on the cellular environment (7). Although toxicity has been thought to be correlated with transformation, a direct relationship has not been shown (6, 13). It is tempting, nevertheless, to consider the possibility that susceptibility to the toxic effects might indicate the potential for achieving transformation, because evidence suggests that both processes may involve metabolism of the hydrocarbons by an inducible aryl hydroxylase enzyme system (6, 9, 10). One product of this system may be a toxic metabolite, while another may be the active carcinogen. This paper provides evidence that, in contrast with previous studies on human fibroblasts in vitro (1, 5), cultures of human epidermal cells do exhibit a toxic response in the presence of MCA and BP. While transformation within these epidermal cultures has not yet been demonstrated, the cytotoxic response suggests that the cells possess a microsomal aryl hydroxylase system, which may also be capable of the conversion of the hydrocarbon to an active carcinogen.

MATERIALS AND METHODS

Pieces of normal adult human abdominal skin, approximately 4 mm square and 1 mm thick, were grown in a clot of chick embryo extract and plasma (Baltimore Biological Laboratory, Baltimore, Md.) on the bottom of plastic Petri dishes, either directly on the plastic or on glass coverslips (8). The tissues were immersed in Eagle's minimal essential medium containing 10% fetal calf serum, penicillin (100 units/ml), streptomycin (100 units/ml), and mycostatin (100 units/ml). Cultures were gassed with a 95% air-5% CO2 mixture and incubated at 37°. The medium was changed every 3 to 4 days. After a period of 10 to 11 days, during which a sizable epithelial outgrowth had appeared, cultures were photographed. The normal medium was then removed and medium containing hydrocarbon was added. The hydrocarbons (Eastman Organic Chemicals, Rochester, N.Y.) were initially dissolved in acetone (1 mg/5 ml) and added to the medium to achieve a final concentration of 1 µg of hydrocarbon and 0.004 g of acetone per ml. The hydrocarbons and their solutions were stored in the dark at 4° and handled under red or amber filtered lighting. The cultures were divided into 4 groups, those containing the carcinogens MCA or BP and controls containing pyrene or normal medium. The controls consisting of only normal culture fluid contained no acetone, because preliminary experiments showed that, in concentrations up to 0.008 g/ml, acetone had no detectable effect on culture growth or cell morphology. The cultures were exposed to the hydrocarbon solutions for 4 days in the dark, after which they were rinsed through 4 baths of normal culture fluid at 37°. After rinsing, the cultures were placed under red or amber filtered lighting. The cultures were divided into 4 groups, those containing the carcinogens MCA or BP and controls containing pyrene or normal medium. The controls consisting of only normal culture fluid contained no acetone, because preliminary experiments showed that, in concentrations up to 0.008 g/ml, acetone had no detectable effect on culture growth or cell morphology. The cultures were exposed to the hydrocarbon solutions for 4 days in the dark, after which they were rinsed through 4 baths of normal culture fluid at 37°. After rinsing, the cultures were placed in fresh fluid. From this point, they were periodically exposed to normal laboratory lighting conditions for examination and photography. The culture medium was changed every 3 to 4 days. Cultures were maintained for 3 months after exposure to the carcinogen.

RESULTS

Controls. The basal cells of the epidermis migrated onto the coverslips from the explant as a sheet of epithelial cells

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2 The abbreviations used are: MCA, 3-methylcholanthrene; BP, benzo[a]pyrene.

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with respect to one another. A total of 55 control cultures was studied, 40 in normal medium and 15 in medium containing pyrene. All showed progressive outward radial growth of the epidermal sheet for 6 to 10 weeks by cell migration and division until reaching a diameter of 1 to 1.5 cm. After 10 weeks, outward growth decreased considerably, although cell division still occurred. Some of the cells in older cultures became much larger than 30 μ in diameter, and the orderly polygonal pattern sometimes became disorganized.

Experimental. Examination after 4 days of exposure to the carcinogen revealed that in 10 of 13 MCA cultures and 14 of 14 BP cultures there was a striking reduction in the amount of outgrowth when compared with that present before treatment (Figs. 1 and 2). This reduction continued for approximately 1 week after the carcinogens were removed. The effect was more pronounced in the BP- than in the MCA-treated cultures. In some of the cultures, following removal of hydrocarbon, a very limited outward growth of a normal-appearing epithelial sheet resumed; in most, no recovery was ever noted. Cultures were followed for 12 weeks. Although outgrowth size generally remained static, the cells appeared viable. Mitoses were present but few in number. No cells with enhanced growth properties were observed. In all cases where outgrowth was reduced, the size was much smaller than that in controls of the same age.

Within 1 week after initial exposure to MCA and BP, the well-ordered arrangement of cells seen before treatment and in the controls diminished, and intercellular relationships became increasingly haphazard. There was an increased tendency for individual cells and islands of cells to become detached from the main body of outgrowth. Also, within 1 week after exposure to the hydrocarbons, the appearance of giant cells ranging in diameter from 100 to 200 μ was noted (Fig. 3). These were especially prominent at the edge of the culture. Once present, giant cells persisted essentially unchanged up to the end of the 12-week observation period. These giant cells were similar to those seen in control cultures, which appeared, however, only after 10 to 12 weeks.

In some carcinogen-treated cultures, fibroblasts became established. Unlike the epidermal cells, these continued to grow rapidly after removal of carcinogen and eventually covered the entire available growth surface.

DISCUSSION

The fact that some normal human cell systems such as fibroblasts in vitro have appeared resistant to the toxic effects of MCA and BP while epidermal cells are sensitive to these hydrocarbons in comparable concentrations points out the importance of recognizing individual cell-type differences. Among similar cell types, the difference in responses of species to hydrocarbon is also an important consideration (4). Although it has been ascertained that the amount of epidermal cell outgrowth is dramatically reduced compared with controls, the reason for this is not entirely clear. Preliminary analysis of mitotic indices suggests that at least part of the reduction is due to depression of cell division. Certainly some reduction may have been due to cell death, but we did not look for specific evidence of this.

Giant cells have been noted in other cell systems exposed to carcinogens in vitro, possibly reflecting a mild toxic effect (5, 15). Their presence in control epidermal cultures, although they were fewer in number and appeared later than in carcinogen-treated material, indicates that they are not specific indicators of a carcinogenic effect. Work with mouse epidermis in vivo has also demonstrated cell enlargement associated with exposure to aromatic hydrocarbons (11, 16).

In none of the cultures during the 12-week observation period did a line of cells emerge with enhanced growth properties. A change in cell morphology and disorganization of growth have been interpreted as criteria of cellular transformation in vitro (2, 3, 12). While the epidermal outgrowths after treatment with carcinogen were more disorganized than usual, similar features were also seen to a lesser degree in 12-week control cultures. The ultimate assertion of transformation can only come with transplantation back into the host. Such work might be carried out with nonhuman primate epidermis.

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Fig. 1. Bright-field light micrograph of culture of normal human epidermis. Dark mass, explant; arrows, edge of outgrowing epithelial sheet. X 33.

Fig. 2. Bright-field light micrograph of same culture as in Fig. 1 after treatment with carcinogen. Note significant shrinkage of the epithelial sheet (arrows). X 33.

Fig. 3. Phase contrast micrograph at edge of carcinogen-treated culture. Compare size of giant cells (arrows) with small cells in the epithelial sheet. X 235.
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