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review of chemical and UV light induced melanomas in experimental animals in relation to human melanoma incidence

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ABSTRACT

Animal data relating to melanoma induction by polycyclic aromatic hydrocarbons (PAHs) were examined for evidence on the likelihood of PAH exposure being responsible for the increase in melanoma incidence seen in workers from a few refineries.

Of the PAHs, only 7,12-dimethylbenz(a)anthracene (DMBA) had been reported to be capable of inducing melanoma, and then only in a few animal species under particular experimental conditions involving high dose levels.

In view of the low level of exposure to DMBA in refineries and the apparent inability of other PAHs or complex hydrocarbon mixtures to produce melanomas, together with the absence of any observed increase in other skin cancers in refinery workers, it was considered unlikely that any increase in melanoma incidence in such workers was the result of PAH exposure.

SUMMARY

In a few epidemiological studies on oil refinery workers, a slight excess of melanoma incidence has been reported. To see if this might be linked to exposure to polycyclic aromatic hydrocarbons (PAHs) contained in refinery streams, a review of animal data on the relationship between PAH exposure and melanoma induction has been carried out and compared with human data.

This revealed that the highly carcinogenic PAH 7,12-dimethylbenz(a)anthracene (DMBA), was capable of inducing melanomas in hamsters, mice and guinea pigs, but only under certain experimental conditions. Evidence suggested, that other carcinogenic PAHs were unable to induce melanomas. As high dose levels of DMBA were generally required to produce melanomas, it was not considered that the amounts present in refinery streams would be sufficient to account for an increase in melanoma incidence in exposed workers.

This conclusion was substantiated by the failure of petroleum-derived complex hydrocarbon mixtures to produce melanomas in animals or man and by drawing attention to the absence of any association between melanoma incidence and the incidence of other skin cancers in man. If PAHs were responsible for an increase in melanoma incidence, and increase in other skin tumours would also be expected.

It was concluded that animal data do not suggest that PAH exposure is likely to be the cause of any elevation in melanomas in refinery workers. It was considered unlikely that further animal work would give any meaningful findings and it was suggested that more detailed epidemiological findings would be required to establish whether any excess incidence of melanomas was due to sunlight, other risk factors or chance occurrences.

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1. INTRODUCTION

There are many reports of human malignant melanoma rates rising in recent years (1,2) and although this has often been attributed to increases in solar radiation (2), there is some concern that environmental or occupational exposure to carcinogenic chemicals might also be implicated (3,4). Associations have been noted between certain occupations and malignant melanoma, but these have principally been in those involving exposure to sunlight (5). Others are suspected of being fortuitous as no clear causative factor can be identified (6).

Against this background an elevation in melanoma incidence in oil refinery workers has been reported in a few locations, whereas in others no such elevation has been observed (6,7,8). In an extensive review, Wong and Raabe (9) concluded that, although there were some inconsistencies, petroleum workers (mostly in refineries) showed no excess of deaths due to skin cancers (including melanomas).

The main occupational exposure in refineries is to hydrocarbons which range from low molecular weight volatile materials to high molecular weight substances. Within this range are many refinery streams that contain various amounts of polycyclic aromatic hydrocarbons (PAHs). Some PAHs are known to have a potential to produce skin cancer in both animals and man if exposure is sufficient, but whether they have the potential to induce melanomas needs to be examined carefully.

The aim of the present review was to examine experimental animal data to see if this provides further information on whether the excesses of melanoma noted in a few refineries could bear any relationship to occupational exposure.

2. MELANOMA INDUCTION IN ANIMALS

The only PAH to have been clearly shown to induce melanomas in experimental animals is 7,12-dimethylbenz(a)anthracene (DMBA). This is regarded as one of the most potent, if not the most potent, carcinogen of the PAHs but it is present only at very low concentrations in crude mineral oils (10).

2.1. DMBA INDUCTION OF MELANOMAS IN EXPERIMENTAL ANIMALS

2.1.1. Hamsters

The species most susceptible to melanoma production appears to be the Syrian (golden) hamster. In this species Della Porta et al (11) and Shubik et al (12) showed that a single topical application of 1% DMBA could induce melanotic lesions together with a few papillomas and carcinomas, but when this dose level of DMBA was given repeatedly only papillomas and carcinomas occurred. Further studies (13) and (14) suggested that the melanotic tumours resulting from a single application of 1% DMBA arose from parafollicular melanocytes. In an electron microscopical study however, Nakai and Rappaport (15) claimed that the melanotic tumours were derived from neuroectodermal mesenchymal cells of the small dermal nerves surrounding the hair follicles and were different from the spontaneous melanomas that occur in golden hamsters. Some doubt remains regarding their origin since Mishima and Oboler (16) subsequently claimed that the melanotic tumours induced by 1% DMBA in golden hamsters were derived from melanocytes which are free in the dermis or around hair follicles.

In order to see if the yield of melanotic tumours from a single topical application of 1% DMBA could be enhanced by promotion, both Della Porta et al (11) and Delgalvis et al (17) applied 5% croton oil twice weekly to the skin after DMBA application, without any apparent effect. More recently, Bernfeld and Homberger (18) using lower dose levels of topical DMBA treatment reported that 12-O-tetradecanoylphorbol-13-acetate (TPA) was similarly ineffective.

Goerttler et al (19), however, reported that TPA did promote DMBA-induced melanomas when DMBA was administered intragastrically as 2 initiating doses of 50 mg/kg within one week. In a later study the same groups of workers (20) reported that intragastric DMBA initiation of melanomas was inhibited by benzoflavone, implying a cytochrome P448 dependent monooxygenase system was required for DMBA activation. Also using the intra-gastric route of DMBA administration, Schweizer et al (21) showed that topical benzoyl peroxide could be used to promote the formation of melanotic tumours.

Hormone balance may be involved in melanoma production in hamsters. Raitschew (22) showed that prior hypophysial transplants (presumably by increasing levels of melanophore stimulating hormone) could increase the incidence of melanomas induced by a single topical application by DMBA. Whether hormonal effects were involved in the induction of melanomas in castrated golden Syrian hamsters by three intrajugular injections of DMBA (23) is uncertain.

2.1.2. Guinea pigs

Edgecomb and Mitchelich (24) reported that the repeated skin painting of 0.5% DMBA at weekly intervals for one year to 32 male and 20 female guinea pigs led to hyperpigmentation, pigmented naevi in both sexes and to malignant melanomas in 4 females. Also found were signs of skin irritation such as epidermal atrophy, hyperkeratosis and alopecia. The latent periods of tumour development were from about a year to over two years. Similar findings were reported by Clark et al (25) and by Gomez et al (26) both of whom applied DMBA repeatedly for a period of 1 to 1½ years, the animals being maintained for up to a further 1½ years; again malignant melanomas were found towards the end of the studies following earlier hyperpigmentation and multiple naevus-like lesions. Signs of severe skin irritation were recorded by Gomez et al (26). Pawlowski et al (27) examined the nature of the pigmented naevi induced by similar treatment at both the light and electron microscope levels and stated that melanin formation took place in epidermal melanocytes and melanocytes of the outer root sheath of the hair follicles and that these melanocytes also form junctional and compound naevi. Schwann cells were not observed to participate in the naevi. Histopathological and electron microscopical examination of malignant melanomas obtained after a longer observation period (28) yielded little further information. Most studies reported that a small number of papillomas were induced as well as naevi, melanotic spots and melanomas.

2.1.3. Mice

The induction of melanomas in mice by DMBA was first demonstrated by Klaus and Winkelmann (29) who showed that when three or four topical applications of 1% DMBA in mineral oil were administered to light and dark pigmented hairless mice an increase in pigmentation occurred in dark mice. This faded after treatment stopped and was followed by the appearance of multiple pigmented nodules considered to arise from dermal melanocytes. Multiple squamous and keratotic tumours also arose. Subsequently Epstein et al (30) showed that, in nude pigmented mice, only a single application of 0.4% DMBA in acetone was necessary to induce the appearance of small pigmented growths (blue nevi) and that some of these could be stimulated to develop into malignant melanomas by UV light. No papillomas or

carcinomas were reported to occur in this study. Other workers (31,32,33 and 34) have shown that a single application of 0.4% or 0.2% DMBA followed by croton oil treatment is effective in producing malignant melanomas in BDFI, CDFI and C57BL mice but not in DBA mice. Kanno et al (35) showed that TPA was also effective at promoting the appearance of melanotic tumours, though the study was insufficiently prolonged to allow them to progress to malignancy. Light microscopic (29) and electron microscopical studies (35) suggest that the tumours arise from dermal melanocytes, the parafollicular melanocytes also being involved in mice with hair. Two of these studies (33 and 34) indicated that a low incidence of papillomas or carcinomas also occurred in studies involving promoters.

2.2. OTHER POLYCYCLIC AROMATIC HYDROCARBONS

There are indications that the strongly carcinogenic PAHs 3-methylcholanthrene (3-MC) and benzo(a)pyrene (BaP) and the weakly carcinogenic PAH chrysene increase the number of dihydroxyphenylalanine (DOPA) positive cells (active melanocytes) in mouse skin but that non-carcinogenic PAHs do not (36). However, since croton oil also induced an increase, this may represent a non-specific response to irritation. The fact that prostaglandins can also stimulate melanocyte proliferation strengthens this argument (37).

When much higher dose levels of BaP or 3-MC were applied in the same way as DMBA these failed to induce melanomas in hamsters (18).

The only evidence of a PAH other than DMBA producing melanotic tumours comes from some old reports of 5,9,10- trimethyl 1,2-benzanthracene producing melanotic lesions (38,39,40).

2.3. COMPLEX HYDROCARBON MIXTURES

The only complex hydrocarbon mixture that has been reported to produce melanotic lesions is coal-tar. The evidence of this is limited to two old studies (41,42), while many subsequent studies on coal-tar and derived products have not shown any evidence of melanotic tumours being induced (43). There are no reports of mineral oils (petroleum or shale derived) or any of their products producing melanomas in mice (44,10).

2.4. NON-PAH CARCINOGENS AND OTHER CHEMICALS

The only other well-studied carcinogen that has been reported to induce melanomas in experimental animals is urethan in hamsters (45,46,47).

In addition to this Fortner et al (48) has reported malignant melanomas arising in Syrian (golden) hamsters treated with sodium taurocholate, sodium taurodeoxycholate, desoxycholate and human bile. The significance of these findings is uncertain as some spontaneous tumours also occurred.

2.5. UV LIGHT

That UV light is involved in human melanoma induction is well established, but its role is not clearly determined (49). The only report of UV light possibly inducing malignant melanoma in animals is by Kripke (49), in which, following the UV irradiation of 40 mice, topical croton oil treatment gave rise to a single melanoma. In the production of malignant melanomas by treatment with DMBA followed by UV light mentioned previously (30), it is uncertain whether the UV light is exerting an effect by inducing genetic damage or by stimulating cell proliferation. It has been shown that UV irradiation of C3H mice prior to innoculation with melanoma cells accelerated the appearance of tumours, suggesting that UV light may have a local effect on the skin site which favours the growth of melanoma cells (50).

2.6. GENETIC FACTORS

The operation of genetic factors in melanoma incidence has been clearly demonstrated in platyfish (51,52) and in miniature swine (53). While studies on the induction of melanotic tumours by DMBA show that some animal species and strains of mice are much more susceptible than others, the reasons for this are uncertain.

3. DISCUSSION AND CONCLUSIONS

Evidence of melanoma induction by polycyclic aromatic hydrocarbons is based almost entirely on animal studies with DMBA. Hence melanoma induction may be a specific property of DMBA rather than a general property of PAHs. This view is supported by the observation that melanomas were not produced in hamsters by the strongly carcinogenic PAHs 3-methylcholanthrene and benzo(a)pyrene, even when these were applied at much higher dose levels (18).

The pattern of exposure to DMBA that gives rise to melanomas varies between species. In hamsters and mice, melanomas are induced by a single high dose (in mice followed by various promoting stimuli), whereas in guinea pigs multiple high doses over a 1 to 2 year period are required.

The concentration of DMBA required to produce melanomas in experimental animals is often in the order of 1%. This level is several order of magnitude higher than the level of DMBA that has been reported for crude petroleum oil (0.9-1.4 ppm) (10). It therefore seems unlikely that the level of DMBA present in refinery streams or other petroleum products would be sufficient to induce melanomas in animals or in man. A great number of complex hydrocarbon mixtures including refinery streams and other oil products have been evaluated for carcinogenic potential in animals. Apart from two old studies of doubtful significance on coal-tar, there are no reports of melanomas being induced by such materials, despite the fact that many had a high PAH content and produced large numbers of other skin cancers (10, 43, 44).

In most situations in which melanomas have been induced by DMBA in experimental animals, other skin tumours (principally papillomas and carcinomas) have also arisen. Furthermore repeated treatments with DMBA in hamsters and mice give rise to other skin tumours instead of melanomas. Hence if PAH exposure in man was responsible for melanoma induction a concurrent increase in incidence of other skin cancers would also be expected. There was, however, no indication of an elevation of other skin cancers in those epidemiological studies on refinery workers in which an increase of melanoma incidence had been observed.

Human data from more than twenty human studies in which skin cancer has been reported in association with exposure to oil products containing PAHs, indicate that such exposure has not produced a noticeable elevation of melanomas even where the incidence of other skin tumours has been high (44). This is in spite of the fact that, an awareness of the problems of working under these conditions has led to active examination for skin tumours. This finding is evidence against exposure to PAHs being the cause of increased melanoma incidence in refinery workers.

In conclusion, the available animal data do not suggest that PAH exposure is the likely cause of the any elevation in melanomas associated with working in oil refineries. It is unlikely that additional animal work on PAHs would help in interpreting the findings of epidemiological studies. It is possible that more detailed epidemiological data on refinery workers might establish whether the excess incidence of melanoma can be attributed to sunlight alone, to other risk factors, or to chance occurrences.

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