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Relative tumor initiating activity of benzo[*a*]fluoranthene, benzo[*b*]fluoranthene, naphtho[1,2-*b*]fluoranthene and naphtho[2,1-*a*]fluoranthene on mouse skin

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Summary

The tumor-initiating activities of benzo[a]fluoranthene (BaF), benzo[b]fluoranthene (BbF), naphtho[1,2-b]fluoranthene (NbF) and naphtho[2,1-a]fluoranthene (NaF) were evaluated on the skin of female CD-1 mice. Each of these polycyclic aromatic hydrocarbons was assayed at total initiation doses of 1.0 and 4.0 μmol/mouse. These hydrocarbons were applied in 10 subdoses administered every other day. Promotion commenced 10 days after the last initiator dose and consisted of thrice weekly application of 2.5 μg of tetradecanoylphorbol acetate for 20 weeks. BbF was the most potent tumor initiator inducing a 100% incidence of tumor-bearing mice with an average of 8.5 tumors per mouse at a total initiator dose of 1.0 μmol. NaF was slightly more active as a tumor initiator than either NbF or BaF. NaF induced a 90% incidence of tumor-bearing mice with an average of 5.9 tumors per mouse at a total initiator dose of 1.0 μmol. BaF and NbF at a total initiator dose of 4.0 μmol exhibited similar tumor-initiating activity with both inducing a 90% incidence of tumor-bearing mice with an average of 4.3 and 6.6 tumors per mouse, respectively. However, at a total initiator dose of 1.0 μmol, BaF and NbF induced a 95% and 65% incidence of tumor-bearing mice with an average of 3.3 and 2.5 tumors per mouse, respectively.

Keywords: non-alternate; polycyclic; tumorigenicity; skin; mice.

Introduction

Polycyclic aromatic hydrocarbons (PAH) represent a major class of carcinogenic environmental pollutants. There are several reviews on their occurrence and tumorigenic potential [1—4]. Studies on non-alternant PAH suggest that their mechanism of activation differs from the bay region theory of activation which appears to apply to several alternant hydrocarbons such as benzo[a]pyrene and chrysene, for which bay region diol epoxide metabolites have been identified as the principal active metabolites that bind covalently to DNA in vivo [5—9].

BbF is a non-alternant PAH which has incorporated within its structure a bay region (Fig. 1). It is a relatively potent carcinogen and common environmental pollutant [10—13]. BbF, however, does not appear to ultimately exert its carcinogenic activity via the formation

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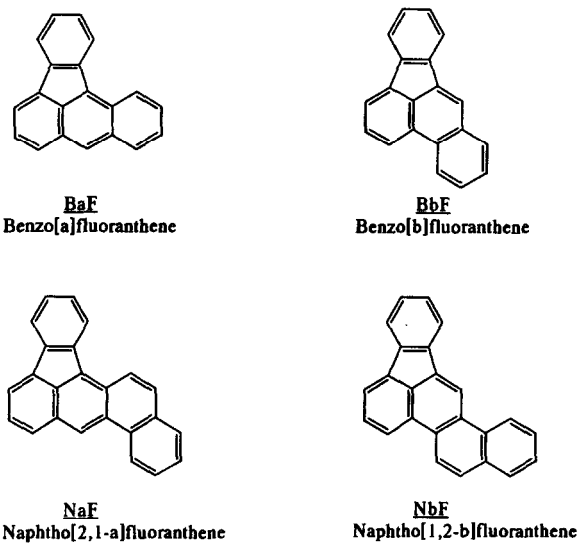

Fig. 1. Structure of BaF, NaF, BbF and NbF.

of a bay region dihydrodiol epoxide [14,15]. In this study the biological effect of incorporating a bay region within a non-alternant hydrocarbon was evaluated (Fig. 1). The tumorigenic activity of BaF, naphtho[1,2-*b*]fluoranthene (NbF) and NaF was evaluated on mouse skin. While the tumorigenic activity of BbF has been extensively evaluated [10—13], the relative tumorigenic activities of the BaF, NaF and NbF have not been determined. With the availability of these compounds for use as reference standards [16], it is anticipated that their contribution to the PAH present as environmental pollutants will be assessed. We report, herein, the tumorigenic activity of these non-alternant hydrocarbons.

Materials and methods

Chemicals

BbF (Benz[*e*]acephenanthrylene) was obtained from Aldrich Chemical Company (Milwaukee, WI). NaF (dibenz[*a,j*]aceanthrylene), NbF (indeno[1,2,3-*hi*]chrysene) and BaF (benz[*a*]aceanthrylene) were synthesized as previously described [14]. In the preceding list of chemicals, the official IUPAC and CA nomenclature for each compound is provided within the subsequent parentheses. Tetradecanoylphorbol acetate (TPA) was obtained from Chemsyn Science Laboratories (Lenexa, KS).

Bioassay

Experimental groups consisted of 20 female CD-1 mice (Charles River Laboratories, Kingston, N.Y.) obtained at an age of 28—35 days. Animals were housed 10 per cage in solid bottom polycarbonate Micro-Isolator cages (Lab Products, Inc., NJ) in ventilated animal racks (Lab Products, Inc., NJ). Hardwood bedding was employed in these studies as obtained from Beta-Chip, North Eastern Products, Warrenburg, NY. Mice were given water and NIH-07 diet adlibitum and were kept under controlled conditions with a 12 h light-dark cycle.

Initiation commenced during the early part of the second telogen phase of the hair cycle (50—55 days old). Each of the non-alternant PAH evaluated in this study were administered at a total initiation dose of 1.0 and 4.0 μmol per mouse. Solutions of these hydrocarbons were applied topically in 100 μl acetone every other day to the shaved backs of the mice using a biopipette employing a total of 10 subdoses. Negative control mice were treated with acetone only. Ten days after the last application of acetone or hydrocarbon, promotion was begun by applying 2.5 μg of TPA in 100 μl acetone thrice weekly for 20 weeks. Mice were shaved as necessary (when regrowth of the hair covered the treated area of the skin) and tumors were counted weekly. The significance of the tumorigenic response was evaluated using the chi-squared test.

Results

The tumor-initiating activities of BaF, BbF, NaF and NbF as determined from topical application to the skin of female CD-1 mice at total initiation doses of 1.0 and 4.0 μmol are summarized in Table 1. While each of these hydrocarbons produced between a 90—100%

Table 1. Tumor-initiating activity of BaF, BbF, NaF and NbF. Female mice were given 10 subdoses of the initiator. Promotion was carried out by thrice weekly administration of 2.5 μg of TPA in 100 μl acetone for 20 weeks.

Compound	Effective no. of mice	Total initiating dose (μmol)	% Skin tumor-bearing mouse	Average skin tumors/mouse
BbF	20	4.0	100*	11.0
	20	1.0	100*	8.5
NaF	20	4.0	100*	7.3
	20	1.0	90*	5.9
BaF	20	4.0	90*	4.3
	20	1.0	95*	3.3
NbF	20	4.0	100*	6.6
	20	1.0	65*	2.5
Acetone	20		10	0.1

*$P < 0.001$.

incidence of tumor-bearing mice at the 4.0 μmol dose, more pronounced differences resulted at a total initiation dose of 1.0 μmol. The results of this bioassay indicate that, among these non-alternant hydrocarbons, BbF is the most active tumor-initiator. This is most clearly seen in the average numbers of skin tumors which were formed at both dose levels (Figs. 2 and 3). At total initiation doses of 4.0 and 1.0 μmol/mouse, BbF produced a 100% incidence of tumor-bearing animals with an average of 11.0 and 8.5 tumors per mouse, respectively.

NaF and NbF also induced a 100% incidence of tumor-bearing mice at a total initiation dose of 4.0 μmol with an average of 7.3 and 6.6 tumors per mouse, respectively. At the lower total initiation dose of 1.0 μmol, NaF and NbF produced a 90% and 65% incidence of tumor-bearing mice. At this dose there was also a difference in tumor multiplicity, NaF-treated mice had an average of 5.9 tumors as compared to NbF-treated mice which developed an average of 2.5 tumors per mouse (Fig. 3). BaF induced a 95% incidence of tumor-bearing mice with an average of 3.3

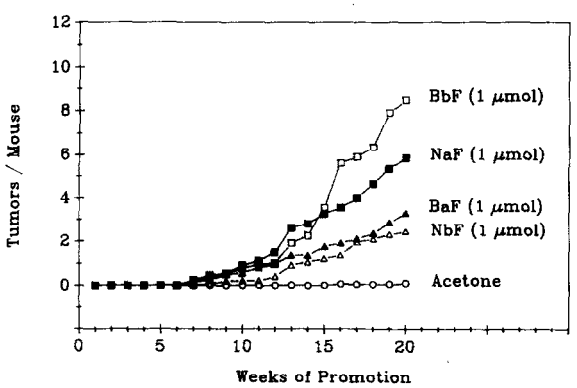

Fig. 2. Average number of tumors per mouse which developed during 20 weeks of promotion with 2.5 μg TPA administered thrice weekly after initiation with a 1.0 μmol total initiator dose of each hydrocarbon.

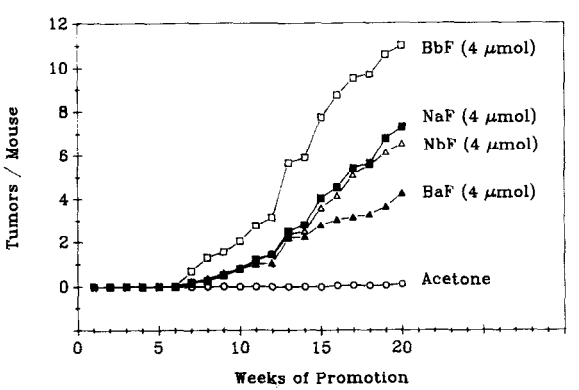

Fig. 3. Average number of tumors per mouse which developed during 20 weeks of promotion with 2.5 μg TPA administered thrice weekly after initiation with a 4.0 μmol total initiator dose of each hydrocarbon.

tumors per mouse at a total initation dose of 1.0 μmol. At the higher initiation dose of 4.0 μmol, however, the incidence of tumor-bearing mice was similar with only a moderate increase in tumor multiplicity. These data suggest that BaF has similar tumor-initiating activity to NaF.

Discussion

The data obtained from the tumor-initiation bioassay clearly indicate that each of the non-alternant PAH evaluated had significant ($P < 0.001$) tumorigenic activity in this assay. Comparison of the relative tumorigenic potential of several benzofluoranthenes have shown that BbF is more active than benzo[*j*]fluoranthene (BjF) and much more active than benzo[*k*]fluoranthene (BkF) as a tumor initiator on mouse skin [8—11]. It is known that BbF is one of the more potent carcinogenic non-alternant PAH in the environment [2]. In this assay for tumor-initiating activity NaF exhibited similar tumorigenic potency to BbF. In comparison to bioassay data obtained under similar assay conditions, NaF also has greater tumorigenic activity than BjF when assayed at a total initiation dose of 1.0 μmol. BjF induced at a total initiator dose of 4.0 μmol a 84% incidence of skin tumors with an average of 7.2 tumors per mouse [10]. At a total initiation dose of 1.0 μmol, BjF induced a 70% incidence of tumor-bearing mice with an average of 3.4 tumors per mouse [17].

BaF resembles BjF in that both compounds have a four-sided bay region or pseudo-bay region incorporated within their structures. BaF at a total initiator dose of 1.0 μmol exhibits similar tumorigenic activity to BjF. In this assay, however, BaF at the 4.0 μmol dose did not produce a substantial increase in tumor multiplicity as previously observed for BjF. It is clear from these data that NaF, which is a benzo-derivative of BaF fused at the 3,4-positions, is more potent as a tumor initiator. The addition of this benzo-ring alters the structure such that a bay region resembling that of benz[*a*]anthracene is formed. Thus, the addition of a bay region may account for the increased tumor-initiating activity of NaF relative to BaF. Dibenzo[*a,e*]fluoranthene is similar to NaF in that it is a benzo-derivative of BaF. While dibenzo[*a,e*]fluoranthene is a tumor initiator on mouse skin, the conditions under which it was assayed differ from those in the current study [18]. Thus, it is not possible to make any comparison of its potency with BaF or any of the other compounds evaluated in this study.

Of the three compounds which have a bay region within their structure, NbF is less active as a tumor initiator than either BbF or NaF. It is evident from this bioassay that the addition of a benzo-ring at the 9,10-positions of BbF, as is the case for NbF, results in a substantial loss of tumor-intitiating activity. While this may implicate that the 9,10-dihydrodiol epoxide of BbF might be associated with its tumorigenic activity, studies performed to date have not supported the involvement of this bay region dihydrodiol epoxide in its activation [12,13].

Both NaF and NbF can be viewed as benzo-derivatives of BaF and BbF, respectively. Further studies are required on the mechanisms by which both BaF and BbF are metabolically activated to more fully elucidate the influence of such benzo-derivatives on their mechanisms of activation.

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References

1 Baum, E.J. (1978) Occurrence and surveillance of polycyclic aromatic hydrocarbons. In: Polycyclic Aromatic Hydrocarbons and Cancer, Vol. 1, pp. 45—70. Editors: H.V. Gelboin and P.O.P. T'so. Academic Press, New York.

2 International Agency for Research on Cancer (1973) Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 3, pp. 69—90, International Agency for Research on Cancer, Lyon.

3 International Agency for Research on Cancer (1983) Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 32, International Agency for Research on Cancer, Lyon.

4 Grimmer, G. (1983) Environmental Carcinogens: Polycyclic Aromatic Hydrocarbons. CRC Press, Inc. Boca Raton, Florida.

5 Conney, A.H. (1982) Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G.H.A. Clowes Memorial Lecture. Cancer Res., 42, 25—29.

6 Sims, P. and Grover, P.L. (1981) The involvement of dihydrodiols and diol-epoxides in the metabolic activation of polycyclic hydrocarbons other than benzo[a]pyrene, In: Polycyclic Aromatic Hydrocarbons and Cancer, Vol. 3, pp. 117—181. Editors: H.V. Gelboin and P.O.P. T'so. Academic Press, New York.

7 Lehr, R.E. et al. (1985) The bay region theory of polycyclic aromatic hydrocarbon carcinogenesis, In: Polycyclic Hydrocarbons and Carcinogenesis, pp. 63—84, Editor: R.G. Harvey. ACS Symposium Series 283, American Chemical Society, Washington, D.C.

8 Harvey, R. (1981) Activated metabolites of carcinogenic hydrocarbons. Acc. Chem. Res., 14, 218—226.

9 Harvey, R. and Geacintov, N.E. (1988) Intercalation and binding of polycyclic hydrocarbon metabolites to nucleic acids. Acc. Chem. Res., 21, 66—73.

10 LaVoie, E.J., Amin, S., Hecht, S.S., Furuya, K. and Hoffmann, D. (1982) Tumour initiating activity of dihydrodiol of benzo[b]fluoranthene, benzo[j]fluoranthene and benzo[k]fluoranthene. Carcinogenesis, 3, 49—52.

11 Wynder, E.L. and Hoffmann. D. (1960) The carcinogenicity of benzofluoranthenes. Cancer, 12, 1194—1199.

12 Habs, M., Schmahl, D. and Misfeld, J. (1980) Local carcinogenicity of some environmentally relevant polycyclic aromatic hydrocarbons after lifelong topical application to mouse skin. Arch. Geschwulstforsch., 50, 266—274.

13 Van Duuren, B.L., Sivak, A., Segal, A., Orris, L. and Langseth, L., (1966) The tumor producing agents of tobacco leaf and tobacco smoke condensate. J. Natl. Cancer Inst., 37, 519—526.

14 Amin, S., LaVoie, E.J. and Hecht, S.S. (1982) Identification of metabolites of benzo[b]fluoranthene. Carcinogenesis, 3, 171—174.

15 Geddie, J.E., Amin, S., Huie, K. and Hecht, S.S. (1987) Formation and tumorigenicity of benzo[b]fluoranthene metabolites in mouse epidermis. Carcinogenesis, 8, 1579—15.

16 Cho, B.P. and Harvey, R.G., (1987) Polycyclic fluoranthene hydrocarbons. 2. A new general synthesis. J. Org. Chem., 52, 5668—5678.

17 Rice, J.E., Weyand, E.H., Geddie, N.G., DeFloria, M. and LaVoie, E.J. (1987) Identification of tumorigenic metabolites of benzo[j]fluoranthene formed *in vivo* in mouse skin. Cancer Res., 47, 6166—6170.

18 Zajdela et al. (1987) Marked differences between mutagenicity in *Salmonella* and tumour-initiating activities of dibenzo[a,e]fluoranthene proximate metabolites; initiation inhibiting activity of norharman. Carcinogenesis, 8, 461—464.