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Benzo[*a*]Pyrene Metabolites: Effects on Viral Interferon Induction

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ABSTRACT

Benzo[*a*]pyrene (BaP) metabolites were assessed, with and without enzymatic activation by rat liver S9, for their inhibitory activities on influenza virus induction of interferon-α/β (IFN-α/β) in mammalian (LLC-MK$_2$) cell cultures. Although BaP *per se* was inactive, metabolized BaP reduced viral IFN induction by approximately 80%. BaP metabolites (phenols, diols, 6-substituted derivatives) exhibited significant inhibitory activity (50% or greater) only when they were activated enzymatically. Although not significant, the diol metabolites without activation mildly reduced IFN induction on the average of 32%. The quinones did not adversely affect the IFN induction process, but three of the seven metabolites tested showed ~30% inhibitory activity in the presence of S9. BaP diol epoxides were direct inhibitors of viral IFN induction while derivatives of these epoxides, tetrols and triols, showed negligible inhibition even with S9. In general, the reported microbial mutagenicities of BaP metabolites could be correlated with their abilities to inhibit IFN induction. That activation-dependent hydrocarbons can be metabolized by S9 added to mammalian cell cultures resulting in the inhibition of viral IFN induction extends the capability and credibility for assessing suspect mutacarcinogens on this basis.

INTRODUCTION

BENZO[*a*]PYRENE (BaP) IS A UBIQUITOUS environmental pollutant representative of a class of compounds, the polycyclic aromatic hydrocarbons (PAH), for which there is increasing concern because of its mutacarcinogenicity as documented by epidemiological and experimental studies.[1-6] Typical of PAH compounds, BaP requires metabolic activation by the mixed-function oxidases (cytochrome P-450), which can result in the formation of approximately 40 metabolites.[7] The primary metabolites of BaP are epoxides, which are transformed to phenols (thence to glutathione conjugates, or quinones and sulfate conjugates), dihydrodiols, and diol epoxides, of which the latter are hydrolyzed to tetrols or reducd to triols.[7] Paradoxically, metabolism may either detoxify chemicals to harmless products or, in the case of BaP, activate the hydrocarbon to form proximate or ultimate products capable of cytotoxicity,[8] enhanced viral oncogenesis,[9] cellular transformation,[10-13] and mutacarcinogenicity.[14-18] Therefore, it is important to know the metabolic pathways and identify the metabolites and their cellular and macromolecular targets, as well as ancillary interactions, to understand cancer-inducing mechanisms.

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The inhibition of interferon (IFN) induction or production has been reported for numerous known mutagens and carcinogens.[19-31] Recently, it was demonstrated that several azo dyes, which require enzymatic activation to elicit mutagenicity in microbial test systems,[32,33] can be also metabolized by the addition of rat liver S9 to mammalian cell cultures, resulting in the inhibition of IFN induction.[23] When BaP, which is inactive *per se*, was enzymatically activated by this procedure, IFN induction was depressed markedly.[24,34] The direct incorporation of rat liver microsomal enzymes with activation-dependent mutacarcinogens into mammalian cell cultures resulting in the inhibition of IFN induction may be potentially as useful as mutagenicity tests in helping to identify biologically reactive metabolites.

It was the purpose of this reported study to assess predominate BaP metabolites (phenols, quinones, diols, diol epoxides), with and without enzymatic activation, for their inhibitory activity on viral IFN induction. Furthermore, an attempt was made to correlate the findings with reported microbial mutagenicities for these metabolites.

MATERIALS AND METHODS

Viruses: The Ao/PR/8/34 influenza and parainfluenza (Sendai) virus strains employed in this study were obtained from the American Type Culture Collection (Rockville, MD). Stock virus pools of each strain were prepared from embryonated chicken eggs in the manner described previously.[35] Influenza and Sendai virus pools contained 5×10^7 and 1×10^9 cell-infecting units of virus per milliliter, respectively, when assayed by the immunofluorescent cell-counting procedure.[35]

Cell Cultures: Rhesus monkey kidney (LLC-MK_2) and human Chang conjunctival (clone 1-5c-4) cell lines obtained from the American Type Culture Collection were used for induction and assay of IFN, respectively. Cell lines were propagated in plastic tissue culture flasks (75 cm^2) with Eagle minimum essential medium fortified with 100 X Essential Vitamin Mixture (10 ml/liter), 200 m*M* solution L-glutamine (10 ml/liter) to which was added sodium bicarbonate (2.2 g/liter), and 10% fetal bovine serum (FBS). Cells were maintained with the aforementioned medium containing only 0.5% FBS.

Reagents: BaP and derivative metabolites were obtained from NCI Chemical Carcinogen Reference Standard Repository (Bethesda, MD). The nomenclature and abbreviations for metabolites are listed in Table 1. Stock preparations of each chemical (400 μg/ml) were made in dimethyl sulfoxide (DMSO) immediately before use and then diluted in maintenance medium to the desired concentration for use in experimental tests. Viability of LLC-MK_2 cell cultures was >95% after 24 h incubation in the presence of 5 μg of these chemicals as determined by the Trypan Blue dye-exclusion procedure. The solvent DMSO had a final concentration of 0.5% in experimental tests and was neither detrimental to cell viability nor viral IFN induction.

Liver homogenate 9,000 g supernatant fraction (S9) was prepared from the livers of male Wistar/Lewis rats (225 g/rat) after induction with Aroclor 1254 (i.p. 100 mg/kg body wt) as described by Ames *et al.*[36] For use in experimental tests, 0.5% suspension of S9 homogenate was prepared in maintenance medium and then passed through a 0.45-μm Nalge Filter Unit (Nalge Co., Rochester, NY) to obtain sterile preparations. The suspension, after filtration, contained 77 μg of protein nitrogen/ml.[37]

IFN Induction; Duplicate experiments were performed and the procedure generally used to study the effects of BaP and its metabolites on IFN induction was carried out as follows: 1 ml (5 μg) of test chemical was added to a 9-ml volume of maintenance medium or 9 ml of medium containing 0.5% S9 which was then added to 75-cm^2 plastic flasks containing confluent LLC-MK_2 cell monolayers ($\sim 2 \times 10^7$ cells). Flasks were incubated at 35°C for 3 h. Thereafter, residual medium was decanted and 2 ml of influenza virus, which had been inactivated by UV irradiation for 45 s at a distance of

TABLE 1. NOMENCLATURE OF BENZO[*a*]PYRENE (BaP). DERIVATIVES AND ABBREVIATIONS

BaP derivative	*Abbreviation*
Phenols	
1-Hydroxybenzo[*a*]pyrene thru 12-hydroxybenzo[*a*]pyrene	1-HOBaP thru 12-HOBaP
Quinones	
1,6-Dihydrobenzo[*a*]pyrene-1,6-dione	1,6-Dione
3,6-Dihydrobenzo[*a*]pyrene-3,6-dione	3,6-Dione
4,5-Dihydrobenzo[*a*]pyrene-4,5-dione	4,5-Dione
7,8-Dihydrobenzo[*a*]pyrene-7,8-dione	7,8-Dione
7,10-Dihydrobenzo[*a*]pyrene-7,10-dione	7,10-Dione
6,12-Dihydrobenzo[*a*]pyrene-6,12-dione	6,12-Dione
11,12-Dihydrobenzo[*a*]pyrene-11,12-dione	11,12-Dione
6-Substituted	
6-Methylbenzo(a)pyrene	6-MeBaP
6-Hydroxymethylbenzo(a)pyrene	6-HOMeBaP
Diols	
cis-4,5-Dihydroxy-4,5-dihydrobenzo[*a*]pyrene	*cis*-4,5-Diol
trans-4,5-Dihydroxy-4,5-dihydrobenzo[*a*]pyrene	*trans*-4,5-Diol
cis-7,8-Dihydroxy-7,8-dihydrobenzo[*a*]pyrene	*cis*-7,8-Diol
(±)-*trans*-7,8-Dihydroxy-7,8-dihydrobenzo[*a*]pyrene	*trans*-7,8-Diol
trans-9,10-Dihydroxy-9,10-dihydrobenzo[*a*]pyrene	*trans*-9,10-Diol
Diol epoxides	
(±)-4,5-Dihydrobenzo[*a*]pyrene-4,5-oxide	4,5-Epoxide
(±)-*r*-7,*t*-8-Dihydroxy-*t*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (anti)	Diol epoxide I
(±)-*r*-7,*t*-8-Dihydroxy-*c*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (syn)	Diol epoxide II
Tetrols and triols	
(±)-*r*-7,*t*-8,9-*c*-9,10-Tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene	7,10/8,9-Tetrol
(±)-*r*-7,*t*-8,*t*-9,*t*-10-Tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene	7/8,9,10-Tetrol
(±)-*r*-7,*t*-8,*t*-9-Trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene	7/8,9-Triol
(±)-*r*-7,*t*-8,*c*-9,*t*-10-Tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene	7,9/8,10-Tetrol
(±)-*r*-7,*t*-8,*c*-9,*c*-10-Tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene	7,9,10/8-Tetrol
(±)-*r*-7,*t*-8,*c*-9-Trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene	7,9/8-Triol

76.2 mm and wavelength of 253.7 nm, was added onto cell monolayers that were then incubated at 35°C for 2 h. The multiplicity of infection (moi) was approximately 2.0. Inoculum was removed and 20 ml of maintenance medium was added to each flask. After incubation, at 35°C for 20 h, supernatant fluid was decanted and centrifuged at 100,000 *g* for 1 h and dialyzed against HCl-KCl buffer pH 2.0 at 4°C for 24 h. Dialysis was continued against two changes of PBS pH 7.1 at 4°C for 24 h. Fluids were passed through 0.22-μm Millex filters GV (Millipore Corporation, Bedford, MA) to obtain sterile preparations. Samples were stored at −80°C until they were assayed for IFN activity. Preparations with antiviral activity possessed the biological and physical properties ascribed to viral IFNs.[38] Controls consisting of cell monolayers that were not treated with chemical solution were handled exactly as described above. Viral induced IFN from cell cultures contained both IFN-α and IFN-β as determined by the procedure of using constant antiserum and varied IFN concentrations.[39] Anti-human IFN-α and IFN-β antisera were obtained from Nutritional Biochemicals (Cleveland, OH).

IFN Assay: An immunofluorescent cell-counting assay that has been described previously was used to determine the IFN potency of test samples.[40] IFN-treated cell monolayers were challenged with 10^4 cell-infecting units of Sendai virus, and infected cells were visualized by direct fluorescent antibody staining. The reciprocal of the IFN dilution that reduced the number of infected cells to 50% of the control served as the measure of IFN activity, *i.e.*, 50% infected cell-depressing dilution ($ICDD_{50}$). With this assay, 0.89 IFN unit corresponds to 1.0 unit of National Institute of Health reference standard HuIFN-β (G-023-902-527). A 50% or greater reduction of IFN induction by test metabolites, which exceeds 98% confidence limits of the assay,[41] was considered significant.

Statistics: Comparative differences of data in Tables 5 and 6 (below) were analyzed by Student's *t* test and analysis of variance with alpha set at 0.05 level. Experimental data are the mean of at least duplicate tests.

RESULTS

Phenol derivatives

The major phenol metabolite of BaP formed by liver microsomal enzymes is 3-HOBaP.[42] Other phenol metabolites that are formed may be either direct hydroxylation products or rearrangement products of the unstable epoxide intermediates.[7] The effects of BaP and phenol metabolites, with and without enzymatic activation by rat liver S9, on viral IFN induction are shown in Table 2. BaP inhibited viral IFN induction by ~80% in the presence of S9. No apparent inhibitory activity of IFN synthesis occurred without enzymatic activation. Similar to results with BaP, all phenol derivatives exhibited negligible inhibition of IFN induction in the absence of S9. However, when S9 was present, all phenol metabolites depressed IFN induction by more than 50% with the exception of 4-and 6-HOBaP.

Quinone and 6-substituted derivatives

For the most part, quinone metabolites (1,6- 3,6- 6,12-diones) are formed by nonenzymatic conversion of BaP phenol derivatives.[7] BaP quinones (Table 3) show no appreciable inhibition of viral IFN induction without enzymatic activation. In the presence of S9, however, 3,6- 4,5- and 7,10-diones reduced IFN production by ~30%.

A membrane-bound microsomal and soluble enzyme of rat liver, hydroxymethyl synthetase, catalyzes the synthesis of 6-HOMeBaP from the parent compound.[43] Viral IFN induction was depressed in the presence of rat liver S9 by both 6-Me- and 6-HOMeBaP, 42.9 and 65.4%, respectively (Table 4). Without enzymatic activation, 6-substituted BaP derivatives had no adverse effect on viral-induced IFN.

TABLE 2. EFFECT OF BENZO[*a*]PYRENE (BaP) AND PHENOL DERIVATIVES WITH AND WITHOUT RAT LIVER S9 FRACTION ON VIRAL IFN INDUCTION[a]

		+S9		−S9	
BaP derivatives	*Quantity (μmoles)*[b]	*IFN yield ($ICDD_{50}$/ml)*[c]	*IFN inhibition (%)*[d]	*IFN yield ($ICDD_{50}$/ml)*	*IFN inhibition (%)*
BaP	0.0198	43	82.5	235	4.1
BaP phenols					
1-HO[e]	0.0186	90	63.3	245	0.0
2-HO	0.0186	52	78.8	242	1.3
3-HO	0.0186	70	71.5	260	0.0
4-HO	0.0186	245	0.0	232	5.4
5-HO	0.0186	65	73.5	245	0.0
6-HO	0.0186	218	11.1	232	5.4
7-HO	0.0186	82	66.6	245	0.0
8-HO	0.0186	55	77.6	226	7.8
9-HO	0.0186	70	71.5	245	0.0
10-HO	0.0186	98	60.0	188	23.3
11-HO	0.0186	44	82.1	242	1.3
12-HO	0.0186	87	64.5	215	12.3
Medium ± S9 (control)		245	0.0	245	0.0

[a]Inducer: Influenza virus added to LLC-MK_2 cell monolayers.
[b]Per 10 ml cell medium.
[c]Reciprocal of 50% infected cell-depressing dilution.
[d](Reciprocal of $ICDD_{50}$/ml of IFN yield/control $ICDD_{50}$/ml IFN) − 1.0 × 100.
[e]Hydroxybenzo[*a*]pyrene.

TABLE 3. EFFECT OF QUINONE DERIVATIVES OF BENZO[*a*]PYRENE (BaP) WITH AND WITHOUT RAT LIVER S9 FRACTION ON VIRAL IFN INDUCTION[a]

		+S9		−S9	
BaP derivatives	*Quantity (μmoles)*[b]	*IFN yield ($ICDD_{50}$/ml)*[c]	*IFN inhibition (%)*[d]	*IFN yield ($ICDD_{50}$/ml)*	*IFN inhibition (%)*
1,6-Dione	0.0177	98	11.0	106	3.7
3,6-Dione	0.0177	73	33.7	110	0.0
4,5-Dione	0.0177	79	28.2	101	8.2
7,8-Dione	0.0177	106	3.7	106	3.7
7,10-Dione	0.0177	74	32.8	108	1.9
6-12-Dione	0.0177	100	9.1	110	0.0
11,12-Dione	0.0177	94	14.6	111	0.0
Medium ± S9 (control)		110	0.0	110	0.0

[a]Inducer: Influenza virus added to LLC-MK_2 cell monolayers.
[b]Per 10 ml cell medium.
[c]Reciprocal of 50% infected cell-depressing dilution.
[d](Reciprocal of $ICDD_{50}$/ml of IFN yield/control $ICDD_{50}$/ml IFN) − 1.0 × 100.

TABLE 4. EFFECT OF 6-SUBSTITUTED DERIVATIVES OF BENZO[*a*]PYRENE (BaP) WITH AND WITHOUT RAT LIVER S9 FRACTION ON VIRAL IFN INDUCTION[a]

		+S9		−S9	
BaP derivatives	*Quantity (μmoles)*[b]	*IFN yield ($ICDD_{50}$/ml)*[c]	*IFN inhibition (%)*[d]	*IFN yield ($ICDD_{50}$/ml)*	*IFN inhibition (%)*
BaP	0.0198	39	84.1	235	4.1
6-MeBaP	0.0187	140	42.9	250	0.0
6-HOMeBaP	0.0177	85	65.4	240	2.1
Medium ± S9 (control)		245	0.0	245	0.0

[a]Inducer: Influenza virus added to LLC-MK_2 cell monolayers.
[b]Per 10 ml cell medium.
[c]Reciprocal of 50% infected cell-depressing dilution.
[d](Reciprocal of $ICDD_{50}$/ml of IFN yield/control $ICDD_{50}$/ml IFN) − 1.0 × 100.

Diol derivatives

The epoxides formed metabolically from BaP by microsome mixed-function oxidases and their hydration by epoxide hydratase results in the formation of 4,5- 7,8-, and 9,10-diols.[7,42] With S9, all diol derivatives were significantly inhibitory (~74%) to IFN induction and were significantly (α = 0.05) more active than unmetabolized diols (Table 5). *cis*- and *trans*-isomers with S9 were not significantly different from each other (α = 0.5). The strong inhibition of IFN production by *trans*-7,8-diol in the presence of S9 could be the result of its conversion to diol epoxide I.

Diol epoxides and 4,5-epoxide

BaP metabolism catalyzed by rat liver microsomal enzymes involves the highly stereoselective oxygenation of (−)-*trans*-7,8-diol to diol epoxides I and II. The ratio of diol epoxides I and II depends on the type and proportions of specific cytochrome P-450 catalyzing the reactions.[7] Of the three epoxides tested (Table 6), diol epoxide I and II and to a lesser extent, 4,5-epoxide, acted directly to inhibit IFN induction. The inhibition exhibited by these epoxides was not significantly different (α = 0.05) either with or without enzymatic activation. Diol epoxide I appeared to show the strongest adverse activity to IFN induction.

Tetrols and triols

Hydrolysis of diol epoxides I and II results in their inactivation and the formation of 7,10/8,9-and 7/8,9,10-tetrols, and 7,9,10/8- and 7,9/8,10-tetrols, respectively. Reduction by nonenzymatic agents accounts for the formation of 7/8,9- and 7,9/8-triols.[7] BaP tetrol and triol derivatives with or without enzymatic activation had no depressing effect on IFN production (Table 7). The inhibitory activity (~25%) noted with 7,10/8,9-tetrol, 7,9/8,10-tetrol, and 7,9/8-triol in the presence of S9 was not significant. In general, these terminal metabolic products of BaP detoxification appeared to be inocuous to the IFN induction system.

TABLE 5. EFFECT OF DIOL DERIVATIVES OF BENZO[*a*]PYRENE (BaP) WITH AND WITHOUT RAT LIVER S9 FRACTION ON VIRAL IFN INDUCTION[a]

		+S9		−S9	
BaP derivatives	*Quantity (μmoles)*[b]	*IFN yield ($ICDD_{50}$/ml)*[c]	*IFN inhibition (%)*[d]	*IFN yield ($ICDD_{50}$/ml)*	*IFN inhibition (%)*
cis-4,5-Diol	0.0174	44	68.6[e]	110	21.5
trans-4,5-Diol	0.0174	38	72.9	79	43.6
cis-7,8-Diol	0.0174	40	71.5[e]	90	35.8
trans-7,8-Diol	0.0174	25	82.2	98	30.0
trans-9,10-Diol	0.0174	35	75.0	95	32.2
Medium ± S9 (control)		140	0.0	140	0.0

[a]Inducer: Influenza virus added to LLC-MK_2 cell monolayers.
[b]Per 10 ml cell medium.
[c]Reciprocal of 50% infected cell-depressing dilution.
[d](Reciprocal of $ICDD_{50}$/ml of IFN yield/control $ICDD_{50}$/ml IFN) − 1.0 × 100.
[e]No significant difference between *cis*- and *trans*-diols at $\alpha = 0.05$ (Student's *t* test).

TABLE 6. EFFECT OF EPOXIDE DERIVATIVES OF BENZO[*a*]PYRENE (BaP) WITH AND WITHOUT RAT LIVER S9 FRACTION ON VIRAL IFN INDUCTION[a]

		+S9		−S9	
BaP derivatives	*Quantity (μmoles)*[b]	*IFN yield ($ICDD_{50}$/ml)*[c]	*IFN inhibition (%)*[d]	*IFN yield ($ICDD_{50}$/ml)*	*IFN inhibition (%)*
BaP	0.0198	43	80.2	213	0.0
4,5-Epoxide	0.0186	96	57.7[e]	128	36.7
(Diol epoxide I)	0.0165	60	72.4	85	58.0
(Diol epoxide II)	0.0165	88	59.5	93	54.0
Medium ± S9 (control)		217	0.0	202	0.0

[a]Inducer: Influenza virus added to LLC-MK_2 cell monolayers.
[b]Per 10 ml cell medium.
[c]Reciprocal of 50% infected cell-depressing dilution.
[d](Reciprocal of $ICDD_{50}$/ml of IFN yield/control $ICDD_{50}$/ml IFN) − 1.0 × 100.
[e]Overall, inhibitory activity between epoxides, +S9 and −S9, were not significantly different, $\alpha = 0.05$.

TABLE 7. EFFECT OF TETROL AND TRIOL DERIVATIVES OF BENZO[*a*]PYRENE (BaP) WITH AND WITHOUT RAT LIVER S9 FRACTION ON VIRAL IFN INDUCTION[a]

		+S9		−S9	
BaP derivatives	*Quantity (μmoles)*[b]	*IFN yield ($ICDD_{50}$/ml)*[c]	*IFN inhibition (%)*[d]	*IFN yield ($ICDD_{50}$/ml)*	*IFN inhibition (%)*
7,10/8,9-Tetrol	0.0156	105	25.0	140	0.0
7/8,9,10-Tetrol	0.0156	134	4.3	141	0.0
7/8,9-Triol	0.0164	131	6.5	143	0.0
7,9/8,10-Tetrol	0.0156	100	28.6	133	5.0
7,9,10/8-Tetrol	0.0156	135	3.6	138	1.5
7,9/8-Triol	0.0164	110	21.5	141	0.0
Medium ± S9 (control)		140	0.0	140	0.0

[a]Inducer: Influenza virus added to LLC-MK_2 cell monolayers.
[b]Per 10 ml cell medium.
[c]Reciprocal of 50% infected cell-depressing dilution.
[d](Reciprocal of $ICDD_{50}$/ml of IFN yield/control $ICDD_{50}$/ml IFN) − 1.0 × 100.

DISCUSSION

We have attempted to correlate the relative mutagenicities of BaP metabolites with their abilities to inhibit viral IFN induction. The mutagenic activity of BaP and its metabolites have been extensively studied using the Ames *Salmonella typhimurium* revertant assay supplemented with activation enzymes derived from a liver microsomal S9 fraction.[45] Because both microbial and mammalian (LLC-MK_2) cells do not possess significant levels of enzymes for metabolizing BaP and its metabolites, this provides conducive conditions for assessing the enzymatic activation of these hydrocarbons with microsomal S9. A high correlation has been reported between carcinogenicity and mutagenicity with a variety of agents in the Ames assay. A positive mutagenic response was predictive of carcinogenicity 69% of the time in the Ames assay. When equivocal carcinogens and borderline mutagens were included, the predictivity increased to 83%.[46] Recently compiled data also indicate an excellent correlation between carcinogenic potential of chemicals and inhibition of IFN-α/β induction.[31]

Ambiquity appears to characterize the data relevant to the mutagenicity of BaP phenol derivatives when assessed by the microbial revertant test which may be the consequence of different *Salmonella* tester strains employed, subtle differences in procedures, and methods used to induce drug-metabolizing liver enzymes. Wislocki *et al.*[15] noted that 6- and 12-HOBaP were fairly strong direct mutagens while 1- and 3-HOBaP were weakly mutagenic. The 1-, 3-, 4-, 7-, and 9-HOBaPs have also been reported to be direct mutagens.[46] It had been suggested that these phenol metabolites and 12-HOBaP were possible ultimate or proximate mutagenic forms of BaP, the latter being converted to ultimate products by bacterial enzyme(s). We did not encounter any significant IFN induction inhibition by any of the phenolic derivatives without enzymatic activation. Our findings are more consistent with those of Nagao *et al.*[14] in that all BaP phenol derivatives, with the exception of 4-, and 6-HOBaP, were mutagenic and inhibited IFN induction only when metabolized by S9. These later metabolites were innocuous because they form quinones.

The quinone derivatives of BaP were not directly inhibitory to IFN induction. Three quinones (3,6-, 4,5-, 7,10-diones) showed some depressive activity (~30%) when activated with S9. For the most part, our results were compatible with findings of microbial mutagenicity reported for these products.[14] Further agreement was noted with tests of 6-Me and 6-HOMe-substituted BaP derivatives in that both products were strongly mutagenic and inhibitory to IFN induction only when enzymatically activated.

Both *cis*- and *trans*-4,5-, and 7,8-diol BaP derivatives as well as *trans*-9,10-diol were significantly inhibitory (>50%) to IFN induction in the presence of S9. They showed inhibitory activity (~32%) without activation, but this was not significantly comparable to metabolized diols. The metabolic conversion of *trans*-7,8-diol to predominately diol epoxide I may account for its strong IFN inhibitory activity and mutagenicity. The *cis* and *trans* isomers of 4,5- and 7,8-diols were not mutagenic without metabolic activation.[15] Nagao *et al.*[14] reported that (−)-*trans*-7,8-diol was a direct mutagen but its (+)-*trans*-7,8-diol isomer was weaker. With S9 activation, both (+) and (−) isomers showed strong mutagenicities.

The arene oxides (4,5-epoxide, diol I and II epoxides) are considered to be possible ultimate forms of BaP.[7] All showed strong mutagenic activity to several strains of *S. typhimurium* without metabolic activation.[14] Diol epoxide I, considered to be a potent carcinogen and tumor initiator, exhibited the strongest mutagenicity. Although more chemically reactive, diol epoxide II was of lesser mutagenic potency.[7] In concurrence with these findings were our results obtained on inhibition of IFN induction by these metabolites (Table 6). The IFN inhibitory activities of these epoxides were direct and not significantly different from each other when S9 was added. It should be noted that in the presence of S9 the depressive action on IFN induction by diol derivatives was not significantly different from the epoxides with S9. In the case of the former, enzymatic activation of these proximate metabolites to diol epoxides could account for their comparable activity on IFN induction. In the absence of activation, however, the BaP epoxides showed significantly greater inhibitory activity than BaP-diol metabolites.

The absence of significant inhibition of IFN induction by tetrol and triol derivatives of BaP of diol epoxides, with or without S9, correlated with their nonmutagenicity in the Ames assay.[14] In general, our assessment of the different BaP metabolites on viral IFN induction in mammalian cells was compatible with microbial mutagenicity data.

The mechanisms by which BaP and its metabolites adversely affect viral IFN induction remains to be elucidated. The focal point of BaP interaction did not appear to involve the cell membrane. We noted that the early stages of virus inducer-cell interactions (virus attachment and penetration) appeared not to be affected by pretreatment of cells with metabolically activated BaP.[44] Multiplication rates of influenza virus were similar in both untreated BaP-treated LLC-MK_2 cells; however, the level of virus growth attained was almost threefold higher in BaP-exposed than in unexposed cells. This appeared to be a reflection of BaP inhibition of IFN induction. This indicates also that complete cessation of host cell protein synthesis by BaP is not occurring and that any impairment of protein synthesis may be highly selective. That metabolized BaP may inactivate IFN was precluded by observations that incubation of the hydrocarbon at 35°C for 24 h with IFN preparations did not decrease IFN potency. It has been suggested that formation of DNA-BaP metabolite adducts could inhibit IFN induction because DNA–cross-linking adversely affects IFN induction.[31]

The presence of BaP did not impede the antiviral defense mechanism of IFN.[44] Pretreatment of cells with activated BaP neither decreased IFN titers when assessed in the usual manner nor impaired the ability of IFN to decrease influenza virus yields as compared to controls. It would appear, therefore, that metabolized BaP has no detrimental effect on preformed IFN-α/β (or the ensuing requirement of protein synthesis) to limit its ability to confer cellular antiviral resistance.

That activation-dependent hydrocarbons can be metabolized *in vitro* by rat liver S9 fraction when added to mammalian cell cultures extends the capability and credibility of assessing suspect mutacarcinogens and their metabolites on the basis of inhibition of viral IFN induction. This procedure and criterion may prove useful in augmenting existing assays for identifying biologically reactive mutacarcinogens.

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