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ANTIOXIDANTS AND THE MUTAGENICITY OF BENZO(A)PYRENE AND SOME DERIVATIVES

Luz M. Calle, Paul D. Sullivan, Mary D. Nettleman, Ignacio J. Ocasio, John Blazyk and Joseph Jollick

Department of Chemistry
Ohio University
Athens, Ohio 45701

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SUMMARY

The mutagenicity of benzo(a)pyrene and some derivatives towards Salmonella typhimurium strain TA 98 is in the order 6-methylbenzo(a)pyrene > 10-methylbenzo(a)pyrene > benzo(a)pyrene > 7,10-dimethylbenzo(a)pyrene. Certain antioxidants inhibit the mutagenicity of these compounds towards strain TA 98 when added to the assay mixture. These results raise some interesting questions concerning the species responsible for the mutagenicity and the effect of antioxidants on them.

INTRODUCTION

The inhibitory effect of antioxidants on the chemical carcinogenesis of BP* has been recognized for several years (1-13). The mechanism of this inhibition may involve components of both a direct and indirect nature. In order to probe possible direct effects the interaction of BP free radicals with antioxidants has already been investigated (14). These studies indicated that only the BP cation radical was reactive towards antioxidants. As another approach towards clarifying possible direct mechanisms the effect of antioxi-

*Abbreviations: BP, benzo(a)pyrene; 6-MeBP, 6-methylbenzo(a)pyrene; 10-MeBP, 10-methylbenzo(a)pyrene; 6-MeOBP, 6-methoxybenzo(a)pyrene; 7,10-DMeBP, 7,10-dimethylbenzo(a)pyrene; DMSO, dimethylsulfoxide; PMS, phenazine methosulfate; PTH, phenothiazine; TMTH, 2,3,6,7-tetramethoxythianthrene; EQ, ethoxyquin (2,2,4-trimethyl-1,2-dihydro-6-ethoxyquinoline); NIT, 4-hydroxy-2,2,6,6-tetramethylpiperidinoxy; 1010, Irganox 1010 (tetrakis [methylene-3-(3',5'-di-t-butyl-4'hydroxyphenyl) proprianate] methane); TBB, 3,3',5,5'-tetra-t-butyl-4,4'-biphenol; 736, Ethyl 736, 4,4'-thiobis-(6-t-butyl-o-cresol); BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene.

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dants on the mutagenicity of BP has been investigated using the Ames test. Such studies may be useful for screening antioxidants and other compounds for anti-mutagenic action and hence for potential anti-carcinogenic effects.

Another aspect of our study has involved the use of the 6-methyl-, 10-methyl-, 6-methoxy-, and 7,10-dimethyl-substituted benzo(a)pyrenes. These compounds may be metabolised differently from BP itself and the effect of antioxidants on their metabolism may provide further evidence for the mechanism of action of the antioxidants.

In this particular report we wish to present our results on the effects of antioxidants on the mutagenicity towards Salmonella typhimurium strain TA 98 of BP, 6-MeBP and 10-MeBP.

EXPERIMENTAL

BP and antioxidant samples were of the highest commercially available purity and were used as received. Samples of 6-MeBP and 6-MeOBP were kindly supplied by Dr. E. Cavalieri and samples of 10-MeBP and 7,10-DMeBP by Dr. M. Newman.

The procedure for the Ames test was essentially that described by Ames et al (15). To 2.5 ml of soft agar at 45°C the following are added; 15 µl of a solution of the hydrocarbon in DMSO, the concentration having been optimized by separate mutagenesis studies on the compound; variable amounts, depending on the experiment, of a freshly prepared solution of the antioxidant being tested, all in DMSO (total volume of DMSO per plate never exceeds 100 µl); 0.1 ml of the overnight culture of strain TA 98 of salmonella typhimurium (kindly supplied by Dr. B. Ames); 0.5 ml of potassium phosphate buffer (pH 7.5); 30 µl of the liver homogenate S-9 fraction (protein ca 38 mg/ml) from β-naptho-flavone induced rats and 2.5 µ moles of NADPH. Mutagenicity assays were carried out using 5 plates per set of conditions and the number of revertant colonies were counted after 48 hours incubation at 37°C. Spontaneous revertants were subtracted to obtain the number of revertants due to added substrates. Checks were also made on the toxicity of the hydrocarbons and antioxidants used.

RESULTS AND DISCUSSION

The mutagenicity of BP and its derivatives towards the TA 98 strain were investigated with the results shown in Table I. 6-MeBP and 10-MeBP were found to be approximately six and two times more mutagenic than BP in this test system, whereas 6-MeOBP and 7,10-DMeBP are considerably less active than BP. These observations can be compared with the reported carcinogenicity of 6-MeBP and

Table I. Number of Revertants per plate for BP and Derivatives. [a.]

Compound	Conc (nmoles/plate)	No. Revertants/plate
BP	6.0	238 ± 9
6-MeBP	0.94	423 ± 6
10-MeBP	2.9	222 ± 8
6-MeOBP	18	20 ± 15
7,10-DMeBP	30	80 ± 30

a. Spontaneous Revertants have been subtracted.

the inactivity of 6-MeOBP when applied topically to mouse skin (16). 10-MeBP is also reported to be inactive when injected subcutaneously in male rats (17). 7,10-DMeBP has not been tested to date. The lack of correlation between the carcinogenicity and mutagenicity tests for 10-MeBP is an interesting result and illustrates the dangers inherent in relying on a single mutagenicity test for assessing the carcinogenic activity of a chemical (18). The result also points out that different active species may be responsible for the mutagenic and carcinogenic responses. Previous studies have indicated that the mutagenicity of BP towards S. typhimurium is probably a sum of contributions from several active species the most important of which are the BP-4,5-oxide, the 7,8-diol-9,10-BP-epoxide and the 3- and 6- BP phenols (19-26). The most important ultimate carcinogenic form of BP appears to be a 7,8-diol-9,10-BP-epoxide (19,27-8). The active species of 10-MeBP in the mutagenicity test have not been identified, however, if one assumes that the methyl group in the 10-position is capable of blocking the formation of a 7,8-diol-9,10-epoxide derivative one might explain the mutagenicity of 10-MeBP in terms of 4,5-oxide and phenol derivatives whose formation should not be affected by the methyl substituent. The lack of formation of a diol-epoxide would then also explain the inactivity of 10-MeBP as a carcinogen. Further experiments to test this hypothesis are in progress.

Table II. Effect of Antioxidants on the Mutagenicity of BP, 6-MeBP and 10-MeBP towards S. typhimurium (Strain TA 98).

Antioxidant (60 nmoles/plate)	No. of Revertants per plate		
	BP (6 nmoles/plate)	6-MeBP (0.94 nmoles/plate)	10-MeBP (2.9 nmoles/plate)
None	238 ± 9	423 ± 6	222 ± 8
PMS	0 ± 4 (100)[a]	10 ± 4 (97)	0 ± 4 (100)
PTH	140 ± 14 (41)	386 ± 72 (9)	101 ± 5 (55)
TMTH	110 ± 6 (54)	324 ± 3 (23)	150 ± 5 (32)
EQ	174 ± 10 (27)	337 ± 21 (20)	131 ± 17 (41)
NIT	187 ± 17 (21)	405 ± 36 (4)	204 ± 12 (8)
1010	226 ± 22 (5)	350 ± 10 (17)	157 ± 3 (29)
TBB	183 ± 12 (23)	190 ± 21 (55)	102 ± 2 (54)
736	203 ± 30 (15)	179 ± 12 (57)	58 ± 5 (73)
BHT	135 ± 27 (43)	272 ± 41 (36)	74 ± 21 (67)
BHA	137 ± 29 (42)	337 ± 35 (35)	177 ± 20 (20)

a. Number in parentheses represents the percent decrease in the number of revertants per plate as compared to the control value.

The effect of various antioxidants on the mutagenicity of BP, 6-MeBP and 10-MeBP towards TA 98 were tested with the results shown in Table II. It should be noted that in these experiments the antioxidant concentration was held constant at 60 nmoles/plate, this resulted in an antioxidant/hydrocarbon ratio of approximately 10, 60 and 20 for BP, 6-MeBP and 10-MeBP, respectively. The obvious conclusion of our study is that the antioxidants do inhibit the in vitro production of mutagenic metabolites from BP, 6-MeBP and 10-MeBP. The exact quantitative determination of these effects and the significance of the observed differences between antioxidants and hydrocarbons should await the results of further studies particularly at different concentrations. However, it should be noted that in the only other reported study of this kind an inhibition of BP mutagenicity of 50 and 61% was noted for BHA and EQ at antioxidant/hydrocarbon ratios of 12.5/1 and 3/1, respectively (29).

Some sepcific comments about the results are perhaps appropriate at this point. PMS was included in our studies since it is known to interfere with the electron transport system and should completely inhibit the metabolism of BP and derivatives, as was experimentally observed. PTH and TMTH may also interfere with electron transport processes and they were also found to produce significant inhibition. EQ is a commercial antioxidant whose mechanism of action may involve a stable nitroxide radical (30). NIT is a stable nitroxide with reported antioxidative properties (31). Of these two compounds EQ appears to be a better inhibitor than NIT. 1010, TBB, 736, BHT and BHA are all phenolic antioxidants which appear to exhibit varying degrees of inhibition.

When the effect of the antioxidants on the overall metabolism of BP was studied by observing the HPLC profiles of metabolites it was found that only BHA, PTH, PMS and EQ decreased the total metabolism of BP. The other antioxidants showed little or no effect on the stable metabolities of BP. This suggests that more than one mechanism exists for the inhibition of BP mutagenicity. The effects of antioxidants on the mutagenicity and stability of specific metabolites of BP and it's derivatives should be studied before further conclusions can be drawn, such studies are in progress.

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