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SHORT COMMUNICATION

Mutagenic Effect of 7,12-Dimethylbenz[a]anthracene-epidioxide on *Salmonella typhimurium*

MEI-HWA TU, DENNIS PERRY AND CHIADAO CHEN

Northwestern University Dental and Medical Schools, Chicago, Illinois 60611

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SUMMARY

TU, M.-H., PERRY, D., & CHEN, C. (1979) Mutagenic effect of 7,12-Dimethylbenz-[a]anthracene-epidioxide ($DMBAO_2$) on *Salmonella typhimurium*. *Mol. Pharmacol.* 15, 189–191.

7,12-Dimethylbenz[a]anthracene-7,12-epidioxide ($DMBAO_2$) generated *in situ* by light-sensitized oxygenation of 7,12-dimethylbenz[a]anthracene (DMBA) is strongly mutagenic to *Salmonella typhimurium*. $DMBAO_2$ generated by rat liver microsomes is only moderately mutagenic. Mutagenicity cannot be shown when $DMBAO_2$ is added directly to the medium. Earlier findings indicated that $DMBAO_2$ is a major metabolite of DMBA by liver cytochrome P-450 oxygenase. Light-sensitized oxygenation of DMBA results in the same product in near quantitative yield with no other detectable product. We thus conclude that $DMBAO_2$ is a mutagen that cannot enter a cell or reach the genetic apparatus before destruction. It can exhibit its maximum effect only when DMBA is allowed to enter the cell first before oxygenation to $DMBAO_2$. These results also lead to the identification of a photoactivated product.

INTRODUCTION

It has been demonstrated that $DMBAO_2$[1] is a major metabolite of the polynuclear hydrocarbon carcinogen, DMBA, by rat liver microsomal cytochrome P-450 oxygenase (1). This epidioxide also can be prepared in excellent yield by light-sensitized oxygenation (1, 2). In both cases, the reactions are reversible. $DMBAO_2$, as is the case with most epidioxides, is highly reactive and can bind covalently to proteins, RNA, and DNA upon short incubation at ambient temperature.[2] Its reactivity with cellular macromolecules suggests the possibility that it may be an ultimate carcinogen. Warshawsky *et al.* succeeded in showing that $DMBAO_2$ causes an increase in cell number in secondary cultures of chick-embryo fibroblasts (3). They also found that it induces morphological changes, and increases glucose uptake and DNA synthesis of these cells. The effect of $DMBAO_2$ was about 3-fold greater than that caused by DMBA. However, Cook and Martin reported that tumors did not result from injection of $DMBAO_2$ in sesame oil into mice (2). Attempts by us to induce mammary cancer in rats with $DMBAO_2$ also failed.[3] The maximally tolerated dose for mice was over 500 mg/kg body weight as compared to 135 mg/kg body weight for DMBA (4), indicating its relative harmless nature. The lack of a pronounced biological effect of this chemically reactive compound in these in-

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[1] The abbreviations used are: $DMBAO_2$, 7,12-dimethylbenz[a]anthracene-7,12-epidioxide; DMBA, 7,12-dimethylbenz[a]anthracene; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine.

[2] Tu, M.-H. and C. Chen, unpublished observations.

[3] Chen, C. and C. Lee, unpublished observations.

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stances may be explained in that it or the bulk of it either cannot enter certain cells, or is destroyed at the membrane or in the cytosol before coming into contact with the somatic gene, or both, rather than its lack of reactivity *per se*. Should this be the case, then the generation of $DMBAO_2$ inside the cell may allow its effects to be exhibited. A modified Ames' test (5, 6) where light was used instead of liver microsomes, was chosen as a preliminary screening device to ascertain if $DMBAO_2$ can be an ultimate carcinogen.

Figure 1 demonstrates the effect of light on the degree of mutagenicity of DMBA on *Salmonella typhimurium* TA 100. When incubations were done in the absence of light, there was an increase in the number of revertants for DMBA in the presence of uninduced microsomes and a NADPH generating system (S-9 Mix) as compared to spontaneous reversion rate. Exposure to room light for 48 hr proved highly mutagenic. Light and large amounts of microsomes appear to have an additive effect as

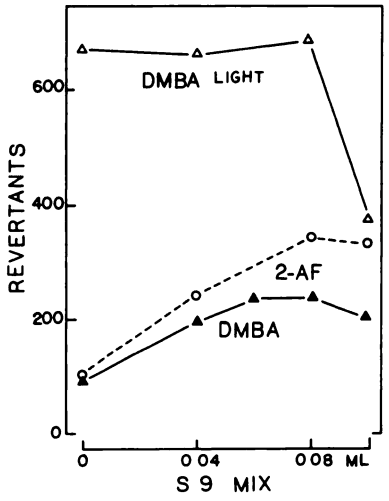

FIG. 1. *The effect of microsomes and light on the number of revertants of Salmonella typhimurium TA100 per plate*

DMBA 20 μg and 2-AF 5 μg per plate. Plates containing DMBA (Δ—Δ) were exposed to two 40 W white fluorescent lights at a distance of 90 cm throughout the incubation time. All other plates were incubated in the dark. The colonies were counted after 48 hours of incubation. Uninduced S-9 Mix was added to all plates at the concentrations indicated. Uncorrected for spontaneous reversion.

this combination caused the death of the microbes. A known mutagen, 2-aminofluorene (2-AF), was used as the control, indicating its need of microsomes for activation. MNNG, another mutagen used as a control, was so effective that at a concentration of 2.5 μg/plate and without S-9 Mix the number of revertant colonies was larger than 1500 and far off the scale.

Figure 2 illustrates the effect of light at various time intervals and different DMBA concentrations. In this and following experiments no S-9 Mix was added. The results show that light alone causes some revertants. Increased reversion can be observed with increased light exposure and DMBA concentration until lethality exceeds mutation. Since we were *previously* able to synthesize $DMBAO_2$ in near quantitative yield with molecular oxygen under light (1, 2), it seems reasonable to suggest that $DMBAO_2$ cannot either freely enter the cell

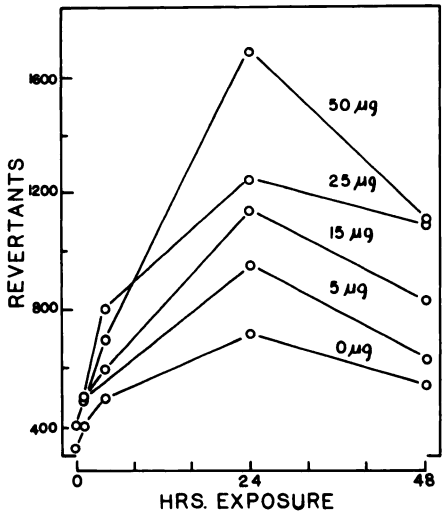

FIG. 2. *The effects of varying DMBA concentrations and the length of exposure to light on the number of revertants per plate*

No S-9 Mix was used. Light exposure began at 0 time. Uncorrected for spontaneous reversion.

or reach the target before destruction.

That $DMBAO_2$ could not reach the genetic apparatus intact is shown in Fig. 3. $DMBAO_2$ exposed to light for 24 hr was no more mutagenic than light alone, though 48 hr exposure to light resulted in more cell death. In this figure DMBA was used to serve as the reference.

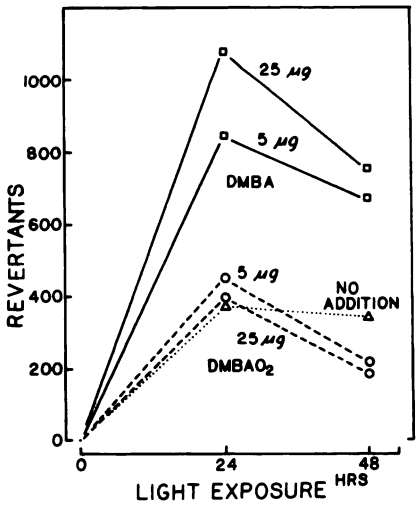

FIG. 3. *The mutagenic effect of DMBA and $DMBAO_2$ exposed to light for 0, 24, and 48 hr*
Corrected for spontaneous reversion.

Though we favor the hypothesis of *in situ* generation of $DMBAO_2$ for the observed mutagenic effect, another possible explanation would be that DMBA can potentiate the mutagenic effect of light. It could serve as a dye in photoactivation of molecular oxygen to its singlet species, which could be directly or indirectly mutagenic.

Photoactivation (acridine) was first shown at the turn of the century (7). Photoactivation of carcinogenic hydrocarbons was reported 35 years later by Lewis (8) using chick embryo cells growing in media containing 1,2,5,6,-benzanthracene, 1,2-benzpyrene, and methylcholanthrene. Epstein (9) has reported the photoactivation of a number of polynuclear hydrocarbons using the death of *Paramecium candatum* as the method of detection. He showed the requirement for oxygen, but could find no evidence of peroxide. These earlier workers provided no indications as to the nature of photoactivated species, nor offered any suggestions that they could be identical with bio-activated species. Our findings suggest that bio-activated ultimate mutagen of DMBA could be $DMBAO_2$, and give evidence that light can activate a mutagen *in situ* to a specific reactive compound.

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