THE MUTAGENESIS ENHANCING ACTIVITY OF 12-O-TETRADECANOYLPHORBOL-13-ACETATE (TPA) IN TWO CHINESE HAMSTER CELL LINES

S.A.Wilson and C.J.Soper, School of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY UK

Tumour promoters, though non-carcinogenic *per se*, act in multistep carcinogenesis to complete a process initiated by primary carcinogens. The presence of tumour promoting agents in the environment may therefore present a significant indirect carcinogenic risk to man. Testing of new and existing compounds for tumour promoting activity is, however, hindered by the lack of rapid screening systems. As the majority of carcinogens are also mutagens (McCann et al 1975), tumour promoters may act *in vitro* to enhance the mutagenic consequences of carcinogen exposure. Demonstration of such mutagenesis enhancing activity might provide the basis for a screening test. Mammalian cell mutation assays have been adapted to assess the mutagenesis enhancing activity of tumour promoters but the data obtained are conflicting (Thompson et al 1980; Trosko et al 1977). Explanations for this have frequently implicated differences in the cell lines and culture conditions used. We have therefore investigated the effects of the potent tumour promoter, TPA, on the induction of ouabain resistance (Oua^R) mutations by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in two Chinese hamster cell lines, CHO-K1A and V79-379A, under different culture conditions.

Mutation to Oua^R was assessed for both cell lines by an *in situ* method of expression time 49 hours, density of cells in selection plates $2x10^5$/10ml medium/ 90mm diameter plate, and concentration of ouabain 1mM. Cells were exposed to MNNG (0.08μg/ml CHO-K1A; 0.04μg/ml V79-379A) for 2 hours, and to TPA (1μg/ml) from the end of mutagen treatment for the remainder of mutation expression and the whole of mutant selection. Cells were grown and treated in one of three different culture media, Ham's F10, Eagle's minimal essential medium(EMEM),or EMEM with $2x10^{-4}$M L-cysteine. Typical results are shown below.

Effects of TPA on MNNG-induced lethality and mutation to Oua^R in CHO-K1A and V79-379A cells under different culture conditions.

MEDIUM	TPA (1μg/ml)	CHO-K1A Plating Efficiency	CHO-K1A Mutants/10^6 Survivors	V79-379A Plating Efficiency	V79-379A Mutants/10^6 Survivors
Ham's F10	-	8.8	113	7.6	78
	+	8.8	197	4.8	248
EMEM	-	32.3	256	24.5	307
	+	32.5	330	17.0	757
EMEM with L-cysteine	-	31.8	74	22.5	335
	+	32.3	146	15.3	744

TPA was shown to enhance MNNG-induced mutagenesis in both cell lines but the extent of the enhancement was dependent upon both the cell line and the culture medium. In both cell lines TPA had no effect on the spontaneous frequency of mutation to Oua^R. The most significant differences in mutagenesis enhancement between the cell lines was observed when they were grown and treated in EMEM which suggests that the effects measured in the two cell lines may arise by different mechanisms. TPA is biologically highly active and the observed mutagenesis enhancing activity may be independent of and unrelated to its tumour promoting activity.

McCann, J. et al (1975) Proc. Natl. Acad. Sci. U.S.A. 72:5135-5139
Thompson, L.H. et al (1980) Cancer Res. 40:3245-3251
Trosko, J.E. et al (1977) Cancer Res. 37:188-193