THE EFFECT OF THE TUMOUR PROMOTER 12-TETRADECANOYLPHORBOL-13-ACETATE (TPA) ON MUTAGENESIS IN MAMMALIAN CELLS

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In multistep carcinogenesis, tumour promoters, though non-carcinogenic per se, act to complete a process initiated by primary carcinogens and produce increases in tumour yield and decreases in the lag time for tumour appearance. Since the majority of carcinogens are also mutagens (McCann et al 1975) we have investigated whether the potent tumour promoter TPA, can enhance mutagenesis induced by chemical and physical agents in cultured mammalian cells. If such a 'co-mutagenic' action could be demonstrated it might enable the development of a rapid and informative screening test for environmental tumour promoters.

An assay system allowing quantification of the frequency of forward, point mutation to ouabain drug-resistance (Oua^R) in a Chinese hamster cell line (CHO-k1A) was designed. The assay was validated with respect to mutation expression time (48h), density of cells in selection plates ($2x10^5$ cells/ 10ml Ham's F10 medium/ 90mm dish), and concentration of selective agent (3mM ouabain). Cells were plated, treated with mutagen, and exposed to promoter throughout mutation expression and selection.

We investigated the effect of TPA ($1.6x10^{-6}$M) on the induction of Oua^R mutations by three known mutagens: the monofunctional ethylating agent ethyl methanesulphonate (EMS); the monofunctional methylating agent N-methyl-N'-nitro-N-nitrosoguanidine (MNNG); and the physical mutagen ultraviolet (UV) radiation, at 254nm wavelength. Representative results are given in Table 1.

Table 1. Effect of TPA on EMS, UV and MNNG induced lethality, and mutation to Oua^R in CHO-k1A cells.

Mutagen treatment	TPA ($1.6x10^{-6}$M)	Survival (%)	Mutants/ 10^6 survivors
EMS, $8x10^{-4}$M, 16h	-	73	99.6
"	+	71	101
UV, 254nm, $25Jm^{-2}$	-	54	63.0
"	+	46	65.2
MNNG, $3.4x10^{-7}$M, 51h	-	9.6	78.4
"	+	10	152

TPA at the test concentration was shown to be non-cytotoxic and to have no effect on the spontaneous frequency of mutation to Oua^R (1-2 mutants/10^6 survivors). It also had no effect on EMS induced lethality or mutagenesis. In combination with UV irradiation, TPA caused a small, consistent reduction in cell viability but again had no effect on the induced mutation frequency. However, TPA significantly enhanced MNNG mutagenesis, increasing the mutation frequency after MNNG treatment by 95% without affecting cell viability. Experiments where TPA was presented to MNNG-exposed cells at various times and for different durations established that TPA enhancement of MNNG-induced mutation to Oua^R required the promoter to be added immediately after the mutagen and to be present throughout mutation expression and selection. A possible interpretation of our findings, based on the proposal that tumour promoters act by gene activation (Boutwell 1978), is that certain types of mutation induced by MNNG, but not by EMS or UV, are normally unexpressed, but can be derepressed or 'activated' in the presence of the tumour-promoting compound.

Boutwell, R.K. (1978), Carcinogenesis Vol.2, Raven Press, New York, pp 49-58
McCann, J. et al (1975) Proc. Natl. Acad. Sci. U.S.A. 72 : 5135-5139

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