PROLIFERATION OF HUMAN LYMPHOCYTES INDUCED BY A SERIES OF TUMOUR-PROMOTING PHORBOL ESTERS

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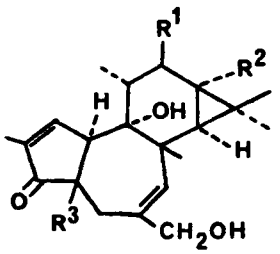
Interest in certain esters of the diterpene phorbol centres upon two actions which they exhibit to varying degrees. The first is the intense inflammation produced upon application to mammalian skin and the second is the tumour promoting activity exhibited on repeated application to mouse skin following a sub-threshold dose of carcinogen. The most potent phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA) induces cell proliferation and subsequent epidermal hyperplasia when applied in nanomolar quantities to mouse skin. These sequential events are recognised as being essential components of TPA's action as a mouse skin tumour promoter (Boutwell 1974). Analogous TPA-induced cell proliferation (mitogenesis) is observed in vitro in human lymphocyte cultures (Estensen et al 1974). As part of an investigation into the mechanisms of action of tumour promoters we have examined the abilities of a series of phorbol esters to induce human lymphocyte proliferation, in order to determine if the activities of these compounds as lymphocyte mitogens correlate with their potencies as tumour promoters.

Mononuclear cells, predominantly lymphocytes, were separated from heparinised human peripheral blood by sedimentation on Ficoll-Isopaque gradients. The cells were washed in phosphate buffered saline and then washed and resuspended at 10^6 cells ml^{-1} in a modified RPMI 1640 medium supplemented with 10% foetal bovine serum. The cell suspension was treated with phorbol ester and dispensed in 0.1 ml volumes ($1x10^5$ cells) into the wells of microtitre plates. The plates were incubated for 72h at 37°C in a humidified 5% CO_2 atmosphere. Proliferative stimulation of lymphocytes was assessed by cellular ^{3}H-thymidine uptake. ^{3}H-thymidine 1μ Ci ml^{-1} was added to each microculture for the final 24h of incubation. The microcultures were harvested onto glass fibre filter discs and the entrained cells washed successively with distilled water, 5% trichloroacetic acid and methanol. The discs were dried thoroughly in glass scintillation vials. Five ml of scintillation cocktail (PPO 6g, POPOP 0.05g l^{-1} toluene) was added to each vial. Counts per minute (cpm) of incorporated ^{3}H-thymidine from a minimum of five replicate microcultures were obtained for each phorbol ester dose.

Table 1. Mitogenic activities of Phorbol Esters.



	R^1	R^2	R^3	Promoter potency	Lowest active conc. (M)
1	Tetradecanoate	Acetate	OH	+++	$1.6x10^{-9}$
2	Decanoate	Decanoate	OH	++	10^{-8}
3	Benzoate	Benzoate	OH	+	$5.0x10^{-8}$
4	Acetate	Acetate	OH	+/-	10^{-6}
5	Tetradecanoate	Acetate	OCH_3	+/-	10^{-6}
6	Decanoate	Decanoate	OH(α)	-	Inactive
7	OH	OH	OH	-	Inactive

In Table 1, for the purposes of comparison, the lowest concentration of a particular ester inducing significant ^{3}H-thymidine uptake is used as a measure of the mitogenic activity of that ester. In dose-response studies phorbol esters with pronounced mitogenic activity (1-3 in Table 1) gave similar maximum levels of stimulated ^{3}H-thymidine uptake. Our data suggest that the mitogenic activities of these esters correlate with their potencies as tumour promoters.

Boutwell, R.K. (1974) CRC Critical Reviews in Toxicology, 419-443.
Estensen, R.D. et al (1974) Control of Proliferation in Animal Cells. Cold Spring Harbor Conferences on Cell Proliferation, Vol.1 pp.627-34.

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