J. Pharm. Pharmacol. 1995, 47: 263
Received September 5, 1994
Accepted December 5, 1994

Letter to the Editor

Sapintoxin D is a Weak Tumour Promoter in Sencar Mouse Skin

J. R. MCLEAN, M. PLANT, D. W. LECUYER, C. DAVIDSON AND J. TRUONG

Health Canada, Radiation Protection Bureau, 775 Brookfield Road, Ottawa, Ontario, Canada K1A 1C1

Sapintoxin D, 12-*O*-[*N*-methylaminobenzoyl]-phorbol-13-acetate, is a naturally fluorescent, 4-deoxyphorbol ester, derived from the fruit oil of *Sapium indicum* Willd (family Euphobiaceae), an evergreen tree indigenous to Bangladesh. Previous investigators have shown that this compound has many properties in common with the strong tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Chopra et al 1958; Miana et al 1977; Edwards et al 1983). Both cause erythema in mouse ear, aggregation of platelets in-vitro, and activation of protein kinase C (PKC). The K_a for PKC activation is reported to be 28 nM for sapintoxin D (Morrice et al 1986) and 8 nM for TPA (Horowitz et al 1981), which suggests that sapintoxin D should be a strong tumour promoter. We now report that sapintoxin D is a weak tumour promoter when compared with TPA in the Sencar mouse 2-stage skin tumorigenesis model. This reduced activity in-vivo may be related to its disposition in skin after topical application.

The shaved dorsal skins (about 5 cm^2) of 48 six-week old Sencar mice were treated with 10 nmol of the tumour initiator 7,12-dimethylbenz[a]anthracene (DMBA) and then mice were randomly assigned to one of two treatment groups. Twice weekly, for 20 weeks, one group received 3·3 nmol TPA in 0·2 mL acetone while the other received an equimolar dose of sapintoxin D. At termination of the experiment, only 13% of mice treated with sapintoxin D developed papillomas compared with 88% for the group exposed to TPA. For mice with papillomas, the mean yield was 6 per mouse for sapintoxin D and 14 per mouse for TPA. In previous experiments we found that papillomas did not develop on mice initiated with DMBA and treated for 20 weeks with acetone (McLean et al 1991). In addition, sapintoxin D did not produce splenic hyperplasia ($0{\cdot}14 \pm 0{\cdot}04$ g, n = 24), in contrast to TPA which produced significant (5% level) splenic hyperplasia ($0{\cdot}30 \pm 0{\cdot}13$ g, n = 24). Sapintoxin D therefore appears to be only a weak tumour promoter in Sencar mouse skin despite some biochemical similarities to the strong tumour promoter TPA.

In another group of mice, skin samples were taken at various times after a single application of sapintoxin D. Frozen sections were examined by fluorescence microscopy using excitation of 356 nm and a filter set to collect emission at 440 nm (the emission maximum of sapintoxin D when exposed to blue light). Under these conditions, sapintoxin D was detected in the sub-epidermal layers at 6 h, but not at 24 h, after sapintoxin D application. This suggests that sapintoxin D may be a weak tumour promoter since it failed to reach the epidermis in sufficient amounts to produce a sustained stimulation of the initiated stem cells.

References

Chopra, R. N., Chopra, I. C., Handa, K. L., Kapur, L. D. (1958) Indigenous Drugs of India. U.N. Dhur, Calcutta, p. 589

Edwards, M. C., Taylor, S., Williamson, E. M., Evans, F. J. (1983) New phorbol and deoxyphorbol esters: isolation and relative potencies in inducing platelet aggregation and erythema of skin. Acta Pharmacol. Toxicol. 53: 177–187

Horowitz, A. D., Greenbaum, E., Weinstein, I. B. (1981) Identification of receptors for phorbol ester tumor promoters in intact mammalian cells of an inhibitor of receptor binding in biological fluids. Proc. Natl. Acad. Sci. 78: 2315–2319

McLean, J. R., Stuchly, M. A., Mitchel, R. E. J., Wilkinson, D., Yang, H., Goddard, M., Lecuyer, D. W., Schunk, M., Callary, E., Morrison, D. (1991) Cancer promotion in a mouse-skin model by a 60 Hz magnetic field: II tumor development and immune response. Bioelectromagnetics 12: 273–287

Miana, G. A., Schmidt, R., Hecker, E., Shamma, M., Moniot, J. L., Kiamuddin, M. (1977) 4 alpha-Sapinine—a novel diterpene ester from *Sapium indicum*. Z. Naturforsch B: 727–728

Morrice, N. A., Ellis, C., Evans, A. T., Evans, F. J., Drummond, A., Aitken, A. (1986) Fluorescent phorbol ester binding to the receptor protein kinase C in-vitro and in-vivo. J. Pharm. Pharmacol. 38 (Suppl.): 30P

Correspondence: J. R. McLean, Health Canada, Radiation Protection Bureau, 775 Brookfield Road, Ottawa, Ontario, Canada K1A 1C1.