IVERMECTIN: AN INHIBITOR OF PROTEIN KINASE C - A POTENTIAL TARGET ENZYME FOR ONCHOCERCIASIS CHEMOTHERAPY

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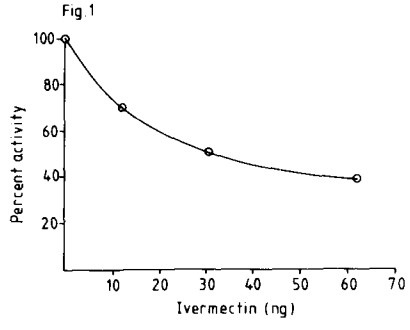
Chemotherapeutic agents potentially suitable for treatment of human onchocerciasis, including the condition known as river blindness, (Chappell, 1983) are being investigated by preparing tissue extracts of the filarial worms Brugia pahangi and Onchocerca gibsoni, to assay and classify their protein kinase activity(ies). Glycolysis, gluconeogenesis and glycogen synthesis are regulated by reversible phosphorylation and since their sole energy source is carbohydrate, studies of protein kinases in these parasites are of importance to evaluate the chemotherapeutic use of their inhibitors.

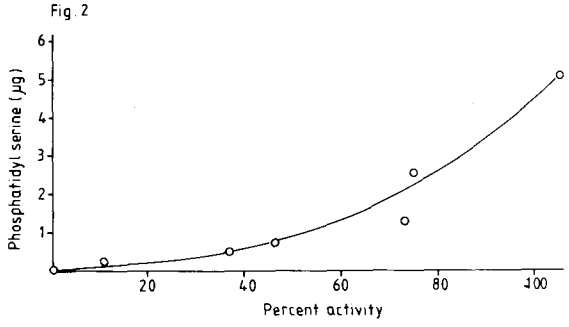
Protein kinase C is a Ca^{2+}-phospholipid dependent protein kinase (Berridge, 1984) present in all mammalian tissues examined. We have preliminary evidence that a similar enzyme may also be present in Brugia pahangi and other filarial worms.

The avermectins are a family of recently discovered drugs with a novel mode of action at very low dosage against a broad spectrum of nematode and arthropod parasites of animals (Campbell et al., 1983). In trials carried out on cattle and sheep, one derivative, ivermectin, was shown to have a better safety profile than other avermectins. Ivermectin is the 22,23 dihydro derivative of avermectin B_1, a macrocyclic lactone produced from the mycelia of an actinomycete, Streptomyces avermitilis.

Protein kinase C was assayed for 10 min. at 30°C using histone III-S as substrate, in a total volume of 40 µl, containing 12.5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 1.25 mg/ml histone, γ-^{32}P ATP, 0.25 µg phosphatidylserine, 0 to 60 ng ivermectin and one unit of enzyme. The reaction was terminated by spotting 30 µl of supernatant onto chromatography paper, washing in 10% trichloroacetic acid and the paper counted in scintillant for ^{32}P incorporation into histone.

We have shown that ivermectin inhibits the activity of protein kinase C isolated from rat brain (Fig.1). This inhibition appears to be competitive with the phospholipid, phosphatidylserine since the activity of protein kinase C could be restored by increasing amounts of phosphatidylserine (Fig.2).





The ability of phorbol esters to enhance the activity of protein kinase C (Aitken 1985) is unaffected by the presence of ivermectin.

Aitken, A. (1985) Chap.10 in "Naturally Occurring Phorbol Esters" (Ed. Evans, F.J.) CRC Press, Boca Raton.
Berridge, M.J. (1984) Biochem. J. 220: 345-360.
Campbell, W.C. et al. (1983) Science 221: 823-828.
Chappell, L.M. (1984) WHO, OCP, Biochem. Soc. Bulletin 5, no.5: 12-14.