INHIBITION OF HERPES SIMPLEX VIRUS TYPE 1 DNA POLYMERASE BY THE NATURAL PRODUCT OOSPOREIN

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Herpesviruses are a common virus infecting man. Two drugs have shown clinical efficacy in the treatment of herpesvirus infections, acyclovir and ganciclovir. Acyclovir has excellent antiviral activity against herpes simplex types 1 and 2[1]; ganciclovir is potent against human cytomegalovirus[2]. Both drugs are nucleoside analogs and are metabolized to their respective triphosphates which then block viral DNA replication by inhibiting the viral DNA polymerase[3,4].

We have developed a natural product screen to search for non-nucleotide inhibitors of viral DNA polymerases. Herpes simplex type 1 (HSV-1) DNA polymerase is a good target since the viral polymerase is well characterized[5] and few natural products are known to interfere with the assay. The strategy of the screen is to identify novel compounds that inhibit HSV-1 DNA polymerase to a significantly greater degree than the enzymes from bacteria and mammalian cells. Nonspecific inhibitors that might interfere with the enzyme-based assay are eliminated by extraction of the fermentation broth with ethyl acetate. High MW species are also eliminated by size exclusion filtration. This screen has led to the identification of the dibenzoquinone, oosporein[6] as a selective inhibitor of HSV-1 DNA polymerase. A structurally related compound, gossypol, was also found to preferentially inhibit HSV-1 DNA polymerase.

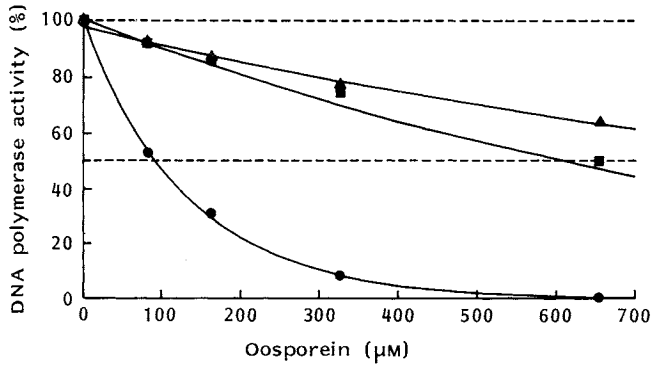
Oosporein is produced by a fungal soil isolate that was identified as *Beauvaria* sp. and deposited as SC15097 in our culture collection. To produce oosporein, a germinator culture was grown in a medium of the following composition: malt extract 1%, yeast extract 1%, peptone 0.1% and glucose 2%. The fermentation proceeded for 72 hours at 25°C on a rotary shaker. This culture then served as the source of inoculum (5%) for the fermentation that was done with the same medium and conditions as used for the germinator. At harvest, the fermentation broth was centrifuged to remove the mycelia, the supernatant adjusted to pH 3 with HCl and extracted with ethyl acetate. The ethyl acetate layer was evaporated *in vacuo* to a residue that was then dissolved in a small volume of methanol. Oosporein crystallized from methanol - water as red needles that were subsequently collected by filtration (mp >300°C, $C_{14}H_{10}O_8$, MW 306, UV λ_{max}^{MeOH} nm 212 and 289). The tetraacetate, tetramethyl ether, trimethyl ether and dimethyl ether derivatives of oosporein were prepared, by previously described methods[6,7].

HSV-1 DNA polymerase was purified from HSV-1 (KOS) infected HeLa S3 cells as previously described[8]. HSV-1 DNA polymerase activity was determined in: 50 mM Tris-HCl pH 8; 5 mM $MgCl_2$; 1 mM DTT; 0.1 M ammonium sulfate; 5 μM (each) dATP, dGTP, dCTP and [^{3}H]dTTP (500 cpm/pmol); 30 μg/ml activated calf thymus DNA; 0.1 mg/ml bovine serum albumin; and HSV-1 (KOS) DNA polymerase. Incubation was for 20 minutes at 37°C. Incorporation of [^{3}H]dTTP into DNA was quantitated by spotting the reaction on Whatman GF/C discs and batch precipitated in cold 5% trichloroacetic acid - 10 mM sodium pyrophosphate, washed 3 times with cold 1 N HCl, once with 95% ethanol, dried and quantitated by scintillation counting. *E. coli* DNA polymerase I (0.06 U) was assayed in the buffer described above. Inhibition of HeLa DNA polymerase activity was determined under the following conditions: 50 mM Hepes pH 7; 5 mM $MgCl_2$; 1 mM DTT; 30 μg/ml activated calf thymus DNA; 0.1 mg/ml bovine serum albumin; 5 μM (each) dATP, dGTP, dCTP and [^{3}H]dTTP (500 cpm/pmol); and HeLa S3 cell extracts[9]. Incubation was for 30 minutes at 37°C and incorporation of radiolabel into DNA was quantitated by trichloroacetic acid precipitation and scintillation counting. Oosporein and gossypol solutions were prepared in DMSO and the final DMSO concentration in polymerase assays was 5%.

Oosporein was found to preferentially inhibit HSV-1 DNA polymerase compared to either HeLa DNA polymerase α or *Escherichia coli* DNA polymerase I (Fig. 1). The oosporein concentration necessary to inhibit 50% (IC_{50}) was 75 μM for HSV-1 DNA polymerase, 610 μM for HeLa DNA polymerase activity and >700 μM for *E. coli* DNA polymerase I using activated calf thymus DNA as a substrate. No inhibitory activity was evident when

Fig. 1. Inhibition of DNA polymerase activities by oosporein.

HSV-1 DNA polymerase (●), DNA polymerase α (■), *Escherichia coli* DNA polymerase I (▲).



Reactions were carried out as described in Materials and Methods except oosporein (dissolved in DMSO) was added at varying concentrations. In the absence of inhibitor, 10 pmol [^{3}H]dTTP was incorporated by HSV-1 DNA polymerase, 100 pmol [^{3}H]dTTP was incorporated by *E. coli* DNA polymerase I and 6 pmol [^{3}H]dTTP was incorporated by HeLa DNA polymerase α.

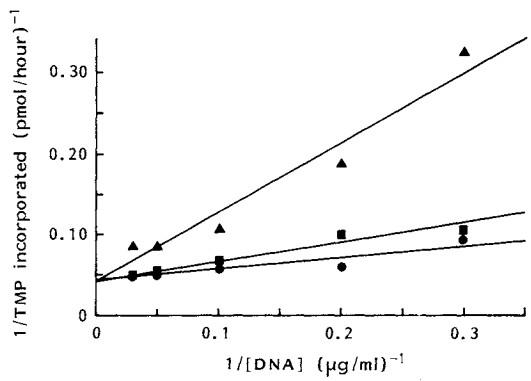
oosporein was tested against an unrelated enzyme, TEM-2 β-lactamase[10], again indicating a specificity of action. Derivatization of 2~4 hydroxyls of oosporein completely destroys the ability to inhibit HSV-1 DNA polymerase; the significance of this is not as yet understood.

Further studies with HSV-1 DNA polymerase were performed to investigate the mechanism of inhibition by oosporein. Oosporein was determined to be a noncompetitive inhibitor of dGTP or dCTP incorporation into DNA (data not shown). In contrast, oosporein is a competitive inhibitor with respect to the DNA substrate (Fig. 2). The inhibition constant for oosporein was determined to be 30 μM for HSV-1 DNA polymerase. The competitive inhibition of HSV-1 DNA polymerase with respect to DNA suggests that oosporein binds to the DNA binding site of the polymerase or intercalates into DNA, thereby disrupting the DNA template. A DNA intercalator should inhibit the DNA relaxing activity of topoisomerase I when closed circular plasmid DNA is used as a substrate; additionally, supercoiling by DNA gyrase is inhibited by DNA intercalators[11]. The lack of inhibition of topoisomerase I and DNA gyrase suggests that this is not the case; neither of these enzymes was inhibited by oosporein up to 100 μg/ml (327 μM) (data not shown). In addition, the gel electrophoretic analysis shows that oosporein does not break phosphodiester bonds in the DNA.

The related compound, gossypol behaves similarly to oosporein; inhibition is noncompetitive with respect to dNTPs and competitive with respect to

Fig. 2. Inhibition of HSV-1 DNA polymerase by oosporein was determined as described in the text with 5 μM dNTPs and the activated calf thymus DNA concentration was varied.

Oosporein concentrations were 0 (●), 98 (■) and 325 (▲) μM.

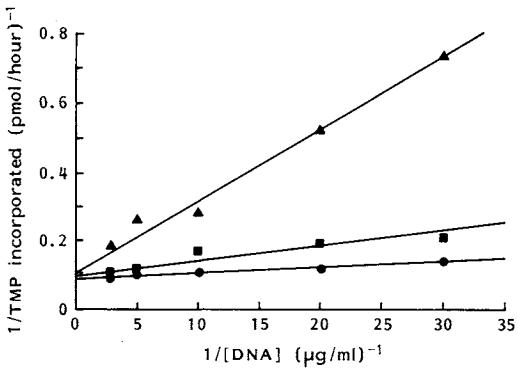


DNA (Fig. 3). The inhibition of HSV-1 DNA polymerase is more potent with an inhibition constant of 4 μM. Gossypol has previously been shown to inhibit HSV-2 growth in cell culture; the mechanism of antiviral action was suggested to occur at the virion envelope since the nonenveloped poliovirus was not affected[12]. Our data suggest the additional possibility that the antiviral effect may be due to inhibition of the viral DNA polymerase.

Other studies with gossypol have indicated that gossypol had antiviral activity versus HSV-2 but was cytotoxic in Vero cells[13]. ROSENBERG *et al.*,[14] have shown that gossypol inhibits HeLa DNA poly-

Fig. 3. Inhibition of HSV-1 DNA polymerase by gossypol was determined with 5 μM dNTPs and various activated calf thymus DNA concentrations.

Gossypol concentrations were 0 (●), 9.8 (■) and 33 (▲) μM.



merase α activity. Gossypol inhibition is noncompetitive with respect to deoxynucleotide triphosphates and competitive with respect to activated calf thymus DNA, the same pattern of inhibition we observe with HSV-1 DNA polymerase. Under our assay conditions, the inhibition we observe with HSV-1 DNA polymerase is much greater than the inhibition of HeLa DNA polymerase activity and suggest that gossypol interacts with HSV-1 and HSV-2 DNA polymerases differently.

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