Eulicin Inhibits Human Immunodeficiency Virus Infection and Replication

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Eulicin, an antifungal antibiotic agent, was previously isolated from a species of Streptomyces and described by Charney et al.1, and the structure of Eulicin (Fig.1) was determined by Herman et al.2. In the present communication, we investigated its effect on human immunodeficiency virus (HIV) infection and replication.

The experiment analyzing effects on primarily infection was performed as follows. H9 cells4 were pretreated with serially diluted eulicin at 37°C for 60 minutes and infected with HIV-1 IIIB at 2x103 TCID₅₀. The cells were incubated for an additional 60 minutes to permit adsorption of viral particles to cells, then diluted with fresh media 1 : 10 and cultured in a 96 well plate. On day 6, the culture was harvested for reverse transcriptase (RT) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assays. The RT assay was performed as described previously5 to estimate the concentration of viral particles from HIV infected cells. The MTT assay5, based on the mitochondrial reduction of MTT, was utilized to measure the number of uninfected and infected living cells after exposure to various concentrations of eulicin. In brief, 100µl of cell suspension was transferred to a new 96 well plate and incubated at 37°C for 4 hours with 10µl of 5mg/ml MTT. 100µl of 10% SDS was then added to lyse cells and solubilize MTT formazan. The absorbance was measured on a microplate reader with a test wavelength of 550nm and reference wavelength of 630 nm.

As shown in Fig. 2, eulicin treatment resulted in a concentration dependent inhibition of HIV replication with slight cytotoxicity at higher concentration. The therapeutic ratio of the effective dose (IC₅₀) to the cytotoxic dose (MTT) was greater than 40. AZT7 treatment also showed concentration dependent inhibition on this assay system (data not shown). Identically treated uninfected cultures studied in parallel showed no change in cellular viability as compared to infected cultures (data not shown).

To exclude the possibility that eulicin directly inhibits reverse transcriptase in this RT assay system, we tested eulicin in the RT assay. No change in RT values was observed at the concentrations as high as 100µg/ml, therefore, this agent did not have an effect on RT. Nevertheless, both infection and replication of HIV were decreased by eulicin.

In order to determine the mechanism of action of eulicin in early stages of HIV infection, we examined
the influence of time of exposure to eulicin. H9 cells were exposed to HIV-1 IIIB at a high multiplicity to ensure that the virus replication step was synchronized in the whole cell population and then incubated at 37°C. Eulicin was added at varying times (−60, 0, +60 minutes) before adsorption or after exposure to H9 cells. Figure 3 shows that any HIV replication was not influenced by the exposure time of eulicin during the virus adsorption step. This suggests that eulicin interferes with events after penetration as the virus goes through its successive replication. This may provide a therapeutic strategy that is useful for HIV infection. Furthermore, this agent may provide a tool for gaining a better understanding of the HIV replication.

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References


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