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]. Efficacy and toxicity studies were performed[2], and the structure of Eulicin (Fig.1) was determined by HERMAN *et al.*[3]. 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 cells[4] were pretreated with serially diluted eulicin at 37°C for 60 minutes and infected with HIV-1 IIIB at 2×10^3 $TCID_{50}$. 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 previously[5] to estimate the concentration of viral particles from HIV infected cells. The MTT assay[6], 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, 100 μl of cell suspension was transferred to a new 96 well plate and incubated at 37°C for 4 hours with 10 μl of 5 mg/ml MTT. 100 μl 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 550 nm 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 (IC_{50}) to the cytotoxic dose (MTT) was greater than 40. AZT[7] 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 100 μg/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

Fig. 1. Chemical structure of eulicin.

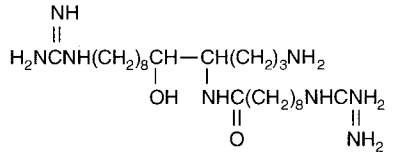
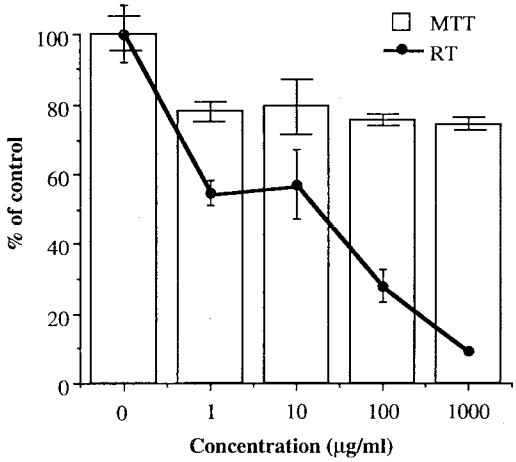
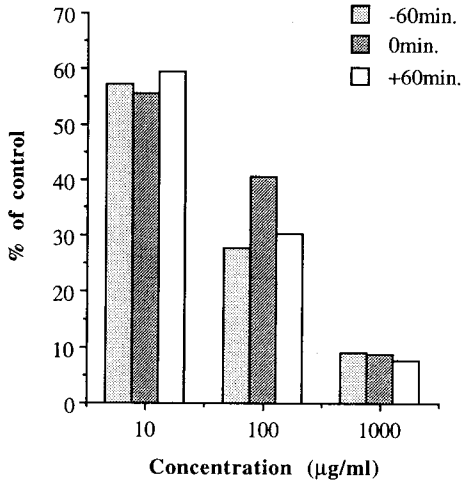


Fig. 2. Inhibitory effect of eulicin on HIV replication.



The data represents the means and standard deviations of triplicate experiments and the values represent % of control without agent.

Fig. 3. Inhibitory mechanism of eulicin during virus adsorption to cells.



H9 cells were inoculated with HIV and eulicin was added either 60 minutes prior to infection, at time zero of infection, or 60 minutes after infection.

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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