INHIBITION BY DOXORUBICIN OF HUMAN IMMUNO-DEFICIENCY VIRUS (HIV) INFECTION AND REPLICATION IN VITRO

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Human immuno-deficiency virus (HIV) also known as human T-lymphotropic virus type III (HTLV-III) / lymphadenopathy-associated virus (LAV) is a newly recognized retrovirus and thought to be the causative agent of the acquired immune deficiency syndrome (AIDS) and AIDS-related complex (ARC). Inhibitors of reverse transcriptase, whose activity is essential for the replication of retroviruses, might be candidates for the chemotherapy of HIV infections. Many compounds have been reported as inhibitors of reverse transcriptase e.g. suramin and HPA, however their clinical efficacy has not been established.

An initial mass survey on a number of antibiotics was carried out in a search for inhibitors of avian myeloblastosis virus (AMV)-reverse transcriptase. Enzyme activity was measured by the incorporation of [3H]TTP into a high molecular fraction using poly(rA)-oligo(dT)12-18 as template-primer in the presence of either 10 or 40 µg/ml antibiotic. Among the potent enzyme inhibitors found, only those of low cytotoxicity have been further tested for their effects on the in vitro replication of HIV using the HTLV-I-carrying cell line, MT-4. One of the virus strains of HIV, HTLV-III was obtained from the culture supernatant of Molt-4/HTLV-III. MT-4 cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 100 IU/ml of benzylpenicillin and 100 µg/ml of streptomycin at 37°C in a CO₂ incubator. MT-4 cells were exposed to virus preparations at a multiplicity of infection (MOI) of 0.002 for 1 hour at 37°C. After infection, the cells were washed and resuspended in fresh medium to give a concentration of 3 x 10⁶ cells/ml. This cell suspension was then cultured in the presence or absence of various concentrations of doxorubicin in a CO₂ incubator. Half of each culture medium was changed every 3 days. The bio-assay system using MT-4 cells for studying HIV infection was developed by HARA et al. Inhibitory effects of sakyomicin A, recombinant human interferon gamma and 3’-azido-3’-deoxythymidine (AZT) on HIV infection were recognized using this assay system.

Approximately 150 antibiotics classified into various groups have been tested for their effects on AMV-reverse transcriptase. Among anthracyclines included in our screening, doxorubicin, daunorubicin and aclarubicin were chosen for the further investigation and the results are given in this note. Doxorubicin and daunorubicin were potent inhibitors of reverse transcriptase. The IC₅₀'s against L5178Y cells were 0.04 µg/ml for daunorubicin and 0.49 µg/ml for doxorubicin. Therefore, daunorubicin was relatively toxic to the cells. Though no marked inhibition of reverse transcriptase was observed by aclarubicin, it was the most cytotoxic of the anthracycline group (IC₅₀ against L5178Y cells: 0.004 µg/ml) (Table 1). In view of the inhibition of reverse transcriptase and cytotoxicity, we further studied doxorubicin and its ability to inhibit HIV infection in MT-4 cells. The cytotoxicity of doxorubicin against HIV-uninfected MT-4 cells, and effects of this chemical on the growth and viability of the cells as well as virus antigen expression induced by HIV infection were assayed on the 3rd and 6th days after infection. Although doxorubicin was cytotoxic against MT-4 cells at concentrations of 10 and 1.0 µg/ml, no significant difference was observed in cell growth be-

Table 1. Inhibition by anthracycline antibiotics of reverse transcriptase from avian myeloblastosis virus.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>IC₅₀ (L5178Y)</th>
<th>Inhibition (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>40 µg/ml</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>70.4</td>
<td>29.8</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>55.5</td>
<td>13.9</td>
</tr>
<tr>
<td>Aclarubicin</td>
<td>29.3</td>
<td>15.3</td>
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(Reduced and adapted from the original text for clarity and conciseness)
Fig. 1. Inhibitory effect of doxorubicin on cytopathic effects of HIV and the expression of HIV-specific antigens.

MT-4 cells (A and B) and HIV infected MT-4 cells (C, D, E and F) were cultured in the presence or absence of various concentrations of doxorubicin. The cells were infected with virus at a multiplicity of infection of 0.002 and incubated for 1 hour at 37°C. After adsorption, infected cells were washed and resuspended in fresh medium to give a concentration of 3 x 10^5 cells/ml. The viable cells with or without HIV infection were determined by trypan blue dye exclusion method 3 days (A and C) and 6 days (B and D) after culture. For the expression of HIV-specific antigens, indirect immunofluorescence (IF) was applied\(^{11-12}\). More than 500 cells were counted and frequency of IF-positive cells was calculated 3 days (E) and 6 days (F) after infection.
between MT-4 cell cultures treated with 0.01 or 0.001 μg/ml of doxorubicin and drug free controls (Fig. 1A and 1B). For example, the number of viable cells in cell cultures treated with 10, 1.0, 0.1, 0.01 and 0.001 μg/ml of doxorubicin were 3, 5, 25, 137 and 167×10⁴ cells/ml, respectively after 6 days culture. At the same time it was 170×10⁴ cells/ml in doxorubicin free control cultures. On the 6th day after infection, although the number of living cells in the culture without doxorubicin was 11×10⁴ cells/ml, those in the cultures with 0.1 and 0.01 μg/ml of this drug were 32×10⁴ and 50×10⁴ cells/ml, respectively (Fig. 1D). In accordance with this observation, induction of HIV-specific antigens was also inhibited by the drug (Fig. 1E and 1F). On the 6th day after infection, frequencies of antigen-positive cells were 24% and 51% in the infected MT-4 cells treated with 0.1 and 0.01 μg/ml of the chemical, respectively. In control cultures as well as those treated with 0.001 μg/ml of doxorubicin 99% of the cells expressed viral antigens. These data show that doxorubicin inhibits the cytopathic effect of HIV and appearance of virus-specific antigens in MT-4 cells at concentrations of 0.1 and 0.01 μg/ml.

To determine the inhibitory effect of doxorubicin on HIV replication quantitatively, a plaque forming assay was performed. The supernatants of HIV infected MT-4 cells which were cultured with various concentrations of drug for 4 days were subjected to a plaque forming assay⁸¹⁹. As shown in Table 2, the supernatants from cell cultures treated with 0.1, 0.01 and 0.001 μg/ml of doxorubicin showed virus titers of 38.7, 38.7 and 54.7×10³ plaque forming unit (PFU)/ml, respectively. Under the same condition the virus preparation from HIV infected MT-4 cells cultured without drug gave 66.7×10³ PFU/ml (Table 2). In cell cultures treated with the higher concentrations of doxorubicin (10 and 1.0 μg/ml), virus antigens were not induced and plaque formation was completely suppressed. However, these effects observed at the higher concentrations appeared to result from the cytotoxicity of doxorubicin against host cells and not on the viruses.

The experimental results described above suggest that doxorubicin inhibits the infectivity and replication of HIV at the concentrations of 0.01 ~ 0.1 μg/ml and might be used as an antiviral agent for AIDS and ARC.

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References

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