69

Characterization of the anti-HIV-1 activity of DABOs.

A.G. Loi°, E. Tramontano°, A. De Montis^, M.E. Marongiu^, M. Artico*, S. Massa*, A. Mai* and P. La Colla°. *Depts. of ^Biologia Sperimentale, Università di Cagliari, *Studi Farmaceutici, Università di Roma "La Sapienza" and °Sardinian Antiviral Research Consortium, Italy*

DABOs are specific inhibitors of HIV-1 targeted at the reverse transcriptase. From the chemical point of the view they are 6-benzyldihydrooxopyrimidines substituted at the position 2 with either alkoxy or alkylthio side chains.

DABOs potently and selectively inhibit in vitro the replication of several HIV-1 laboratory strains and clinical isolates in a variety of T and monocytic cells. Effective concentrations (EC_{50}) are in the range 0.3-1.0 μM, whereas doses up to 300 μM are not cytotoxic. No activity against HIV-2 can be demonstrated at doses up to 100 μM.

In enzyme assays DABOs inhibit HIV-1 recombinant RT at doses comparable to those inhibiting virus multiplication in cell cultures; their kinetic pattern of inhibition shows that they do not compete with natural substrates.

Highly resistant mutants to the most potent DABOs can be selected after 3 passages in the presence of high drug doses (50 μM). Mutants are sensitive to AZT, ddI or ddC and partially cross-resistant to nevirapine and E-BPU-S (2 and 10 fold increase in EC_{50}, respectively). The identification of the mutation responsible for DABO resistance is currently under investigation.

Combinations of DABOs with AZT or ddI result in additive/slightly synergistic anti-HIV-1 effects.

Long-term treatments of acutely infected MT-4 cells with combinations of a DABO derivative (2.5-10 μM) with AZT (0.08-0.32 μM) result in no viral breakthrough after 45 days. On the contrary virus breakthrough can be observed when DABO (10 μM) is combined with ddI (up to 80 μM) or ddC (up to 24 μM).

We are currently investigating whether integrated provirus is present in there surviving HIV-infected, drug-treated cultures.

This work was supported in part by ISS grants n. 9204-68 and 9204-05.

70

Anti-HIV Activity of Bovine Seminal RNase.

P. La Colla^, D. Lichino^, A.G. Loi^ and G. D'Alessio°

Depts. of ^Biologia Sperimentale, Università di Cagliari and °Chimica Organica e Biologica, Università Federico II di Napoli, Italy.

BS-RNase (bovine seminal RNase), an enzyme acting both on single- and double-stranded RNA, is a versatile biological effector with special biological actions that include a potent anticancer activity both *in vitro* and *in vivo* (D'Alessio 1993, Trends Cell Biol. **3**, 106-109).

Recently, BS-RNase has been shown to inhibit the HIV-1 replication in H9 cells at non-cytotoxic concentrations (10^{-7} M). Other homologous RNases, such as RNase A (the well known model protein devoid of any special biological actions) and EDN (an RNase from human eosinophil granules with a selective cytotoxicity for certain neurons), have shown no anti-HIV-1 activity. On the contrary, onconase (an RNase with anticancer activity (Mikulski et al., 1990, J.Natl.Cancer Inst. **82**, 151-152) from amphibian eggs) has been found to potently inhibit the HIV-1 replication.

We tested RNase A (as a reference compound) and BS-RNase for cytotoxicity and anti-HIV-1 activity in MT-4 and C8166 cells. In both cell lines, RNase A was non-cytotoxic at doses up to $7x10^{-5}$ M. On the contrary, CC_{50}s of BS-RNase for cell growth were 10^{-7} M and $4x10^{-7}$ M in MT-4 and C8166, respectively.

When tested in acutely infected cells, RNase A resulted always ineffective, whereas the behaviour of BS-RNase was different according to the virus/cell system used. When evaluated in MT-4 cells, BS-RNase was ineffective in preventing the HIV-1-induced CPE but inhibited virus multiplication, as evaluated by p24 assay, with an EC_{50} of $5x10^{-8}$ M. In acutely infected C8166 cells, and in C8166-H9/III_B cocultures, BS-RNase was effective in reducing both syncytium formation and p24 levels.

This work was supported by ISS grants n. 9204-68 and 9204-47.