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Inhibition of HIV-1 Replication by SDZ NIM 811, a Non-Immunosuppressive Cyclosporin A Analog: Mode of Action Studies.

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(Me-Ile-4) Cyclosporin (SDZ NIM 811) is a representative of a group of 4-substituted Cyclosporins being devoid of immunosuppressive activity while retaining full binding capacity to cyclophilin, and exhibiting potent anti-HIV-1 activity. Compound SDZ NIM 811 inhibits selectively HIV-1 replication in T4 lymphocyte and monocytic cell lines and in primary T4 lymphocytes and monocytes. SDZ NIM 811 was found to be equally effective against several laboratory HIV-1 strains and against clinical isolates from geographically distinct regions, when tested in primary T4 cells and/or monocytes. Studies on mechanism of action revealed that there is no correlation of anti-HIV activity with immunosuppression, no inhibition of proviral gene expression and no inhibition of virus-specific enzyme function by SDZ NIM 811, either free or bound to cyclophilin. The compound does not influence CD4 expression and does not inhibit fusion between virus-infected and uninfected cells. PCR analysis of early viral DNA showed that reverse transcription of incoming viral RNA took place normally, but no virus-specific DNA could be detected in the high molecular weight fraction of cellular DNA. Thus, SDZ NIM 811 seems to block a step after reverse transcription and before or at integration. Furthermore, SDZ NIM 811 was found to inhibit formation of infectious particles from chronically infected cells, a result indicating a second point of interference in the viral life cycle for this compound. It has been described recently that cyclophilin A and B bind to the HIV-1 gag protein. In a cell-free system CsA was demonstrated to block the interaction of the gag protein p24 with cyclophilin A. In our hands, too, CsA as well as SDZ NIM 811 block the formation of this complex. Further experiments will help to elucidate whether the interference of SDZ NIM 811 with cyclophilin-gag protein interaction can explain the antiviral effects.

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Sequence Comparison of Different Subpopulations of Proviral and 'Viral' HIV-1 Reverse Transcriptase from Patients on Long-term AZT Treatment

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Objectives: To analyse the sequences of HIV-1 Reverse Transcriptase (RT) from single patients on AZT therapy, using limiting dilution double PCR, and to compare the variety and *de facto* ratio of provirus and actually expressed virus (reverse transcribed cDNA from serum) from different tissues. To investigate, if the pool of resistant/sensitive sequenced RT on the viral level, can be related to the clinical progression of patients on long-term AZT treatment.

Material and Methods: Sequential blood samples were taken from patients on long-term AZT (or ddI) treatment. For preparation of proviral DNA, PBMCs were isolated from 10 ml of heparinised blood. DNA was extracted using PCI and precipitated with $Ethanol_{abs}$ at 4°C. After centrifugation, the nucleic acid pellet was suspended in TE buffer and the DNA concentration quantified. Viral RNA was extracted from 200 µl of serum with lysis buffer and $CHCl_3$. The RNA was precipitated with Isopropanol after chloroform extraction, dissolved in dH_2O and reverse transcribed into cDNA. Limiting dilution double PCR was then used to amplify RT at the cut off point, so that a variety of different provirus and 'virus' sequences from one patient isolate could be analysed.

Results: We found amino acid (aa) substitutions in the two areas between aa positions 62 to 93 and 201 to 215. Amino acid changes in RT from patients harbouring resistant strains of HIV-1 could be specified so far at positions 62 (Ala→Phe), 93 (Gly→Pro), 201 (Glu→Lys), 210 (Leu→Ser, Trp), 211 (Arg→Lys), and 214 (Leu→Phe) in addition to position 215 (Thr→Tyr) described previously. Although the patient had received AZT treatment for more than nine months, wild type 'AZT sensitive' RTs co-existed with differently mutated 'AZT resistant' RTs in the same patient sample.