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Effect of suramin on *in vitro* HIV DNA integration reaction

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Retroviral growth requires as obligatory step the integration of a DNA copy of the viral RNA into the genomic DNA of the host (Varmus & Brown in "Mobile DNA", 1989). The integration event is catalyzed by a single viral protein, the integrase enzyme (IN) (Farnet & Haseltine J. Virol. 65: 1910, 1991) which is one of the products of the *pol* gene. Structure-activity relationship study has demonstrated that the deletion of 45 residues completely suppresses both processing and strand transfer (Drelich et al., Virology 188:459, 1992). It is of interest to consider that this protein domain contains about 20% of basic amino acids such as Arg and Lys. This value is close to the one observed for basic proteins such as histones and leads to the occurrence of a high density of positive charges. This feature makes the IN protein a privileged target for polyanionic drug such as suramin which has been found to inhibit HIV replicative cycle *ex vivo* (Mitsuya et al., Science, 226:172, 1984). We have expressed recombinant human immunodeficiency virus type I (HIV-1) integrase (IN) in *Escherichia coli* (Bushman & Craigie, PNAS, 88: 1339, 1991) and purified the protein to homogeneity. The purified protein efficiently catalyses the overall *in vitro* integration reaction namely the processing of the LTR ends and the strand transfer reaction. Using as substrate the 3' end of synthetic oligonucleotides which match the termini of HIV-I U5 LTR and supercoiled pSP65 DNA as target (Carteau et al. Arch. Biochem. Biophys. in press), we have investigated the effect of suramin on the catalytic activity of the IN protein. It was found that suramin displays at stochiometric concentrations a strong inhibitory effect on both processing and strand transfer reactions. As evidenced by a filter binding assay, this inhibition is related to the decrease of the IN protein binding efficiency to the U5 LTR end DNA fragment. The inhibition of integration could be involved for a large part in the anti-HIV effect of this drug previously observed in cultured cells.

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QUANTITATIVE PCR ANALYSIS AS AN IMPORTANT TOOL FOR THE EVALUATION OF THE ACTIVITY OF ANTIVIRAL DRUGS IN HIV-INFECTED MONOCYTES/MACROPHAGES

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Quantitative polymerase chain reaction (PCR) was settled to assess the utility of HIV-DNA analysis for the in vitro evaluation of antiviral drugs and to investigate the mechanisms of activity of various compounds in human monocytes/macrophages (M/M). Drugs as AZT, adriamycin (ADR), U-75875 protease inhibitor, and two cytokines (granulocyte-macrophage colony-stimulating factor, i.e. GM-CSF, and macrophage-colony stimulating factor, i.e. M-CSF) were evaluated for their effect upon HIV infection and replication. AZT (an inhibitor of virus reverse transcriptase) induced a comparable level of inhibition of both virus replication and HIV-DNA when given before virus challenge, while it was completely ineffective in chronically-infected M/M. Interestingly, 0.05ug/ml ADR did not induce cell toxicity nor inhibition of virus reverse transcriptase, while it did cause >60 fold decrease of the amount of HIV-DNA. Also in the case of ADR, no activity whatsoever was detected in chronically-infected M/M, thus overall suggesting that inhibition of HIV replication occurs after virus retrotranscription and before RNA synthesis. In the case of U-75875 protease inhibitor, no detectable modulation of the amount of HIV-DNA was observed neither in de novo infected- nor in chronically-infected M/M, despite the dramatic reduction of virus production observed in the supernatants of such cells. This suggests that the activity of U-75875 is essentially directed against late stages of virus replication. In the case of GM-CSF and M-CSF, both cytokines increase virus replication in M/M. However, while M-CSF induced a weak increase of the number of HIV-DNA copies, GM-CSF caused a significant decrease, thus suggesting that the GM-CSF-mediated mechanism of virus enhancement acts mainly after HIV-DNA synthesis. These data suggest that quantitative PCR analysis is able to provide informations about the efficacy and the mechanisms of action of anti-HIV drugs. Further studies are currently applying PCR analysis in the assessment of drug-mediated modulation of virus integration in host cells.