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Liposome Encapsulated Anti-HIV Agents: A New Approach in AIDS Therapy (in Vitro Studies). Beshkov Danail, Gabev E. E. *, Gabev E. B., Argirova R. M. Central AIDS Lab., National Center of Infectiuos and Parasitic Diseases, Sofia, Bulgaria. * Instiut of Parasitology, Bulgarian Academy of Sciences, Sofia, Bulgaria.

OBJECTIVE: To surmount the side effects of longterm antiretroviral therapy a study based on liposome drug encapsulation was undertaken. Two liposome preparations were tested and compared to AZT in vitro: 1. Liposome encapsulated AZT(L/AZT) and 2. Liposome encapsulated AZT in combination with PEBA-presumably enzyme blocking agent (L/AZT/PEBA). METHODS: MT4 cell line was infected with HIV (H9/HTLV-IIIB) at m.o.i. 0.02. The cells were exposed to the liposome preparations once at the oncet of the experiments. Final concentration of AZT in all cases was 4.5 microM/l and PEBA 1.5 miliM/l. Toxicity was evaluated by MTT assay. Anti-HIV activity was measured as the inhibition of p24 Ag (Diagnostics Pasteur and Du Pont). Both assays were performed once weekly for 21 days. RESULTS: No toxicity was observed. At day 7 the preparations showed well expressed but similar anti-HIV effect. On day 14 L/AZT was at least two times more effective than AZT-free, while for L/AZT/PEBA this effect was more than 60 times higher. On day 21 L/AZT/PEBA and L/AZT still showed anti-HIV effect while AZT-free was no more active. CONCLUSION: Liposome encapsulation enhances and prolongs in vitro anti-HIV effect of AZT especially in combination with PEBA. This approach is perspective for dose diminishing and combination of synergically acting agents as evdent in this study.

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Antibody-targeted Immunoliposomes Containing Antisense Oligonucleotide Mediate A Specific Antiviral Effect On H9 Cells Infected With HIV-1

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The sequence specific suppression of HIV-1 replication using HIV-1 antibody-targeted liposomes containing antisense phosphorothioate oligonucleotides is described. Liposomes were generated, which encapsulated the 20 mer sequence of the rev HIV-1 regulatory gene in the form of a phosphorothioate oligonucleotide. Specific targeting was accomplished by conjugating purified HIV-1 positive human IgG to the surface of the liposomes to form an immunoliposome. HIV-1-infected H9 cells incubated with the immunoliposomes were rendered less permissive for viral multiplication. As compared with the positive control infected with HIV-1, HIV-1 replication was reduced by nearly 95 percent in antisense immunoliposome treated cells. Inhibition of HIV-1 replication was not observed using empty or immunoliposomes containing random phosphorothioate oligomer sequences. These immunoliposomes exhibit dual specificity: a targeting antibody on the surface of the liposome specific for infected cells, and inside the immunoliposome, an oligomer with antiviral activity that is complementary to a specific portion of the mRNA of the infected cell. The antiviral activity of the free as well as the encapsulated oligonucleotides were assessed by p24, RT assays, Western Blot, immunofluorescence and PCR analysis. Liposome preparations demonstrated minimal toxicity in H9 cell culture experiments. The altered HIV-1 mRNA specifically induced by targeted antisense liposomes suggests that the mechanism for the inhibition of viral expression is its interaction with the rev regulatory gene resulting in translation arrest. These *in vitro* culture results demonstrate the potential efficacy of drug-encapsulating immunoliposomes in the treatment of AIDS and AIDS related complex.