Magnetoliposomes May Be Useful for Elimination of HIV from Infected Individuals

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A simple method is proposed for the treatment of the acquired immunodeficiency syndrome (AIDS) by introducing into the blood stream magnetoliposomes with coupled human immunodeficiency virus (HIV) receptor proteins. After some time lag, needed for binding of HIV and HIV-infected cells to magnetoliposomes, the arterio-venous shunt, as used with dialysis patients should be inserted and the patient's blood should be passed through multiple tubes filled with stainless steel wool, each of which is surrounded by a coil producing a strong non-homogeneous magnetic field, which may result in a substantial reduction of HIV and HIV-infected cells in the infected body.

Introduction

Very high efficiency of the binding of liposomes to human immunodeficiency virus type 1 (HIV) and to chronically HIV-infected cells (T4-lymphocytes) which express the virus envelope proteins on their surface was achieved by either reconstituting the recombinant transmembrane CD4 receptor in the liposome membrane (Cudd *et al.*, 1990, by covalent coupling soluble CD4 to a liposome surface (Flasher *et al.*, 1994) or by coupling to liposomes a synthetic peptide corresponding to a region responsible for interaction of envelope protein gp 120 with its cellular receptor (Slepushkin *et al.*, 1996). Encapsulated antiviral drugs in liposomes may be used subsequently for the destruction of HIV bound.

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Recently we have suggested to use magnetoliposomes (liposomes loaded besides the drug with a magnetic particles) in the treatment of cancer (Babincová, 1993; Babincová and Babinec, 1995), and this approach has been shown to be feasible both *in vitro* (Viroonchatopan *et al.*, 1995) and *in vivo* (Viroonchatopan *et al.*, 1996) for treatment of hepatocellular carcinoma. In this case two advantages of magnetoliposomes (MLs) became evident: a locally applied constant magnetic field is used to bind the MLs to the appropriate site (e.g. tumor) and subsequently release the drug at a given site by microwave radiation or a high-frequency magnetic field (Babincová and Babinec, 1997).

Materials and Methods

Small magnetite (Fe_3O_4) particles (with diameter ~15 nm) were prepared by co-precipitation of $FeCl_2$ and $FeCl_3$ (Sigma, USA) in the presence of excess of ammonia. 15 mg of soybean phosphatidylcholine (Sigma, USA) was dissolved in chloroform/methanol (2:1 v/v) in a round bottom flask (Rosensweig, 1985). 10 mg of magnetite was added to the same flask, and the solvent evaporated in a rotary evaporator, so that a thin lipid film with embedded magnetic particles was formed. This film was hydrated by 1 ml of Tris buffer [20 mM tris-(hydroxymethyl)-aminomethane, Radelkis, Budapest, Hungary], including 100 mM NaCl; pH 7.4) and the flask was vigorously shaken. This procedure resulted in the formation of MLs. Non-encapsulated magnetite particles were removed by magnetic decantation.

Magnetic responsiveness of MLs has been quantified using a turbidimetric method with the spectrophotometer SPECOL 210 (Carl-Zeiss Jena, Germany) working at 600 nm.

Results and Discussion

Magnetic beads with immobilized antibodies are commonly used in a very efficient separation of microorganisms (Safarik *et al.*, 1996). For these purposes MLs are also efficient, as shown by Fig. 1. A tissue culture flask with 50 ml of MLs suspension was placed on a commonly available permanent magnet (alloy of Co and Sm, from an

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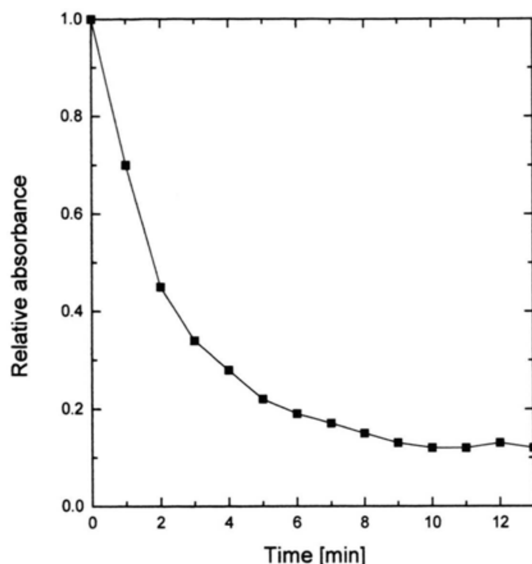

Fig. 1. Time course of removal of magnetoliposomes from a water suspension (see text for details). Relative absorbance is defined as $I(t)/I(0)$ where $I(t)$ is intensity of light transmitted through the cuvette with MLs suspension at the time t. Magnetic field was applied at the time t=0.

older computer hard disk, with a magnetic strength of ~0.3 Tesla). At regular time intervals 0.5 ml samples were taken 2 cm above the surface of the magnet. Since absorbance of light is proportional to the MLs concentration, the time dependence of absorbance descrease is a measure of magnetic separation efficiency. As can be seen, even using a magnetic field of moderate strength, MLs are efficiently removed from the top to the bottom of the culture flask. MLs may be more efficiently separated using highly non-homogeneous magnetic fields (De Cuyper, 1996). We have formed them by putting a plug of stainless steel wool into the glass tube with a diameter 0.3 cm. Steel wool becomes magnetic in the presence of an external magnet and produces extremely strong field gradients. A MLs suspension was pumped slowly through the tube and MLs are separated with an almost 90% efficiency. They are concentrated on the steel wool and may be easily removed by flushing the tube in the absence of a magnetic field.

To eliminate HIV and HIV-infected cells from the blood stream we therefore suggest the following combination of above mentioned techniques: In the first step MLs with reconstituted CD4-derived proteins are injected intravenously to the organism and after some time lag (needed to bind HIV and HIV-infected cells to MLs) the arterio-venous shunt, as used with dialysis patients should be inserted and the patient's blood should be passed through multiple tubes filled with stainless steel wool, each of which is surrounded by a coil producing a strong non-homogeneous magnetic field. All the MLs and MLs with bound HIV and HIV-infected cells are retained in the portion of tube affected by magnetic field and MLs free blood goes back to the patient.

We look forward to finding an experimental opportunity to check our methods with a sick patient.

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