

Liposzómák terápiás alkalmazásai (szisztémás terápia)

Dr. Voszka István

b) Szisztémás alkalmazások

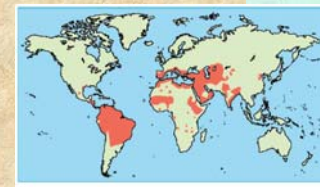
Általában akkor, ha az adott gyógyszernek súlyos mellékhatásai vannak.

1. A RES-t érintő betegségek kezelhetők

C-liposzómába zárt gyógyszerekkel.

Pl. a leishmaniasis kezelhető

liposzómás antimon-származékokkal



2. Antibiotikumok

Főképp, ha az adott gyógyszer terápiás és toxikus koncentrációja között kicsi a különbség

Liposzómában - a szükséges gyógyszer mennyiség lecsökken

- a kezelés hatásfoka javul

Baktériumellenes pl. aminoglikozid típusú antibiotikumok
brucellózis, vesemedence-gyulladás kezelésére (streptomycin, gentamycin, stb.)

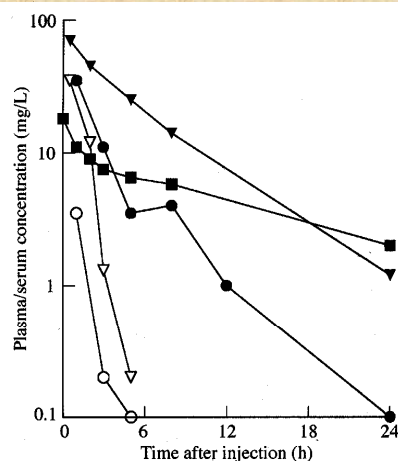


Figure 1. Circulation kinetics of conventional liposome encapsulated aminoglycosides (closed symbols) and free aminoglycosides (open symbols). Aminoglycoside concentrations at indicated time-points after injection of a single dose of gentamicin 20 mg/kg in rats (triangles),⁶⁶ amikacin 40 mg/kg in mice (circles)⁶⁸ or gentamicin 5.1 mg/kg in AIDS patients (squares).⁸⁰



Gombaellenes pl. Amphotericin B

(első törzskönyvezett liposzómás gyógyszerkészítmény – gyári neve AmBisome®)

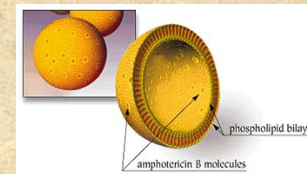
telített PC / Chol / DSPG (2: 1: 0,8) + 10 mol% AmB ~80 nm-es SUV-okban)

A toxicitás behatárolja az adható mennyiséget. Gyakran a toxikus koncentráció kisebb, mint a minimális terápiás.

Liposzómában kevesebb mellékhatás (mellékhatások: láz, izomfájdalom, thrombophlebitis, vesekárosodás, anaemia)

Feltételezett ok: a liposzómák affinitása sokkal kisebb a humán sejtek koleszterinjéhez, mint a gombasejtek ergosterinjéhez

Más törzskönyvezett liposzómás Amphotericin B készítmény: Abelcet®



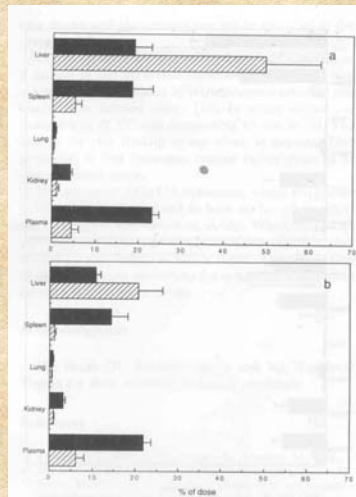


Fig. 2. Biodistribution of liposomes in normal mice at 12 h after intravenous administration. Mice were injected with PGlcUA-liposomes (closed bar) and DPPG-liposomes (hatched bar) as described in Materials and Methods. Data show the percent injected dose per tissue and S.D. The radioactivity of $[^3\text{H}]$ inulin (a) and that of cholesteryl $[^{14}\text{C}]$ oleate (b) are shown.

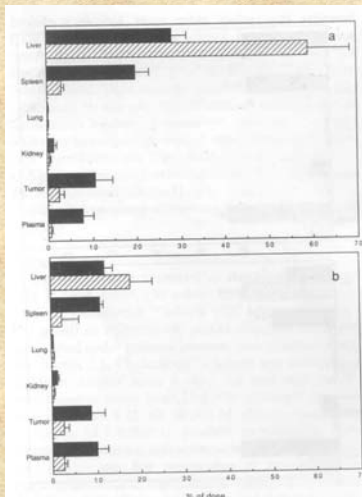


Fig. 3. Biodistribution of liposomes in tumor-bearing mice at 12 h after intravenous administration. Tumor-bearing mice were injected with PGlcUA-liposomes (closed bar) and DPPG-liposomes (hatched bar) as described in Materials and Methods. Data show the percent injected dose per tissue and S.D. The radioactivity of $[^3\text{H}]$ inulin (a) and that of cholesteryl $[^{14}\text{C}]$ oleate (b) are shown.

Stealth liposzómában (PEG, glukuronsav, szíálsav a felületen) – nagyobb szelektivitás és hatékonyság

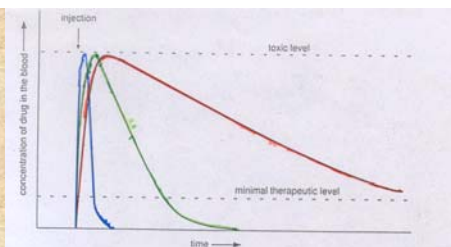
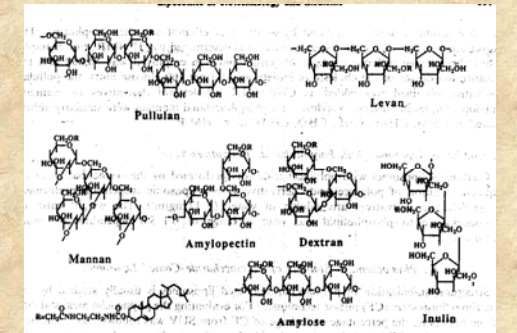
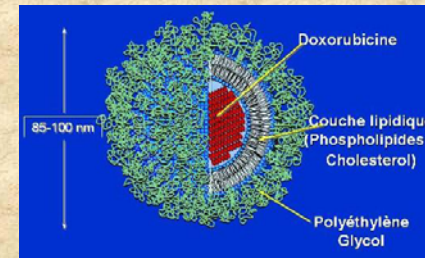


Figure 11. Concentration of a drug injected into the bloodstream depends on the form in which it is delivered. In treating a disease the usual goal is to maintain a therapeutic but nontoxic level of the drug in the blood for as long as possible. A free drug (delivered without a carrier) is usually present at therapeutic concentrations in the blood for a very short period (blue). Encapsulating the drug within a conventional liposome increases its duration in the blood (green), whereas drugs carried by stealth liposomes (red) may remain at therapeutic levels hundreds of times longer than a free drug.

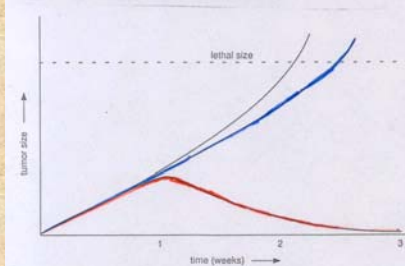
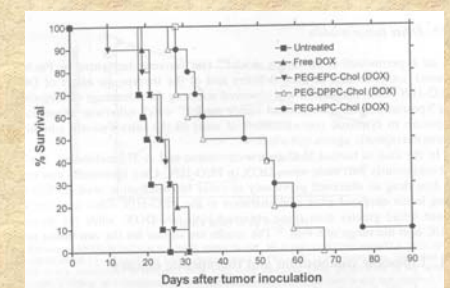
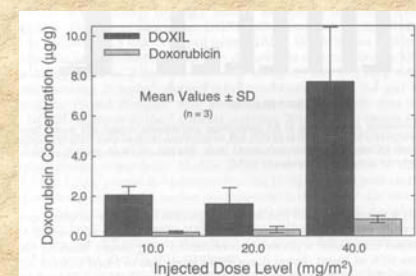
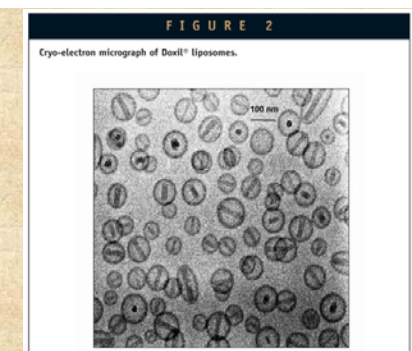
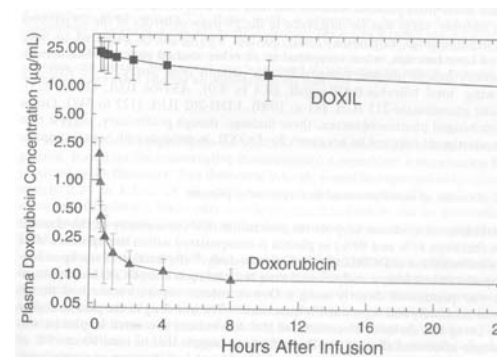


Figure 12. Size of a cancerous tumor in a laboratory animal is reduced with the use of an anti-tumor agent encapsulated within a stealth liposome (red). In cases where the animal is left untreated (blue) or the drug is administered in its free form (blue) the tumor will continue to grow until it reaches a lethal size. (Adapted from Topoloff et al. 1991.)



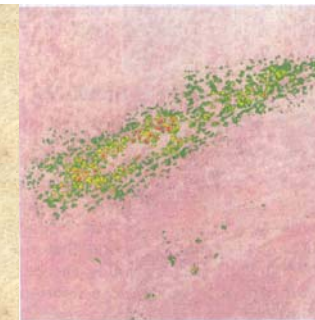
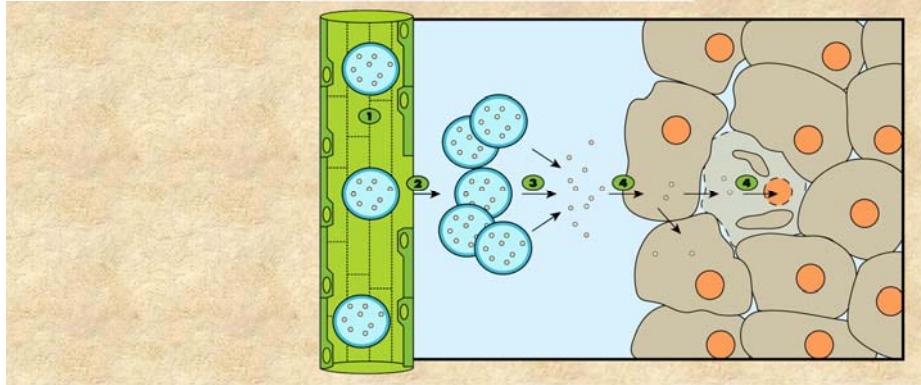
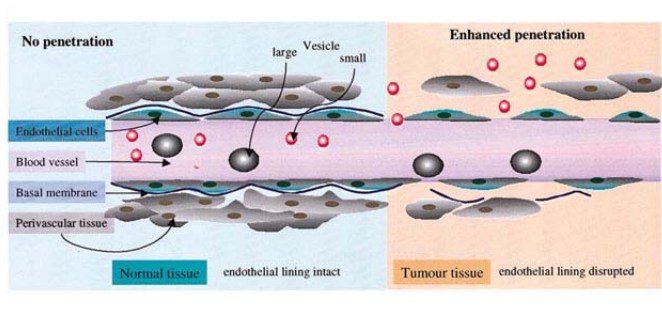


Figure 2

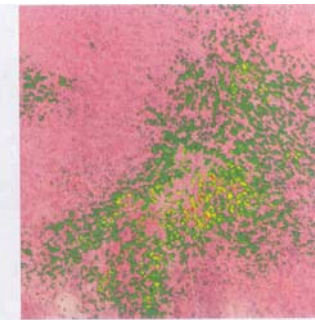


Figure 3

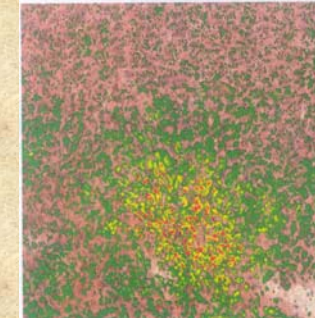


Figure 4

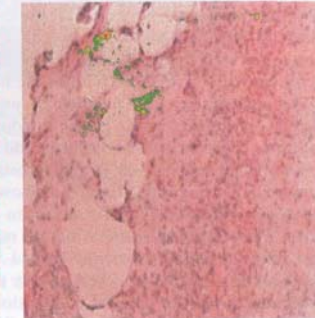


Figure 5

LDL-be zárt citosztatikumok

A daganatsejtek felszíni LDL-receptorainak száma a malignitással arányosan nő → szelektív bejuttatás a daganatba.

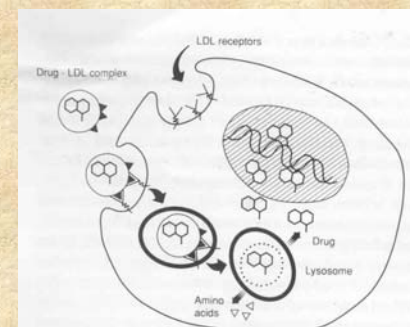
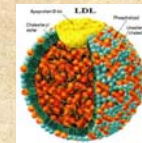


FIGURE 1
Proposed scheme (not to scale) for the receptor-mediated endocytosis of a lipophilic drug incorporated into LDL.

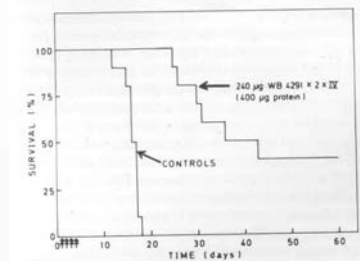
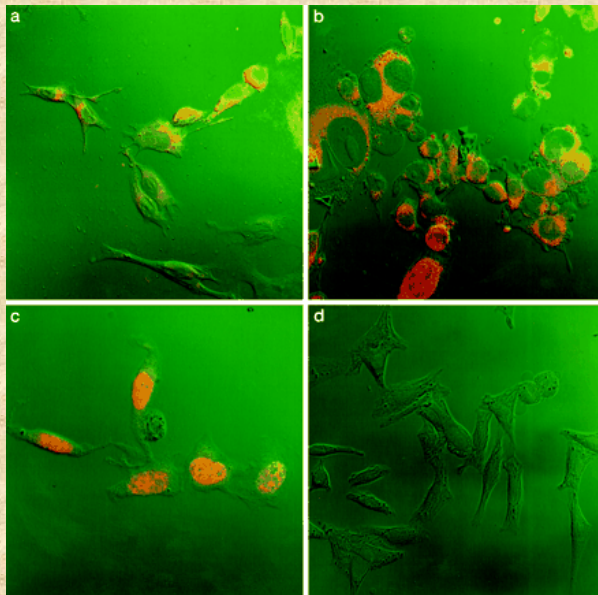
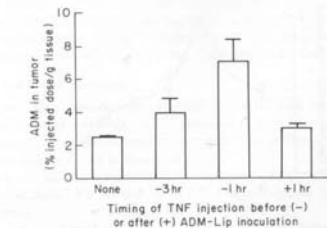
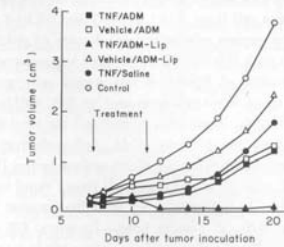
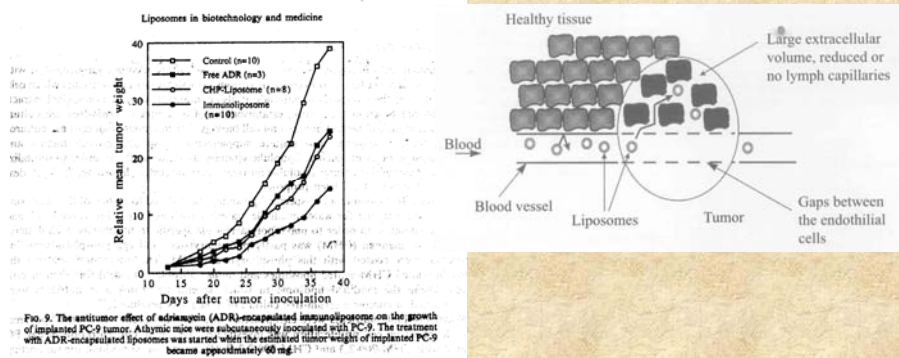


FIGURE 2
Survival of leukemic BALB/c mice after treatment with a WB 4291-LDL complex administered by intraperitoneal injection. The mice were inoculated intraperitoneally on day 0 with 10^5 WEHI-3B cells (a murine myelomonocytic leukemia cell line) and the WB 4291-LDL complex was given on days 1-4. There were ten animals in each group. WB 4291 is a highly lipophilic nitrogen mustard derivative. (Reprinted, with permission, from *Br. J. Cancer* 62: 724-729, 1990.)



Confocal microscopy of liposomal DOX uptake by M109R-HiFR cells cultured in folate-depleted RPMI 1640 at 37°C. *a*, *b*, and *c*, binding, internalization, and nuclear localization of DOX delivered by FTL after a 30-min, 60-min, and 90-min incubation, respectively. *d*, cells exposed for 4 h to nontargeted PEG-coated liposomes encapsulating DOX (Doxil).

Immunliposzómák: szelektív kötődés a daganatsejthez. Toxin vagy citosztatikum zárható bele. TNF együttes adása segíti a liposzómák átlépését az erekből a tumorba.

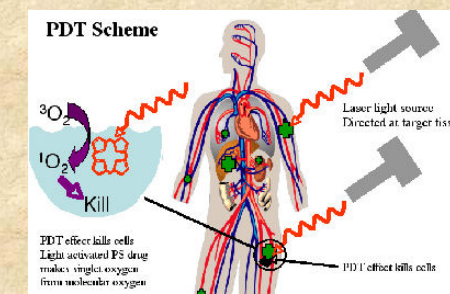
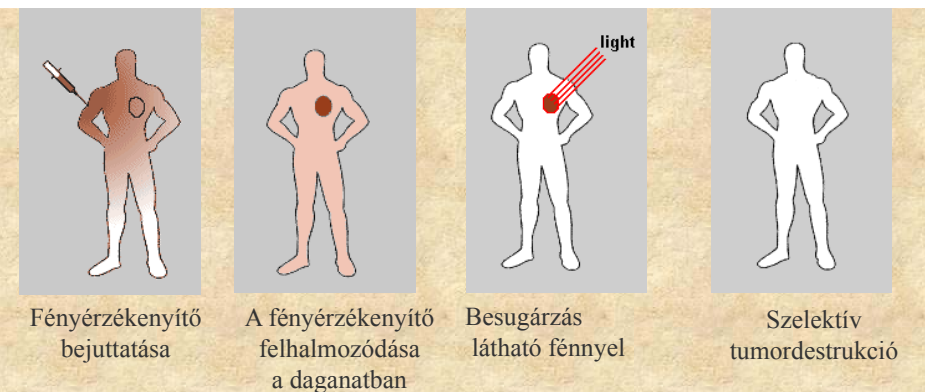
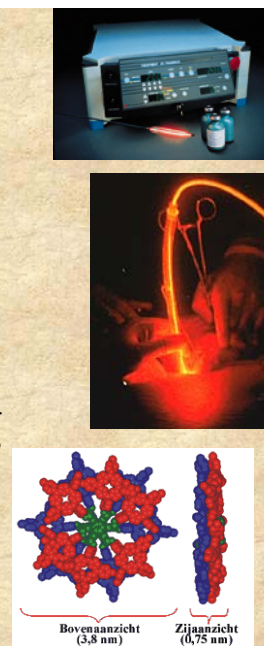


Fotodinámiás terápia (PDT)

Fényérzékenyítő anyagot (hematoporfirin, ftalocianin származékok, stb.) juttatnak a tumorsejtekbe. Megvilágítják megfelelő hullámhosszú fénnel → reaktív oxigéngyökök képződnek → tumorsejtek pusztulása.

A hatás függ a fényérzékenyítő anyag jellemzőitől. Minél hidrofílebb (pl. szulfonált forma) annál inkább a tumorban lokalizálódik. De a daganatpusztító hatás fordítottan arányos a szulfonáltsággal.

Megoldás pl.: a liposzómába zárt fényérzékenyítőt LDL-lel asszociáltatva juttatják a tumorba.



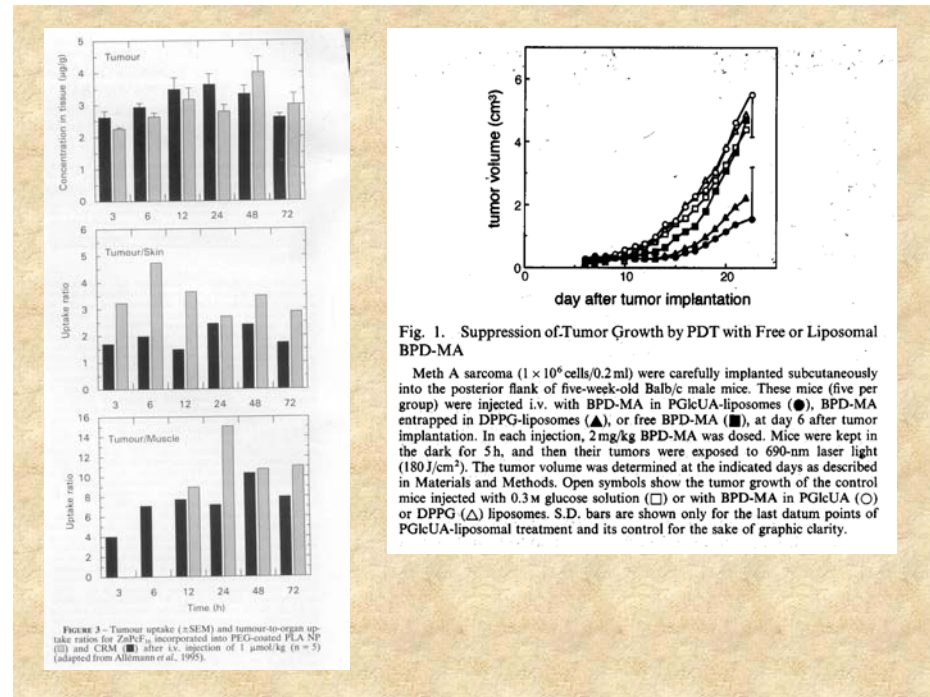
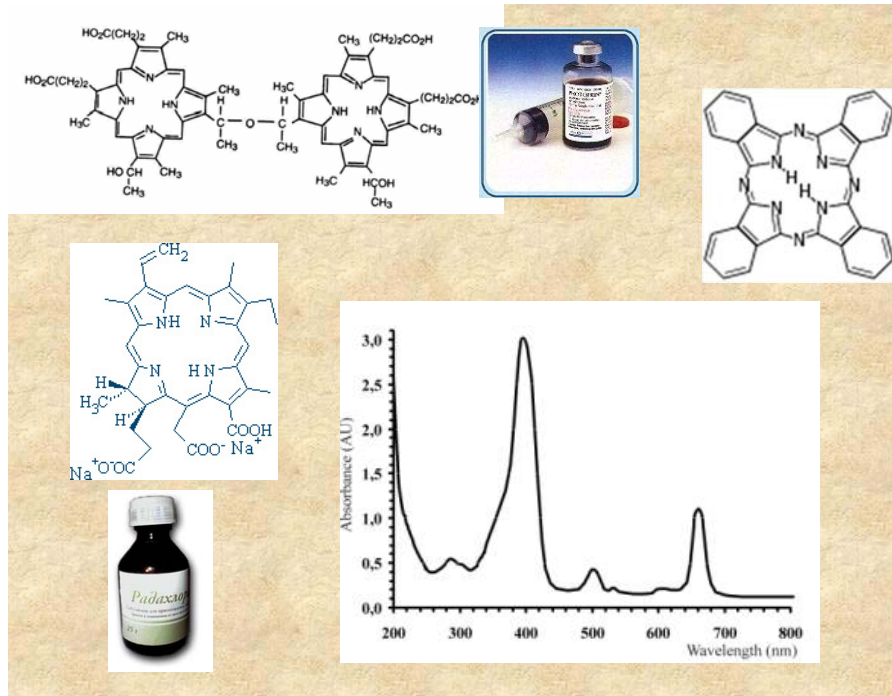
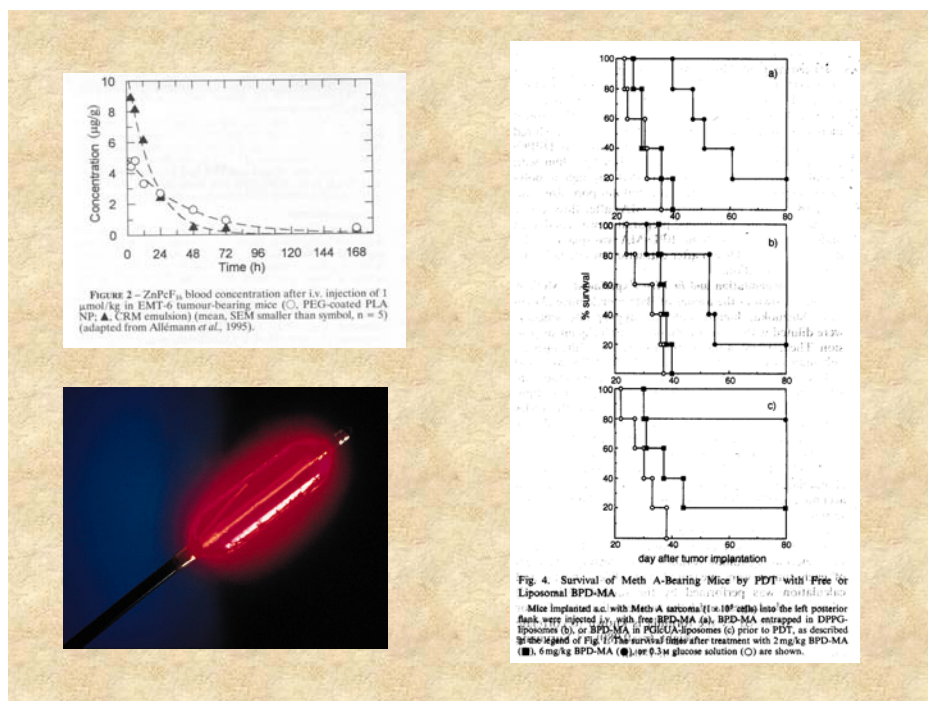
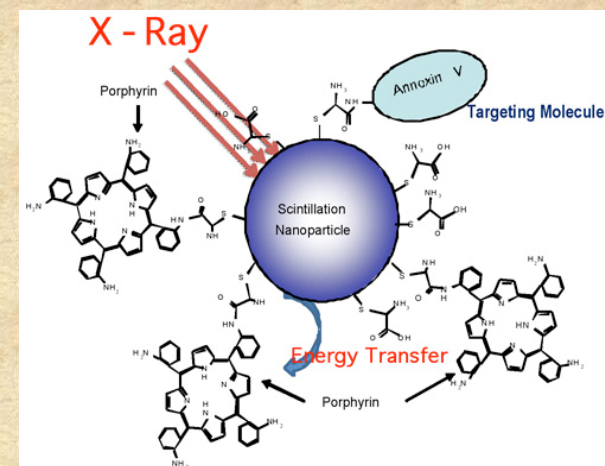


Fig. 1. Suppression of Tumor Growth by PDT with Free or Liposomal BPD-MA

Meth A sarcoma (1×10^6 cells/0.2 ml) were carefully implanted subcutaneously into the posterior flank of five-week-old Balb/c male mice. These mice (five per group) were injected i.v. with BPD-MA in PGlcUA-liposomes (●), BPD-MA entrapped in DPPG-liposomes (△), or free BPD-MA (□), at day 6 after tumor implantation. In each injection, 2 mg/kg BPD-MA was dosed. Mice were kept in the dark for 5 h, and then their tumors were exposed to 690-nm laser light (180 J/cm^2). The tumor volume was determined at the indicated days as described in Materials and Methods. Open symbols show the tumor growth of the control mice injected with 0.3 M glucose solution (□) or with BPD-MA in PGlcUA (○) or DPPG (△) liposomes. S.D. bars are shown only for the last datum points of PGlcUA-liposomal treatment and its control for the sake of graphic clarity.



Röntgensugárzással indukált PDT



4. AIDS

Mind a celluláris (főleg a CD4⁺ limfociták számának csökkenése miatt) mind a humorális immunválasz csökkent → védekezési képtelenség a fertőzésekkel szemben (opportunisták fertőzések)

Okozója a HIV vírus – reverz transzkriptázzal rendelkező RNS vírus. A kezelés alapja a reverz transzkriptáz gátlása dezoxinukleozid-analógokkal, melyekben a 3'-OH- csoportot H-, azido-, vagy más csoport helyettesíti → nem képeznek foszfodiészter kötetést. Hosszú távon alkalmazandók, ezért toxicitásukat figyelembe kell venni. Kombinációjuk célszerű – kevésbé szokik hozzá a vírus, kisebb toxicitás (a purin és pirimidin analógok más-más úton hatnak)

Pl. 3'-azido-3'-deoxitimidin (AZT) = zidovudin (ZDV)

Hosszabb kezelés során csontvelői toxicitása van. Liposzómába zárással kivédhető. A plazmában tartózkodás ideje is nő.

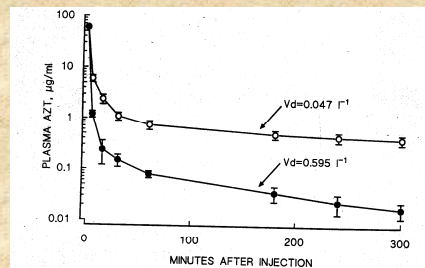
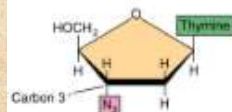


Fig. 7. Plasma AZT levels following the administration of AZT (●) or liposomal AZT (○). Plasma AZT levels (as ³H-methyl-AZT) were determined at 15-300 min following the i.v. administration of a bolus of AZT or liposomal AZT at a dose of 60 μg/mouse (2 mg/kg body weight). Results shown are the mean ± SD of groups of 5 mice. Volumes of distribution (V_d) were calculated using the equation V_d = A/C where A = 60 μg and C = plasma concentration at time zero (obtained by extrapolation of the elimination phase line to zero time).



Table III. Tissue distribution of AZT and liposomal AZT.

Tissue	AZT	% Injected dose/g tissue ^(a)	
		DPFC/DMPG	Liposomal AZT DPFC/PS
Liver	0.59 ± 0.06	3.10 ± 0.21 ^(b)	2.10 ± 0.15 ^(bc)
Spleen	0.60 ± 0.10	2.30 ± 0.08 ^(b)	2.15 ± 0.12 ^(b)
Kidney	7.00 ± 1.45	2.30 ± 0.41 ^(b)	1.10 ± 0.08 ^(b)
Lung	0.30 ± 0.19	1.90 ± 0.17 ^(b)	4.60 ± 0.35 ^(bc)
Muscle	0.40 ± 0.11	0.50 ± 0.14	0.45 ± 0.17
Brain	0.15 ± 0.09	0.18 ± 0.10	0.16 ± 0.06
Bone marrow ^(d)	0.02 ± 0.003	< 0.0001 ^(b)	< 0.0001 ^(b)
Urine	59.9 ± 4.8	23.8 ± 3.6 ^(b)	21.9 ± 2.7 ^(b)

^(a) Groups of 5 CD1 mice were treated with AZT (2 mg/kg body weight) or 2.5 μmol liposomes containing AZT (2 mg/kg body weight) spiked with ³H-methyl-AZT. DPFC/DMPG liposomes were formulated in the molar ratio 10/1; DPFC/PS liposomes were formulated in the molar ratio 7/3. Tissue distribution was determined 60 min after i.v. injection using a dose volume of 200 μl.

^(b) Significantly different from AZT treatment (P < 0.01, Student's t-test for unpaired data).

^(c) Significantly different from DPFC/DMPG liposomes (P < 0.01).

^(d) Results for bone marrow are expressed as percent dose/10⁶ nucleated cells. The limit of detection using ³H-methyl-AZT was 2.5 pg AZT.

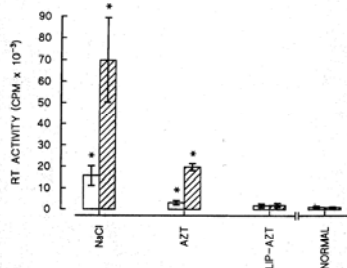


Fig. 10. Effect of treatment with AZT or liposomal AZT on the development of plasma RT activity in LP-BM5-infected C57BL/6 mice.

Groups of 5 LP-BM5-infected mice were treated for 6 weeks as described in the legend to figure 8. Plasma RT activity was determined after 3 and 6 weeks treatment. Blank columns: 3 weeks' treatment; hatched columns: 6 weeks' treatment; * = significantly different from control, mock-infected mice (P < 0.001, Student's t test for unpaired data).

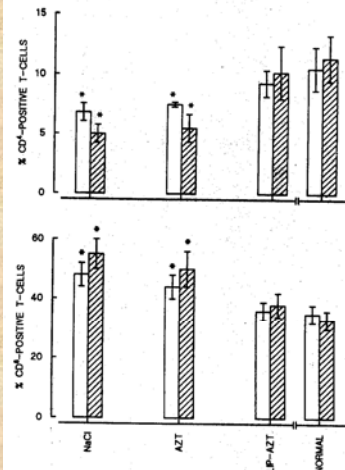


Fig. 9. Effect of treatment with AZT or liposomal AZT on splenic CD4⁺ and CD8⁺ T-cell populations.

Groups of 5 LP-BM5-infected mice were treated for 3 or 6 weeks as described in the legend to figure 8.

Upper panel: proportion of CD4⁺ T cells; lower panel: proportion of CD8⁺ T cells; blank columns: 3 weeks' treatment; hatched columns: 6 weeks' treatment; * = significantly different from control, mock-infected mice (P < 0.01, Student's t test for unpaired data).

Adjuváns hatás

Adjuváns: bármely ágens, amely nem specifikusan fokozza az immunválaszt egy specifikus antigénnel szemben.

A liposzómák hatékony adjuvánsként működnek, így pl. az AIDS során lecsökkent immunválaszt is fokozzák.

Előnyük: - nem toxikusak

- egyszerűen előállíthatók

- összetételük szükség szerint változtatható

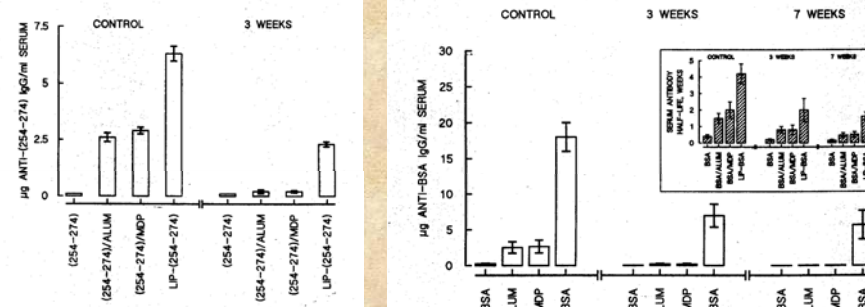


Fig. 4. Immunoadjuvant activity against peptide (254-274) in normal mice and in mice with MAIDS.

Table IV. Bone marrow toxicity of AZT and liposomal AZT.

Treatment (mg/kg/day)	Bone marrow cells/femur (x 10 ⁶)	Leucocytes ml/blood (x 10 ⁹)	RBC ml/blood (x 10 ⁹)
Vehicle control	13.9 ± 1.6	10.3 ± 2.1	9.8 ± 0.7
Liposomes	14.2 ± 1.1	10.6 ± 0.9	10.1 ± 0.7
AZT			
0.08	11.8 ± 0.9	9.1 ± 1.7	8.7 ± 0.6
0.4	9.0 ± 1.4 ^(a)	7.9 ± 0.8	7.3 ± 0.4 ^(a)
2.0	6.6 ± 1.8 ^(a)	6.1 ± 0.9 ^(a)	6.3 ± 0.4 ^(a)
10.0	6.3 ± 1.3 ^(a)	5.1 ± 1.6	6.0 ± 0.6 ^(a)
50.0	5.9 ± 0.8 ^(a)	5.2 ± 1.9	5.4 ± 0.7 ^(a)
Liposomal AZT			
0.08	14.0 ± 2.1	10.0 ± 1.3	9.6 ± 0.8
0.4	13.6 ± 1.6	10.4 ± 1.3	9.8 ± 0.5
2.0	13.3 ± 1.8	10.6 ± 0.9	9.7 ± 0.8
10.0	13.9 ± 1.2	9.8 ± 1.3	9.2 ± 0.6

Groups of 5 CD1 mice were treated daily by the i.v. route with vehicle (0.85% NaCl), liposomes (2.5 μmol), AZT or liposomal AZT in a dose volume of 200 μl. Bone marrow cellularity, peripheral blood leucocyte and RBC numbers were determined after 5 treatments. The results shown are the mean ± SD.

^(a) Significantly different from control treatment (P < 0.05, Student's t test for unpaired data).

^(b) Significantly different from control treatment (P < 0.01).

5. Vaccinatio

Kihasználható a liposzómák adjuváns hatása (liposzómában bevitt antigénnel az immunválasz fokozható)

Specifikus antigének zárhatók liposzómába, vagy köthetők a felszínére (a hatás az Ag elhelyezkedésétől függ.)

- Bezárt Ag → rövid távú válasz, főleg IgG1 termelődik.
- Felületen kötött Ag → hosszú távú válasz, IgG1, IgG2a, IgG3, IgM termelődik.

A két esetben a hatásmechanizmus különböző.

TABLE 1

Entrapment of Peptides and Proteins in DRV Liposomes

Material	Amount used (mg)	Phospholipid used (μmol)	Entrapment (% of used)
Tetanus toxoid	2.00	16	40-82
Bovine serum albumin	2.00	16	40-45
RIVE	0.05	16	29-31
A/Sichuan	0.05	16	38-45
rHBsAg	0.20	16	31-33
LV39	0.20	16	74-82
Interleukin-2	Up to 10 ⁶ units	16	60-70
Poliiovirus 1-VP2 peptide	0.22	16	74-82
Poliiovirus-VP2 peptide	0.22	16	62-68
HBsAg S peptide	1.00	32	42-45
HBsAg pre-S ₁ peptide	1.00	32	46-48

Note. Materials were entrapped as described in the text. RIVE, reconstituted influenza virus envelopes; A/Sichuan, strain influenza virus hemagglutinin and neuraminidase; rHBsAg, recombinant hepatitis B surface antigen; LV39, *Leishmania major* antigen (mixed isolate); HBsAg, full-length hepatitis B surface antigen synthetic S peptide had a 110-137 amino acid sequence (30); synthetic pre-S₁ peptide, sequence was 15-48 (30).

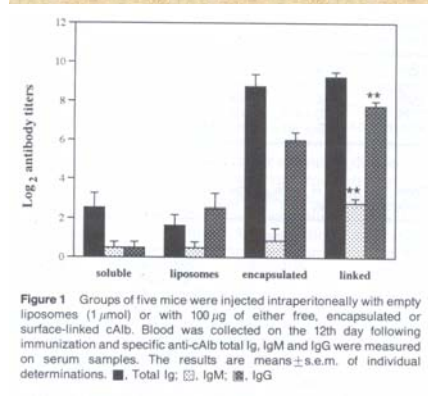


Figure 1 Groups of five mice were injected intraperitoneally with empty liposomes (1 μmol) or with 100 μg of either free, encapsulated or surface-linked cAlb. Blood was collected on the 12th day following immunization and specific anti-cAlb total Ig, IgM and IgG were measured on serum samples. The results are means ± s.e.m. of individual determinations. ■, Total Ig; □, IgM; ▨, IgG

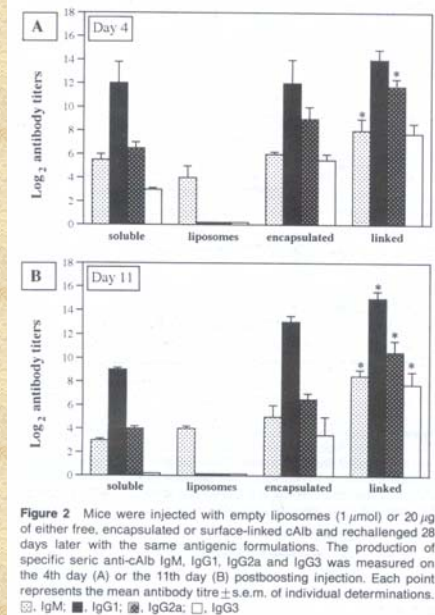


Figure 2 Mice were injected with empty liposomes (1 μmol) or 20 μg of either free, encapsulated or surface-linked cAlb and rechallenged 28 days later with the same antigenic formulations. The production of specific anti-cAlb IgM, IgG1, IgG2a and IgG3 was measured on the 4th day (A) or the 11th day (B) postboosting injection. Each point represents the mean antibody titre ± s.e.m. of individual determinations. □, IgM; ▨, IgG1; ▩, IgG2a; ■, IgG3

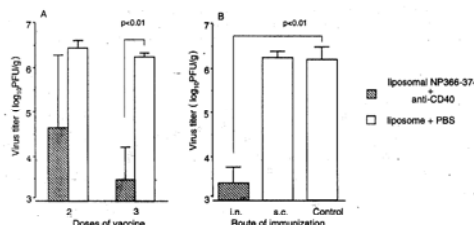


Fig. 1. Protective effects of two or three immunizations with liposomal NP366-374 together with anti-CD40 mAb. Three to four mice in each group were immunized intranasally twice or three times at 2-week intervals. Subcutaneous immunization provided no effective immunity against virus replication in the lung (B). Three to four mice in each group were intranasally or subcutaneously immunized three times at 2-week intervals. Mice were challenged intranasally with A/Aichi/2/68 (H3N2) a week after the last immunization. In both experiments, control mice were given liposome alone. Five days later, mice were sacrificed and lungs were collected. Lung virus titers were determined as described in Section 2. Bars and error bars represent mean titers and standard deviations for each group.

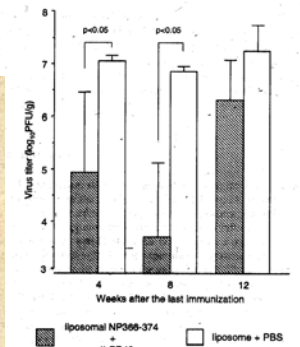
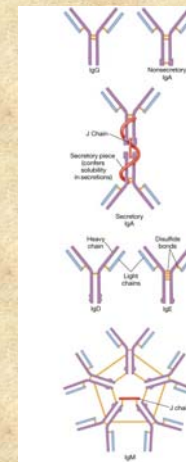
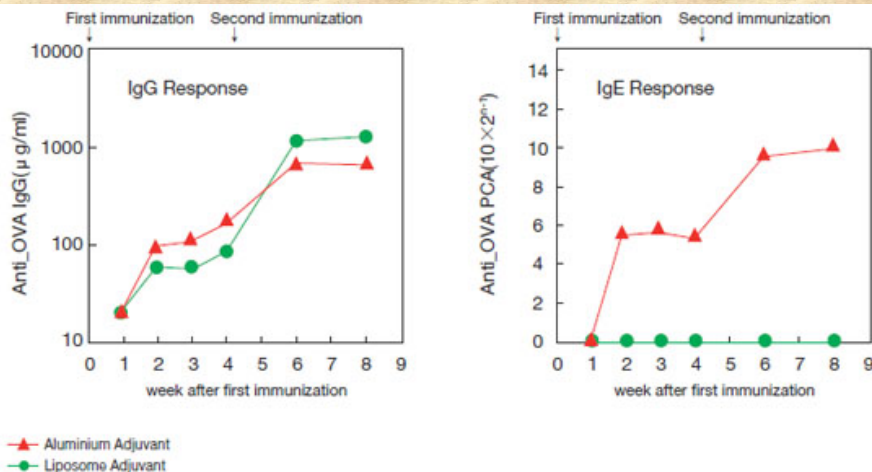


Fig. 2. Duration of the protective effect of immunization with liposomal NP366-374 together with anti-CD40 mAb. Three to four mice in each group were immunized intranasally three times at 2-week intervals. Mice were challenged with A/Aichi/2/68 (H3N2) 4, 8, or 12 weeks after the last immunization. Five days later, lungs of mice were collected to calculate virus titers. Bars and error bars represent means and standard deviations for each group.

6. Allergia kezelése

Az allergiás reakció során IgE termelődik. Megfelelő összetételű liposzómába zárva az antigént csökkenthető az IgE és fokozható az IgG termelődése → allergiás betegségek immunterápiája.



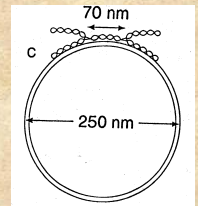
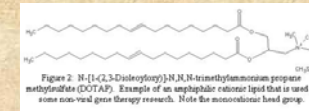


7. Génátvitel

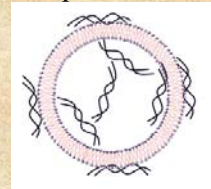
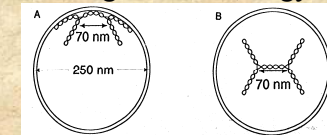
Megvalósítható DNS-darab bezárása, vagy a liposzóma felszínén való megkötése útján (főleg pozitív töltésű liposzómák esetén)

A kötődés lehet:

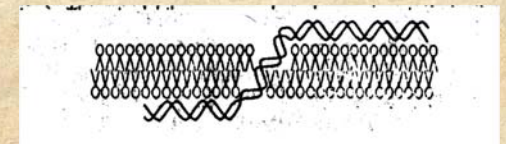
- elektrosztatikus kapcsolódás a külső felszínhez



- a liposzóma üregébe zárva, vagy a belső felszínhez kapcsolódva

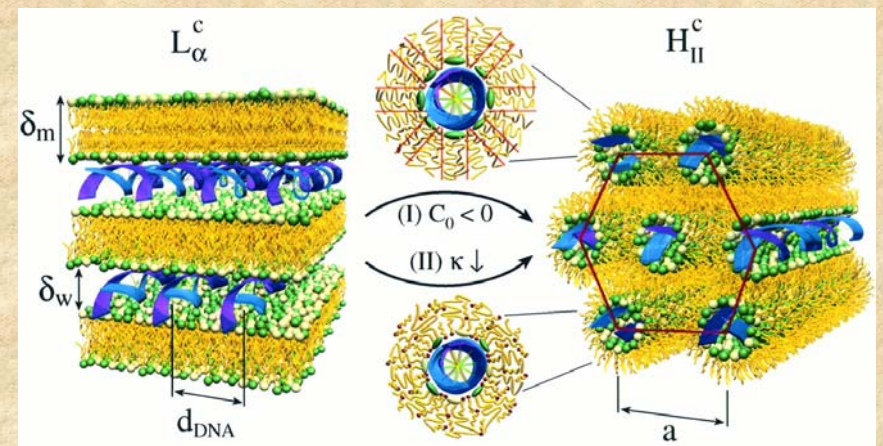
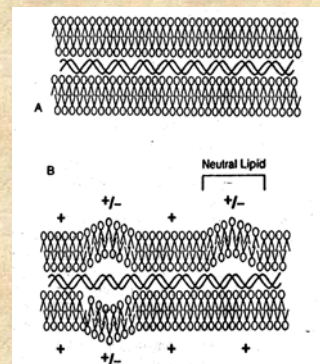
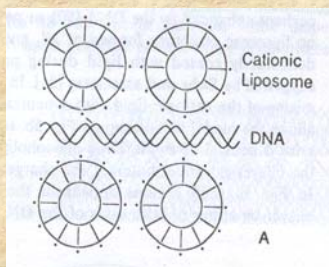


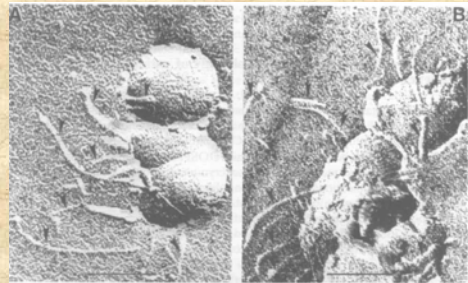
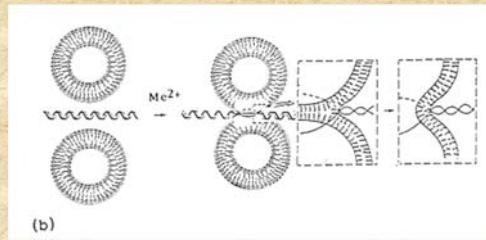
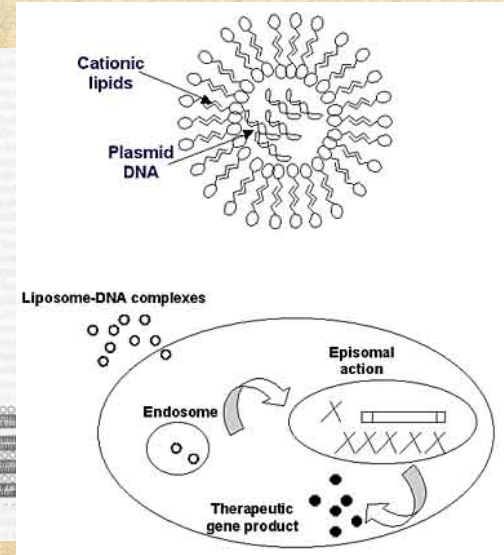
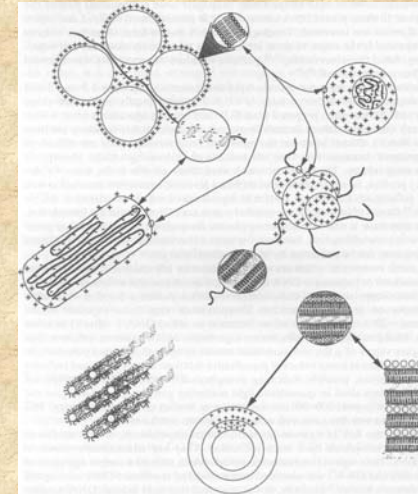
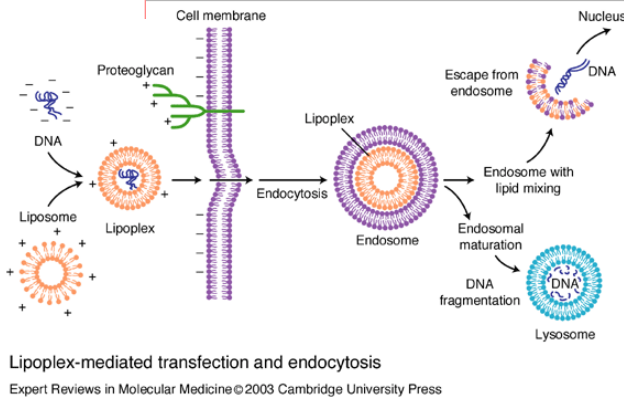
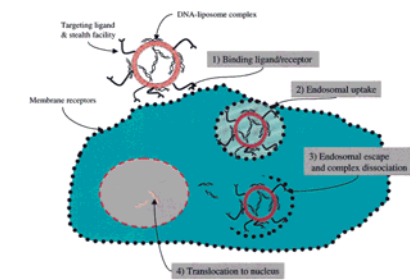
- részben belül, részben kívül



- fúzió során két bilayer közé kerülhet a DNS
A sejtbe juttatáshoz legcélszerűbb a liposzóma és a sejtmembrán fúziója.
A liposzóma felszíni markereitől függően más-más sejtek vehetik fel, pl. galaktóz – májsejtek; mannóz – fehérvérsejtek

A DOPE általában alkotóeleme az ilyen célú liposzómáknak membrándestabilizáló hatás (ahhoz szükséges, hogy a DNS kiszabaduljon az endoszómából)





	Calcium phosphate precipitation	Direct injection	Retroviral-mediated	Lipofection
Viable <i>in vivo</i>	-	+	?	+
Efficient means of delivery	-	+	+	+
Not disruptive to cell membrane	-	-	+	+
Transfects many cell types	+	+	?	+
Non carcinogenic	+	+	-	+
Metabolizable delivery agent	-	-	-	+
Reproducibility	-	+	+	+
Ability to target to specific tissues <i>in vivo</i>	-	+	-	+
Low cost	+	+	-	-