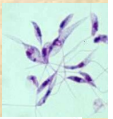
Liposzómák terápiás alkalmazásai (szisztémás terápia)

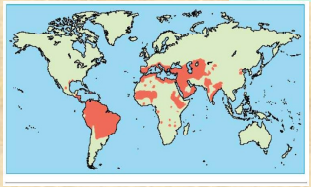
Dr. Voszka István

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 gyógyszermennyiség lecsökken
- a kezelés hatásfoka javul

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

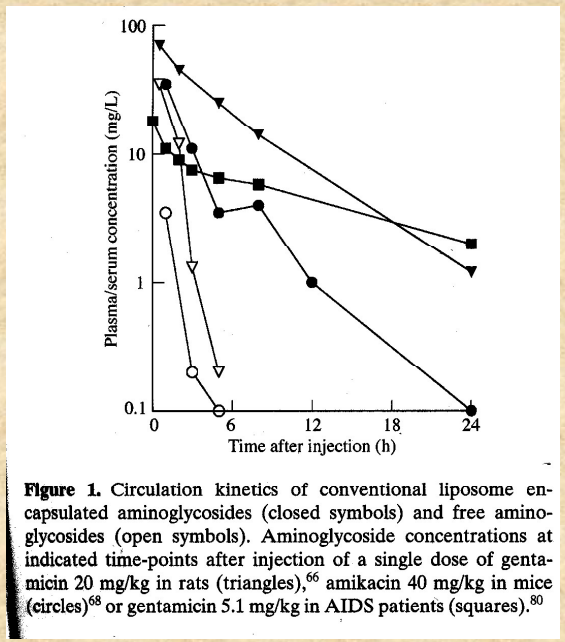
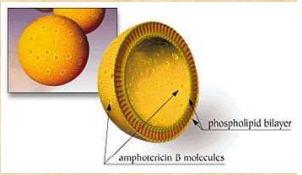


Figure 1. Circulation kinetics of conventional liposome encapsulated aminoglycosides (closed symbols) and free aminoglycosides (open symbols). Aminoglycoside concentrations at indicated time-points after injection of a single dose of gentamicin 20 mg/kg in rats (triangles),[66] amikacin 40 mg/kg in mice (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 ergoszterinjéhez

Más törzskönyvezett liposzómás Amphotericin B készítmény: Abelcet®

ABELCET 5 mg/ml szuszpenziós infúzió

Hatóanyag: **Amphotericin b**

Kiszerelés: **10x20 ml**

•vényköteles

Fogyasztói ár: **430496 Ft** Támogatott ár: **430496 Ft**

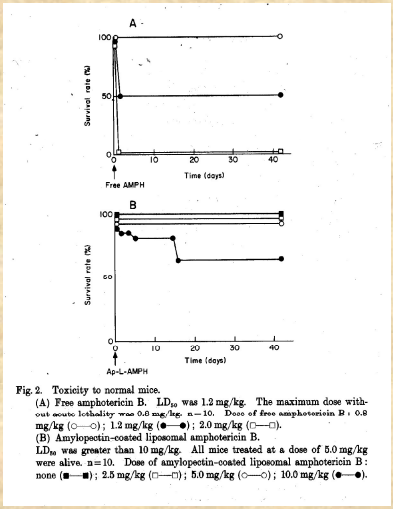


Fig. 2. Toxicity to normal mice.
(A) Free amphotericin B. LD_{50} was 1.2 mg/kg. The maximum dose without acute lethality was 0.6 mg/kg. n=10. Dose of free amphotericin B: 0.8 mg/kg (○—○); 1.2 mg/kg (●—●); 2.0 mg/kg (□—□).
(B) Amylopectin-coated liposomal amphotericin B.
LD_{50} was greater than 10 mg/kg. All mice treated at a dose of 5.0 mg/kg were alive. n=10. Dose of amylopectin-coated liposomal amphotericin B: none (■—■); 2.5 mg/kg (□—□); 5.0 mg/kg (○—○); 10.0 mg/kg (●—●).

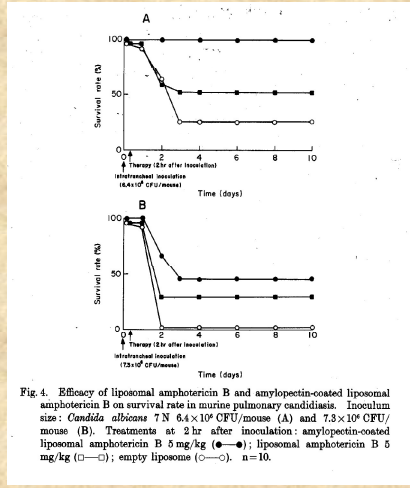


Fig. 4. Efficacy of liposomal amphotericin B and amylopectin-coated liposomal amphotericin B on survival rate in murine pulmonary candidiasis. Inoculum size: *Candida albicans* 7 N 6.4×10^6 CFU/mouse (A) and 7.3×10^6 CFU/mouse (B). Treatments at 2 hr after inoculation: amylopectin-coated liposomal amphotericin B 5 mg/kg (●—●); liposomal amphotericin B 5 mg/kg (□—□); empty liposome (○—○). n=10.

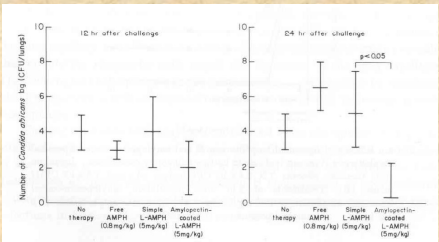
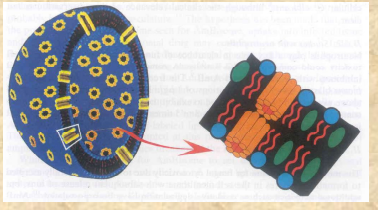


Fig. 3. The number of *Candida albicans* in lungs at 12 and 24 hr after injection of amphotericin B, liposomal amphotericin B and amylopectin-coated liposomal amphotericin B in murine pulmonary candidiasis. Inoculum size was 9.3×10^6 CFU/mouse. n=6. Data represent the mean±S.D.



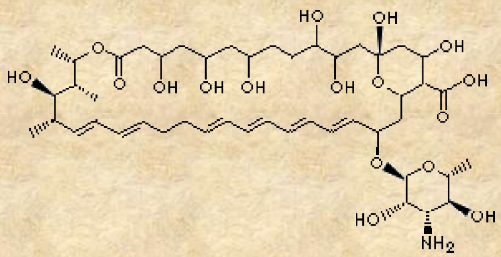
Polysaccharide-Coated Liposomal Amphotericin B for Pulmonary Candidiasis 487

TABLE 1. *Organ concentration of amphotericin B after injection of free, simple liposomal, and amylopectin-coated liposomal amphtericin B in normal mice*

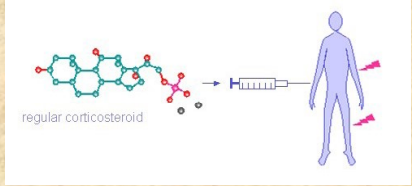
	Free-AMPH[a]		Simple-Lipo-AMPH[b]		Ap-Lipo-AMPH[b]	
	30 min (μg/g)	24 h (μg/g)	30 min (μg/g)	24 hr (μg/g)	30 min (μg/g)	24 h (μg/g)
Brain	ND[c]	ND	ND	ND	ND	ND
Heart	0.12±0.02[d]	ND	1.64±0.41	0.85±0.22	1.13±0.46	0.35±0.06
Lungs	0.34±0.05	ND	25.42±7.78	2.36±0.5	55.33±0.71	2.5±0.38
Liver	0.94±0.12	0.29±0.07	14.2±2.36	14.7±1.28	10.46±1.16	12.47±1.07
Spleen	0.81±0.17	0.23±0.10	15.56±2.93	13.61±3.65	10.17±0.76	10.39±0.8
Kidneys	0.36±0.07	0.09±0.03	3.7±1.05	1.00±0.36	2.85±0.18	0.43±0.23

[a]Amphotericin B 0.8 mg/kg weight.
[b]Amphotericin B 5.0 mg/kg weight.
[c]Not detectable (less than 0.09 μg/g tissue).
[d]The mean±S.D.

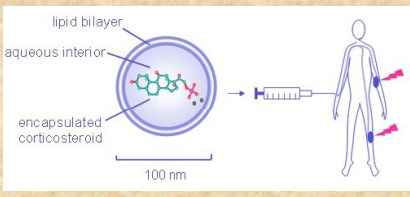
Nyotran (liposzómás nystatin) foszfolipid liposzómában



Szteroidok



Nanocort®: a terápiás elv
Kortikoszteroid SUV-ba zárva. Szelektíven dúsul a gyulladásokban és a daganatokban az érfalak fokozott permeabilitása miatt.
A liposzómákat foszfolipid és koleszterin alkotja.



3.Daganatterápia
Liposzómák alkalmazásával csökkenthetők a gyakran igen súlyos mellékhatások

Pl. doxorubicin (Doxyl®, Caelyx®, Myocet®) – szívizom-károsodás
methotrexate – máj-, vesekárosodás, kopaszság
vincristin (Onco TCS) – perifériás idegek károsodása, kopaszság
Ara-C (DepoCyt®) – csontvelő-, bélfal-károsodás
Daunorubicin (DaunoXome®) – szívizom-, csontvelő-károsodás

Közönséges liposzómák → RES-t érintő daganatok ill. áttétek kezelése

Caelyx 2 mg/ml koncentrátum infúzióhoz (1x10 ml) gyógyszer adatai

Gyártó: Janssen-Cilag International
Hatóanyag: doxorubicin
Kiszerelés: 1x10 ml
Fogyasztói ár: 146393 Ft
Támogatott ár: 146393 Ft

Normatív TB támogatás: 0%
Közgyógyellátásra adható: igen
EÜ támogatásra adható: nem
EÜ 100% támogatásra adható: nem

Kiadhatóság:
Szakorvosi/kórházi diagnózist követően, folyamatos szakorvosi ellenőrzés mellett alkalmazható készítmények.

MYOCET 50 mg por és előkeverékek liposzómás diszperziós infúzió készítésére szánt koncentrátumhoz

Kiszerelés	Kiadhatóság	Fogyasztói ár	Fizetendő ár
2 db 50 mg-os sorozat	vényköteles	379 737 Ft	379 737 Ft

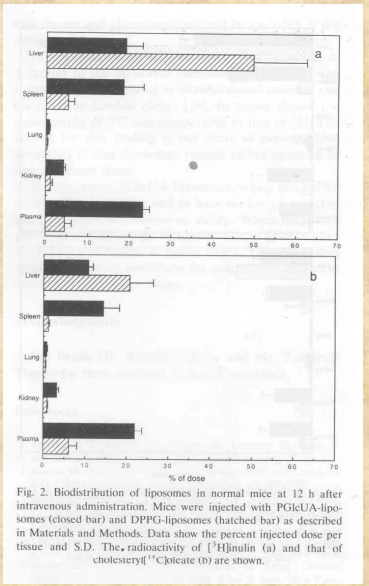


Fig. 2. Biodistribution of liposomes in normal mice at 12 h after intravenous administration. Mice were injected with PGlcUA-liposomes (closed bar) and DPPG-liposomes (hatched bar) as described in Materials and Methods. Data show the percent injected dose per tissue and S.D. The radioactivity of [^{3}H]inulin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

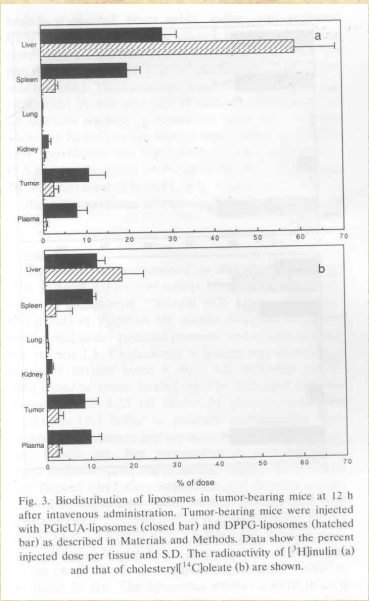
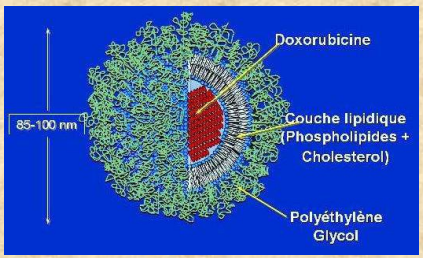
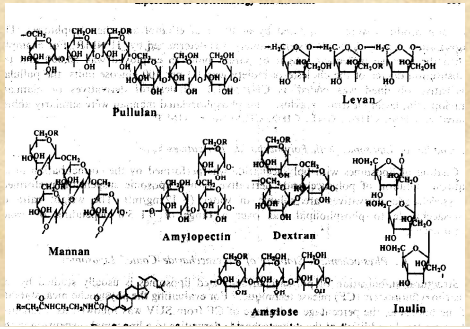


Fig. 3. Biodistribution of liposomes in tumor-bearing mice at 12 h after intravenous administration. Tumor-bearing mice were injected with PGlcUA-liposomes (closed bar) and DPPG-liposomes (hatched bar) as described in Materials and Methods. Data show the percent injected dose per tissue and S.D. The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

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





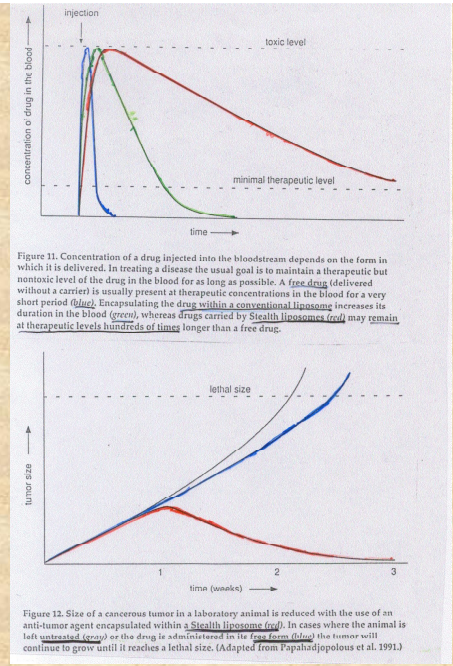
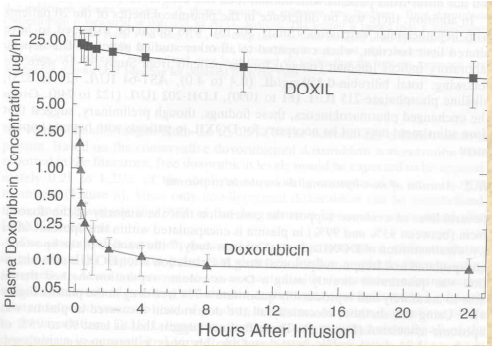
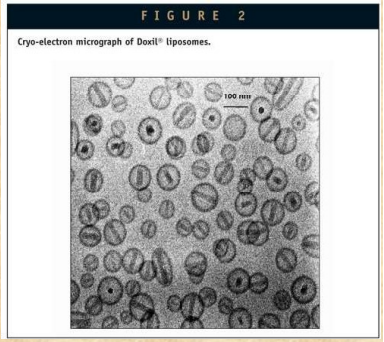
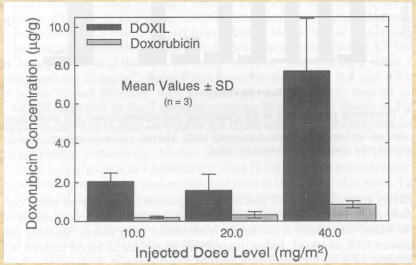


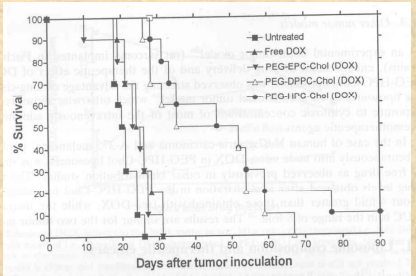
Figure 11. Concentration of a drug injected into the bloodstream depends on the form in which it is delivered. In treating a disease the usual goal is to maintain a therapeutic but nontoxic level of the drug in the blood for as long as possible. A free drug (delivered without a carrier) is usually present 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.

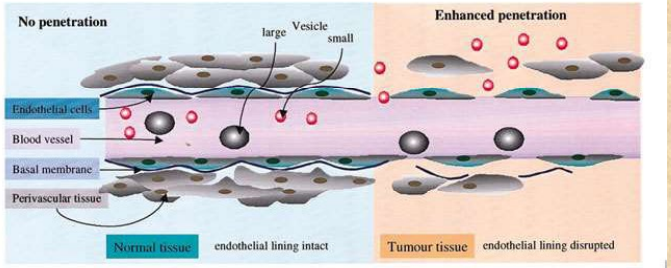
Figure 12. Size of a cancerous tumor in a laboratory animal is reduced with the use of an anti-tumor agent encapsulated within a Stealth liposome (*red*). In cases where the animal is left untreated (*gray*) or the drug is administered in its free form (*blue*) the tumor will continue to grow until it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

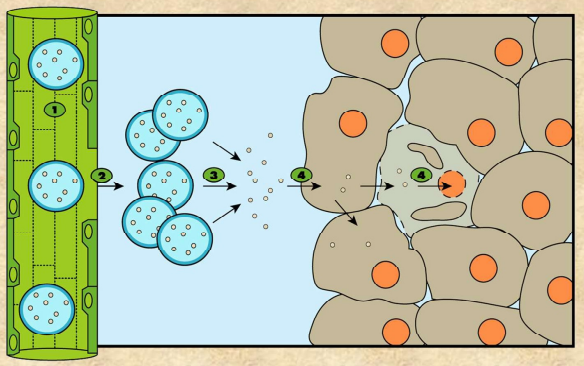












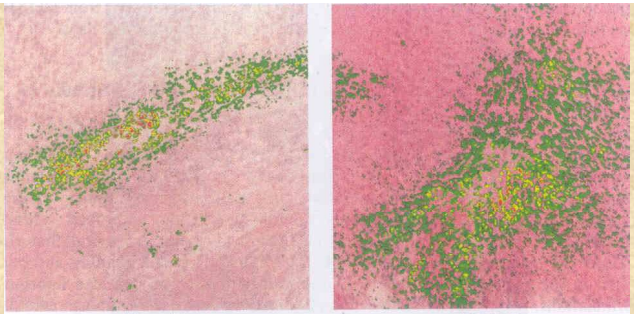


Figure 2

Figure 3

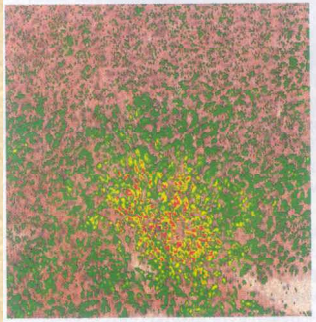


Figure 4

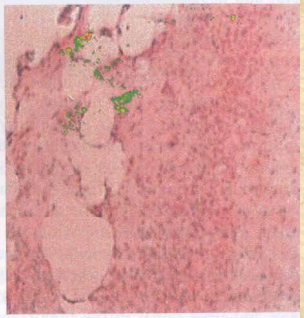
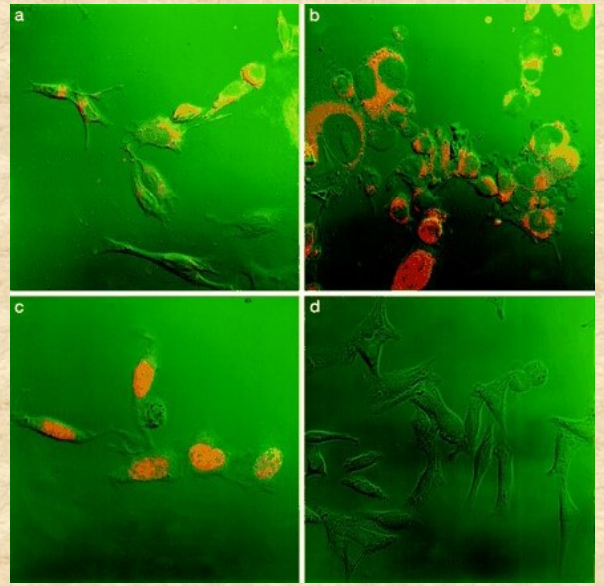


Figure 5

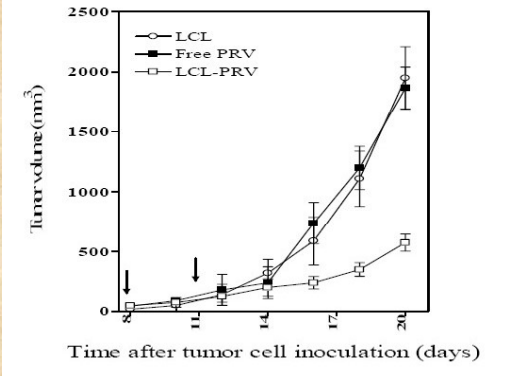


Confocal microscopy of liposomal DOX uptake by M109**R**-HiFR cells cultured in folate-depleted RPMI 1640 at 37°C. *a*, *b*, and *c*, binding, internalization, and nuclear localization of DOX delivered by FTL after a 30-min, 60-min, and 90-min incubation, respectively. *d*, cells exposed for 4 h to nontargeted PEG-coated liposomes encapsulating DOX (Doxil).

Sztatinok a tumorterápiában

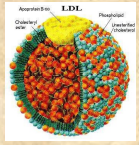
Sokkal nagyobb dózis szükséges, mint a koleszterinszint csökkentéséhez, ezért célszerű liposzómába zárni.
(PRV = pravastatin)

egér melanóma modell



LDL-be zárt citosztatikumok

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



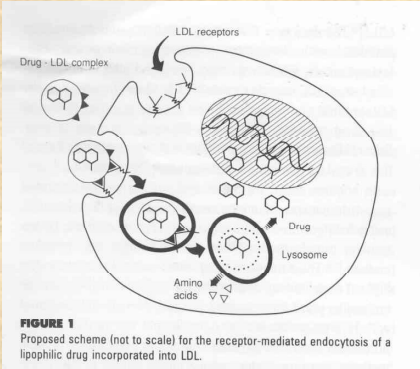


FIGURE 1
Proposed scheme (not to scale) for the receptor-mediated endocytosis of a lipophilic drug incorporated into LDL.

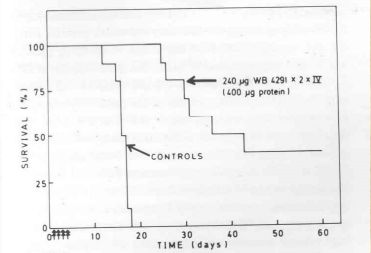


FIGURE 2
Survival of leukemic BALB/c mice after treatment with a WB 4291–LDL complex administered by intraperitoneal injection. The mice were inoculated intraperitoneally on day 0 with 10^6 WEHI-3B cells (a murine myelomonocytic leukemia cell line) and the WB 4291–LDL complex was given on days 1–4. There 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.)

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

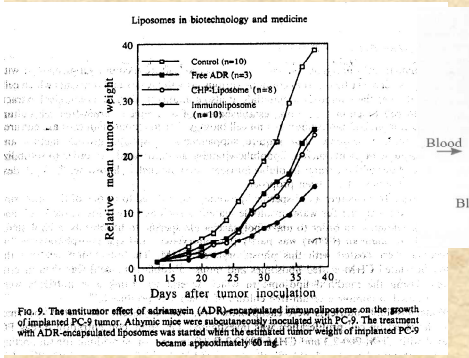
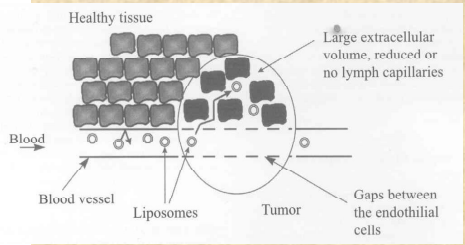


Fig. 9. The antitumor effect of adriamycin (ADR)-encapsulated immunoliposome on the growth of implanted PC-9 tumor. Athymic mice were subcutaneously inoculated with PC-9. The treatment with ADR-encapsulated liposomes was started when the estimated tumor weight of implanted PC-9 became approximately 60 mg.



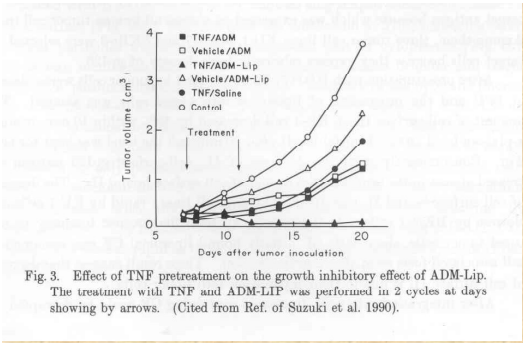


Fig. 3. Effect of TNF pretreatment on the growth inhibitory effect of ADM-Lip. The treatment with TNF and ADM-LIP was performed in 2 cycles at days showing by arrows. (Cited from Ref. of Suzuki et al. 1990).

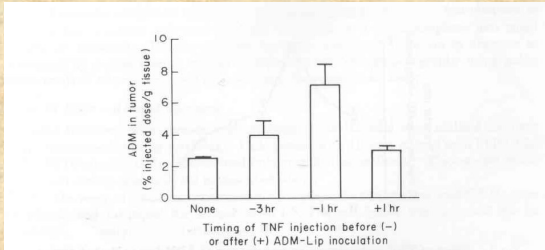


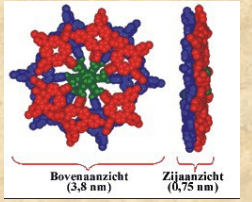
Fig. 2. Effect of injection time schedule of TNF treatment on the distribution to tumor. The indicated time after TNF injection, ADM-Lip were injected. (Cited from Ref. of Suzuki et al. 1990).

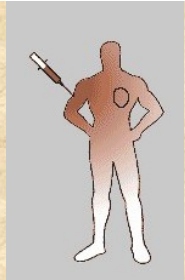
Fotodinámiás terápia (PDT)

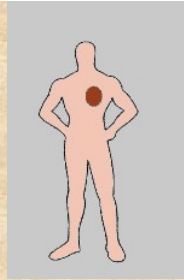
Fényérzékenyítő anyagot (hematoporfirin, ftalocianin származékok, stb.) juttatnak a tumorsejtekbe. Megvilágítják megfelelő hullámhosszú fénnyel → reaktív oxigéngyökök képződnek → tumorsejtek pusztulása.

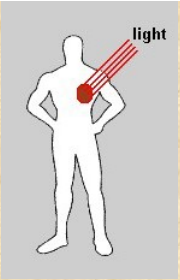
A hatás függ a fényérzékenyítő anyag jellemzőitől. Minél hidrofilebb (pl. szulfonált forma) annál inkább a tumorban lokalizálódik. De a daganatpusztító hatás fordítottan arányos a szulfonáltsággal.

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

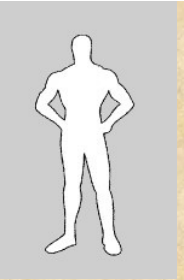


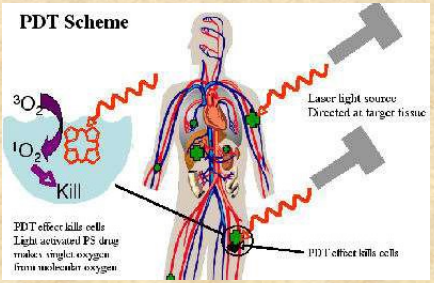

Fényérzékenyítő bejuttatása

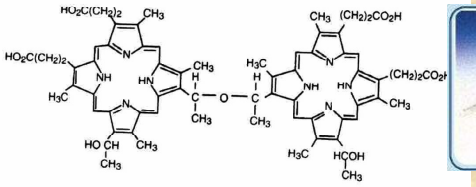

A fényérzékenyítő felhalmozódása a daganatban

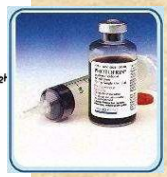


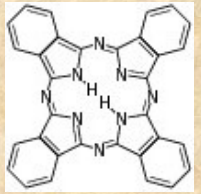
Besugárzás látható fénnyel

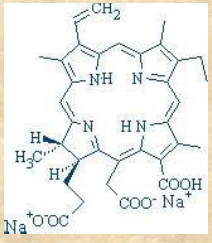

Szelektív tumordestrukció

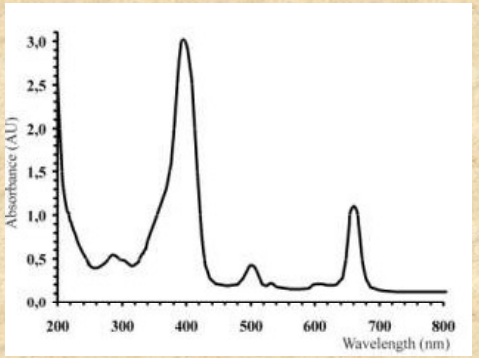












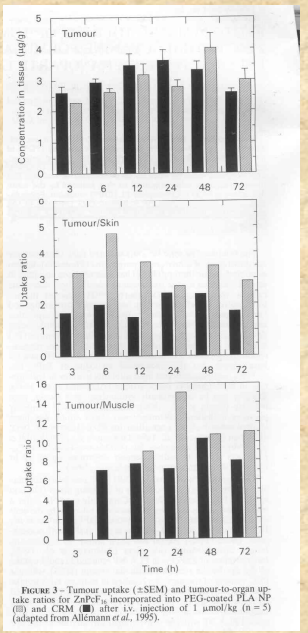


FIGURE 3 – Tumour uptake (±SEM) and tumour-to-organ uptake ratios for $ZnPcF_{16}$ incorporated into PEG-coated PLA NP (▨) and CRM (■) after i.v. injection of 1 µmol/kg (n = 5) (adapted from Allémann *et al.*, 1995).

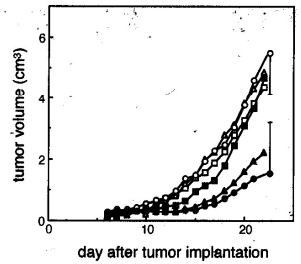


Fig. 1. Suppression of Tumor Growth by PDT with Free or Liposomal BPD-MA

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/cm²). 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.

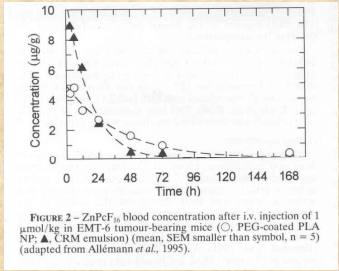


FIGURE 2 – $ZnPcF_{16}$ blood concentration after i.v. injection of 1 µmol/kg in EMT-6 tumour-bearing mice (○, PEG-coated PLA NP; ▲, CRM emulsion) (mean, SEM smaller than symbol, n = 5) (adapted from Allémann *et al.*, 1995).

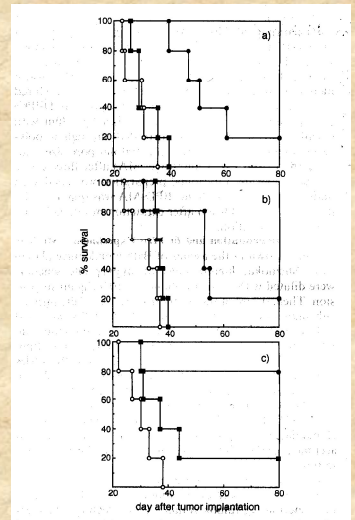
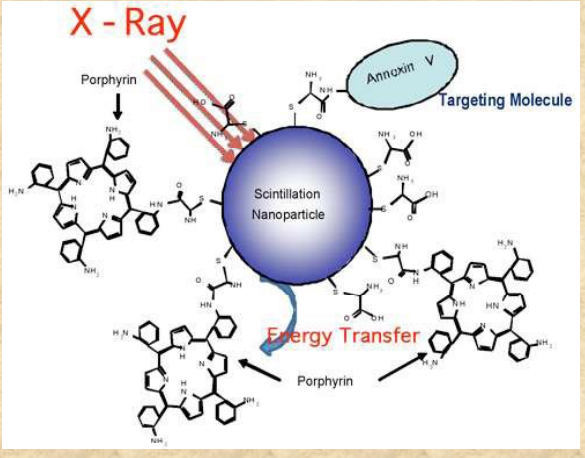


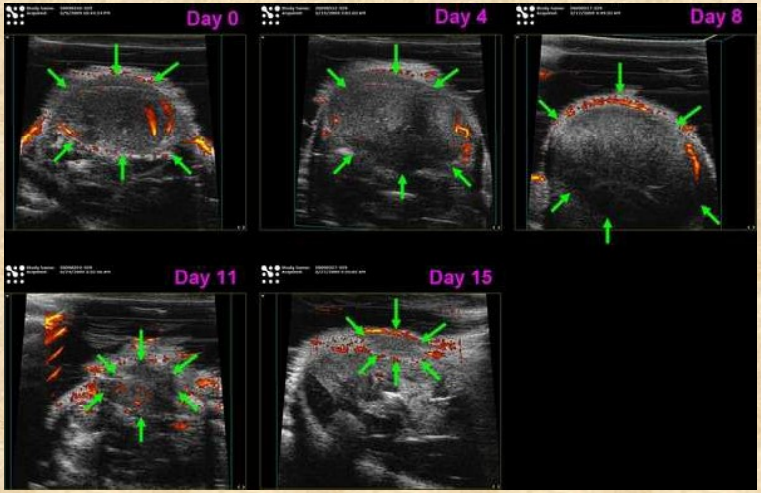
Fig. 4. Survival of Meth A-Bearing Mice by PDT with Free or Liposomal BPD-MA

Mice implanted s.c. with Meth A sarcoma (1×10^6 cells) into the left posterior flank were injected i.v. with free BPD-MA (a), BPD-MA entrapped in DPPG-liposomes (b), or BPD-MA in PGlcUA-liposomes (c) prior to PDT, as described in the legend of Fig. 1. The survival times after treatment with 2 mg/kg BPD-MA (■), 6 mg/kg BPD-MA (●), or 0.3 M glucose solution (○) are shown.

Röntgensugárzással indukált PDT



Radioterápia célzott izotópkezeléssel (Re-188)



Longitudinal power Doppler ultrasound Imaging. The images were acquired at 0, 4, 8, 11 and 15 days after injection of 188Re-liposome (29.6 MBq). Locations of color signals indicative of blood vessels. The arrow marks an area of tumor. The imaging showed a decrease in the tumor volume and number of blood vessels.

Forgalomban lévő liposzómás daganatellenes gyógyszerek

Név	Hatóanyag	Gyártó	Egyéb	Indikáció
DepoCyt	citarabin	SkyePharma	-	malignus lymphomás meningitis
DaunoXome	daunorubicin	Gilead Sciences	-	Kaposi-sarcoma
Doxil/Caelyx	doxorubicin	Ortho Biotech, Schering-Plough	PEGilált	Kaposi-sarcoma, metasztázisos mell- és petefészekrák
Myocet	doxorubicin	Zeneus	-	ciklofoszfamid terápiával kombinálva metasztázisos mellrákban
Visudyne	verteporfirin	QLT, Novartis	-	PDT időskori macula degeneráció, patológiás myopia és szemészeti hisztoplazmózis
Marqibo	vincristin	Talon Therapeutics	-	Philadelphia kromoszóma negatív felnőtt ALL
Lipusu	paclitaxel	Luye Pharma Group	-	petefészekrák, mellrák és nem-kissejtes tüdőrák

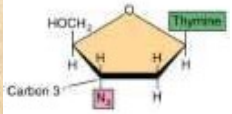
4.AIDS

Mind a celluláris (főleg a $CD4^+$ limfociták számának csökkenése miatt) mind a humorális immunválasz csökkent → védekezési képtelenség a fertőzésekkel szemben (opportunista 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 kötést. 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'-dezoxitimidin (AZT) = zidovudin (ZDV)

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



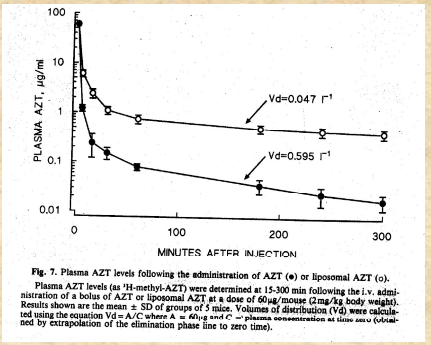


Fig. 7. Plasma AZT levels following the administration of AZT (●) or liposomal AZT (○).

Plasma AZT levels (as ^{3}H-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 (Vd) were calculated using the equation Vd = A/C where A = 60 μg and C = plasma concentration at time zero (obtained by extrapolation of the elimination phase line to zero time).

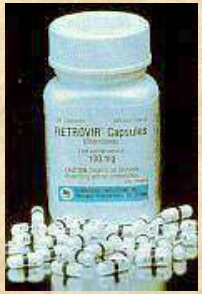


Table III. Tissue distribution of AZT and liposomal AZT.

Tissue	% Injected dose/g tissue[a] AZT	Liposomal AZT DPPC/DMPG	Liposomal AZT DPPC/PS
Liver	0.59 ± 0.06	3.10 ± 0.21[b]	2.10 ± 0.13[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 (3 mg/kg body weight) spiked with ^{3}H-methy-AZT. DPPC/DMPG liposomes were formulated in the molar ratio 10/1; DPPC/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 DSPC/DMPG liposomes ($P < 0.01$).
[d]Results for bone marrow are expressed as percent dose/10^7 nucleated cells. The limit of detection using ^{3}H-methyl-AZT was 2.5 pg AZT.

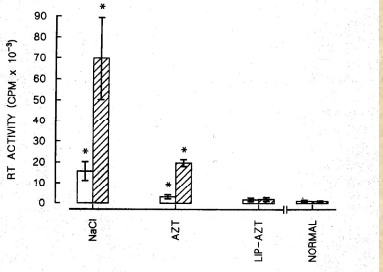


Fig. 10. Effect of treatment with AZT or liposomal AZT on the development of plasma RT activity in LP-BM5-infected C57BL/6 mice.

Groups of 5 LP-BM5-infected mice were treated for 6 weeks as described in the legend to figure 9. 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).

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

Treatment (mg/kg/day)	Bone marrow cells/ femur ($\times 10^6$)	Leucocytes ml/blood ($\times 10^6$)	RBC ml/blood ($\times 10^6$)
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[b]	6.1 ± 0.9[a]	6.3 ± 0.4[a]
10.0	6.3 ± 1.3[b]	5.1 ± 1.6	6.0 ± 0.6[a]
50.0	5.9 ± 0.8[b]	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 leukocyte 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 ($P < 0.01$).

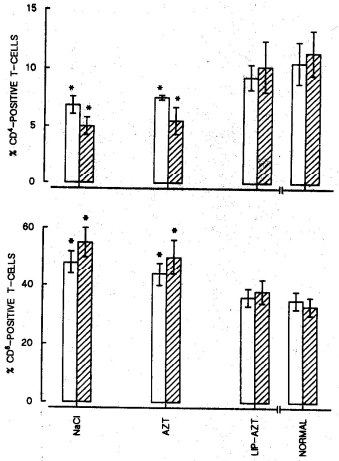


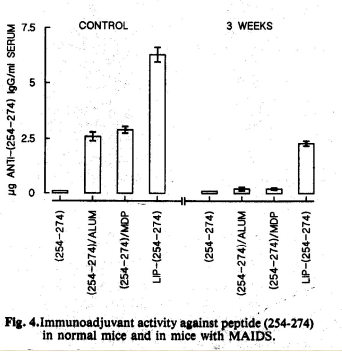
Fig. 9. Effect of treatment with AZT or liposomal AZT on splenic $CD4^+$ and $CD8^+$ T-cell populations.

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



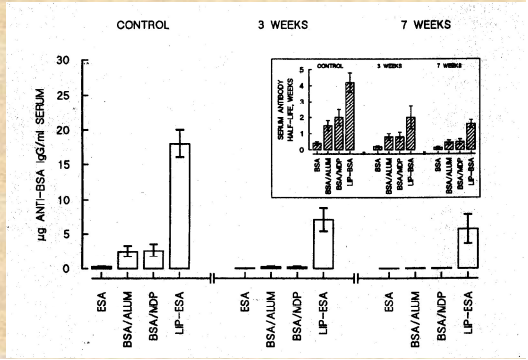


Fig. 4. Immunoadjuvant activity against peptide (254-274) in normal mice and in mice with MAIDS.

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
Poliovirus 1-VP2 peptide	0.22	16	74–82
Poliovirus-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-S_1 peptide, sequence was 15–48 (30).

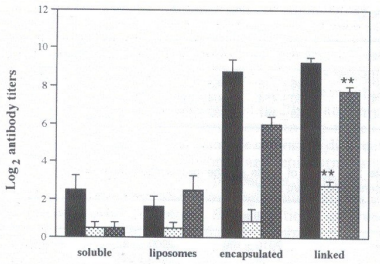


Figure 1 Groups of five mice were injected intraperitoneally with empty liposomes (1 μmol) or with 100 μg of either free, encapsulated or surface-linked cAlb. Blood was collected on the 12th day following immunization and specific anti-cAlb total Ig, IgM and IgG were measured on serum samples. The results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

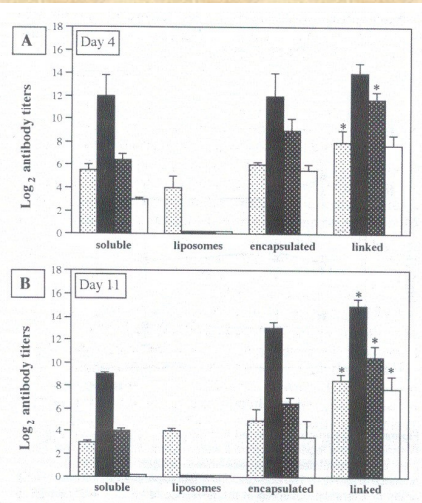


Figure 2 Mice were injected with empty liposomes (1 μmol) or 20 μg of either free, encapsulated or surface-linked cAlb and rechallenged 28 days later with the same antigenic formulations. The production of specific seric anti-cAlb IgM, IgG1, IgG2a and IgG3 was measured on the 4th day (A) or the 11th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

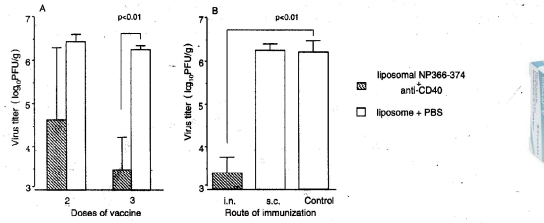


Fig. 1. Protective effects of two or three immunizations with liposomal NP366–374 together with anti-CD40 mAb (A). Three to four mice in each group were immunized intranasally twice or three times at 2-week intervals. Subcutaneous immunization provided no effective immunity against virus replication 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.

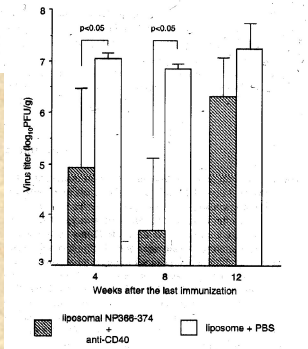
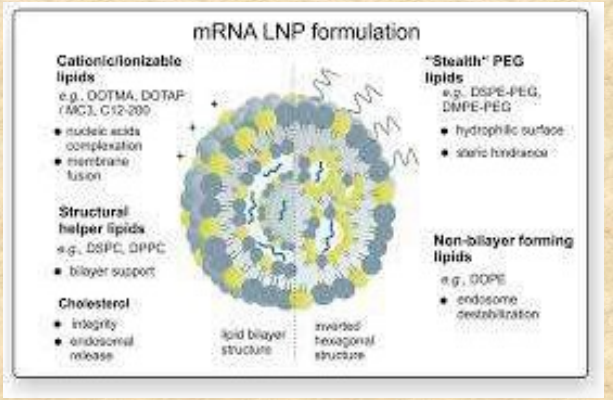


Fig. 2. Duration of the protective effect of immunization with liposomal NP366–374 together with anti-CD40 mAb. Three to four mice in each group were immunized intranasally three times at 2-week intervals. Mice were challenged with A/Aichi/2/68 (H3N2) 4, 8, or 12 weeks after the last immunization. Five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

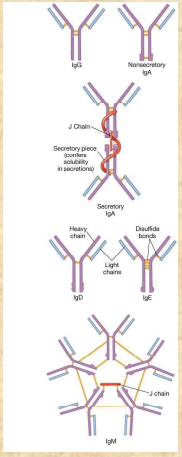
Pfizer-BioNTech, illetve Moderna COVID-19 vakcina

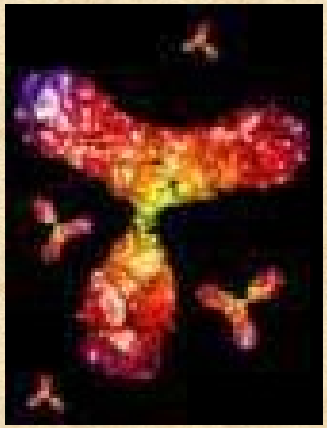
mRNS lipid nanoparticulumban

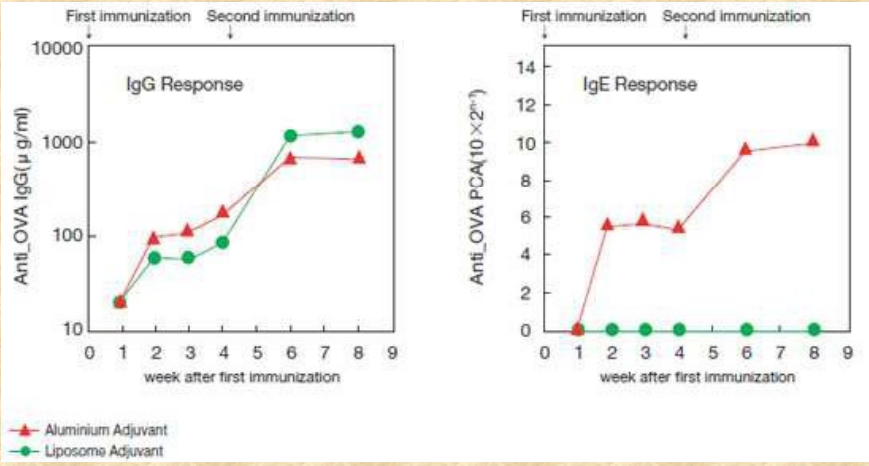


6.Allergia kezelése

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

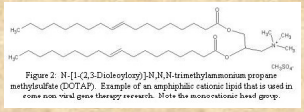
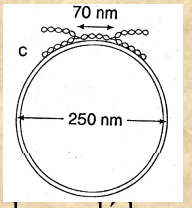
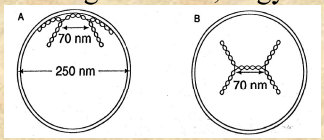
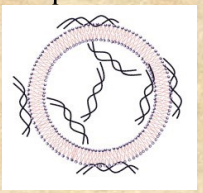


Figure 2: N-[1-(2,3-Dioleoyloxy)]-N,N,N-trimethylammonium propane methylsulfate (DOTAP). Example of an amphiphilic cationic lipid that is used in some non viral gene therapy research. Note the monocationic head group.

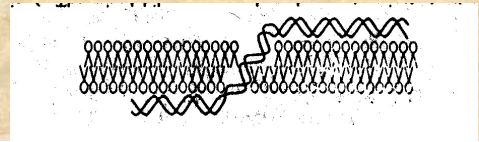


- a liposzóma üregébe zárva, vagy a belső felszínhez kapcsolódva





- részben belül, részben kívül

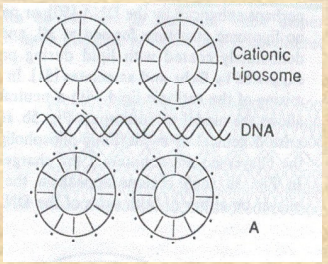


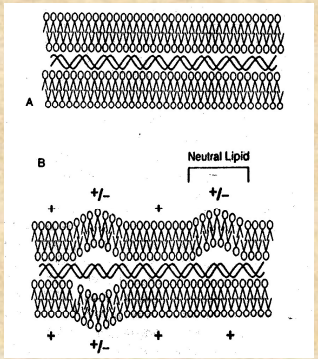
- fúzió során két bilayer közé kerülhet a DNS

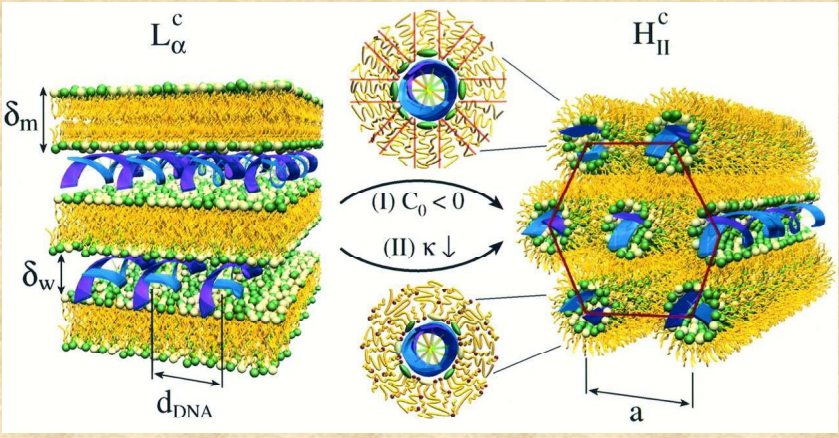
A sejtbe juttatáshoz legcélszerűbb a liposzóma és a sejtmembrán fúziója.

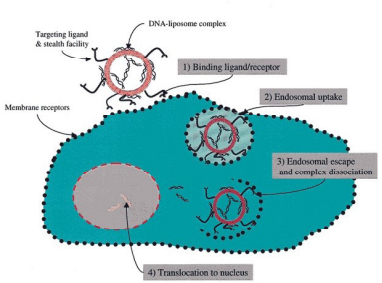
A liposzóma felszíni markereitől függően más-más sejtek vehetik fel, pl. galaktóz – májsejtek; mannóz – fehérvérsejtek

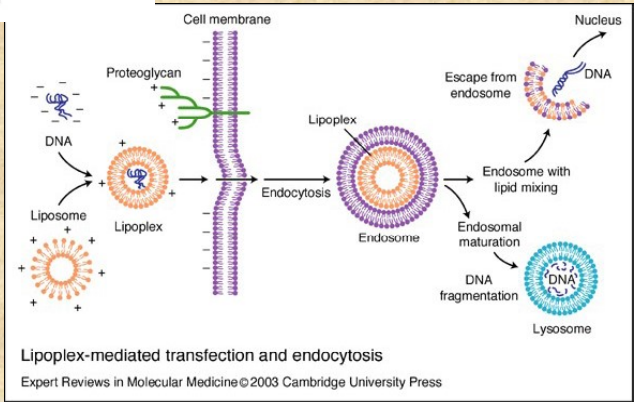
A DOPE általában alkotóeleme az ilyen célú liposzómáknak membrándestabilizáló hatás (ahhoz szükséges, hogy a DNS kiszabaduljon az endoszómából

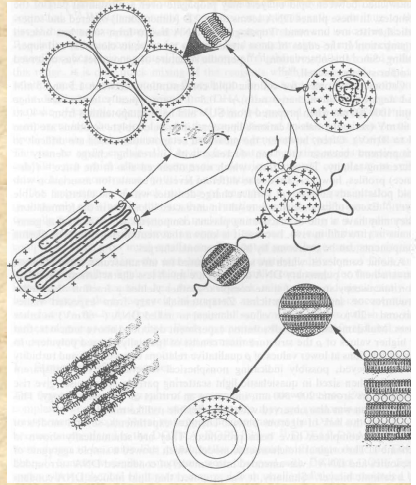


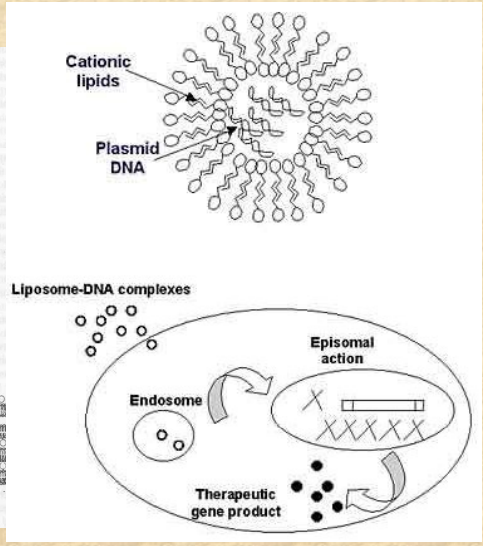


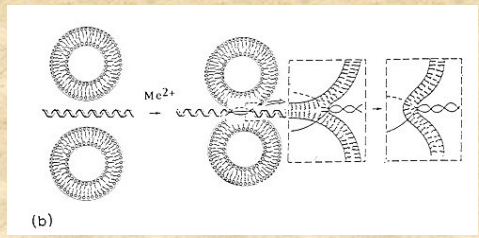


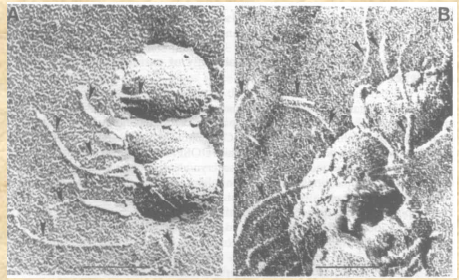




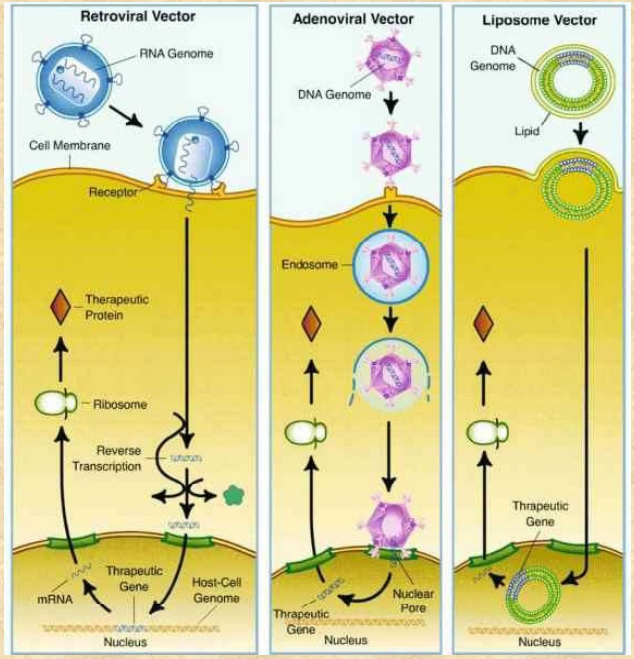








	Calcium phosphate precipitation	Direct injection	Retroviral mediated	Lipofection
Viable *in vivo*	–	+	?	+
Efficient means of delivery	–	+	+	+
Not disruptive to cell membrane	–	–	+	+
Transfects many cell types	+	+	?	+
Non carcinogenic	+	+	–	+
Metabolizable delivery agent	–	–	–	+
Reproducibility	–	+	+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



13.3 Liposomes in gene therapy

- Recombinant DNA tech., studies of gene function & gene therapy all depend on delivery of nucleic acids(genetic material) into cells in vitro & in vivo.
- Gene can be viral (adenovirus, retrovirus) & non viral(liposomes & lipid based systems, polymers & peptides)

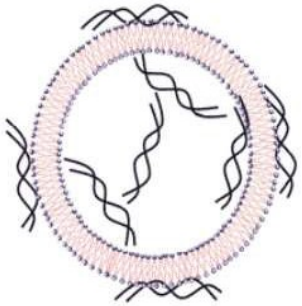
Type of vectors	Advantages	Disadvantages
Viral vectors (Adenovirus, retrovirus & adeno-associated virus)	Relatively high transfection efficiency	▪Immunogenicity, presence of contaminants & safety ▪Vector restricted size limitation for recombinant gene
Non viral vectors (liposomes/lipid based systems, polymers & peptides)	▪ Favorable, pharmaceutical issue-GMP, stability, cost ▪Plasmid independent structure ▪Low immunogenicity ▪Opportunity for chemical/physical manipulation	▪ low transfection efficiency

DNA delivery of Genes by Liposomes

Cheaper than viruses

No immune response

Especially good
for in-lung delivery (cystic fibrosis)



100-1000 times more plasmid DNA needed
for the same transfer efficiency as for viral vector