

Liposzómák terápiás alkalmazásai (szisztémás terápia)

Dr. Voszka István

b) Szisztémás alkalmazások

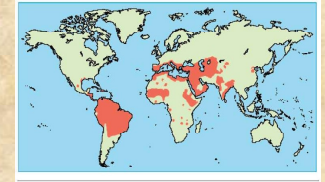
Általában akkor, ha az adott gyógyszernek súlyos mellékhatásai vannak.

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.)

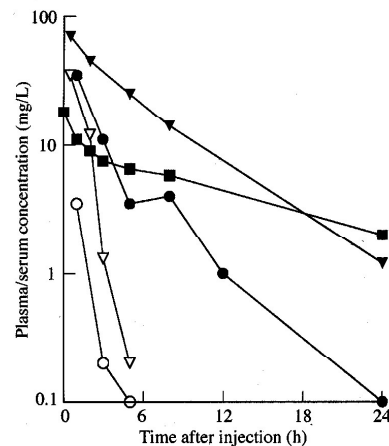


Figure 1. Circulation kinetics of conventional liposome encapsulated aminoglycosides (closed symbols) and free aminoglycosides (open symbols). Aminoglycoside concentrations at indicated time-points after injection of a single dose of gentamicin 20 mg/kg in rats (triangles),⁶⁶ amikacin 40 mg/kg in mice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



Gombaellenes pl. Amphotericin B

(első törzskönyvezett liposzómás gyógyszerkészítmény – gyári neve AmBisome®)

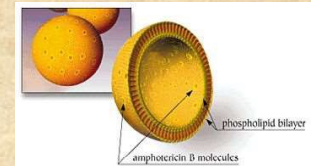
telített PC / Chol / DSPG (2: 1: 0,8) + 10 mol% AmB ~80 nm-es SUV-okban)

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

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®



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

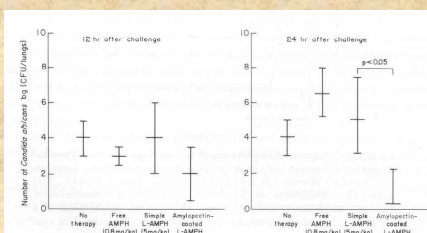
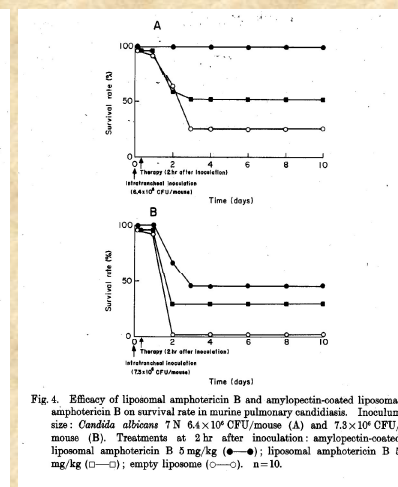
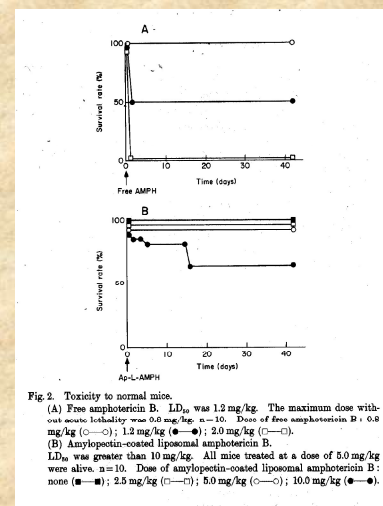
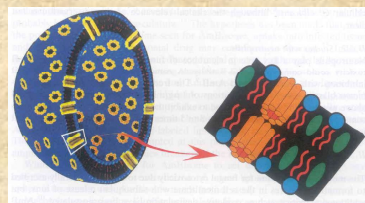


Fig. 3. The number of *Candida albicans* in lungs at 12 and 24 hr after injection of amphotericin B, liposomal amphotericin B and amphotericin-coated liposomal amphotericin B in murine pulmonary candidiasis. Inoculum size was 9.3×10^6 CFU/mouse. n=6. Data represent the mean \pm s.d.



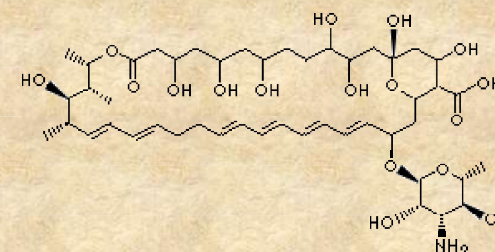
Polysaccharide-Coated Liposomal Amphotericin B for Pulmonary Candidiasis 487

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

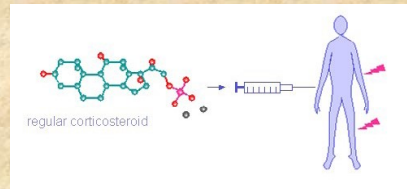
	Free-AMPH ^a		Simple-Lipo-AMPH ^b		Ap-Lipo-AMPH ^c	
	30 min ($\mu\text{g/g}$)	24 h ($\mu\text{g/g}$)	30 min ($\mu\text{g/g}$)	24 hr ($\mu\text{g/g}$)	30 min ($\mu\text{g/g}$)	24 h ($\mu\text{g/g}$)
Brain	ND ^d	ND	ND	ND	ND	ND
Heart	0.12 ± 0.02 ^e	ND	1.64 ± 0.41	0.85 ± 0.22	1.13 ± 0.46	0.35 ± 0.06
Lungs	0.34 ± 0.05	ND	25.42 ± 7.78	2.36 ± 0.5	55.33 ± 7.1	2.5 ± 0.38
Liver	0.94 ± 0.12	0.29 ± 0.07	14.2 ± 3.26	1.74 ± 1.28	14.6 ± 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	1.08 ± 0.36	2.05 ± 0.18	0.43 ± 0.23

^cThe mean \pm s.d.

Nyotran (liposzómás nystatin) foszfolipid liposzómában



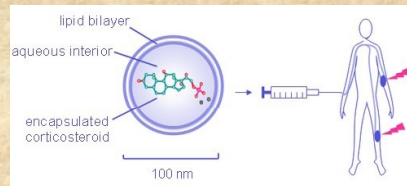
Szteroidok



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%

Közfgyógyellátásra 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

2 db 50 mg-os

sorozat

Kiadhatóság

vényköteles

Fogyasztói ár

379 737 Ft

Fizetendő ár

379 737 Ft

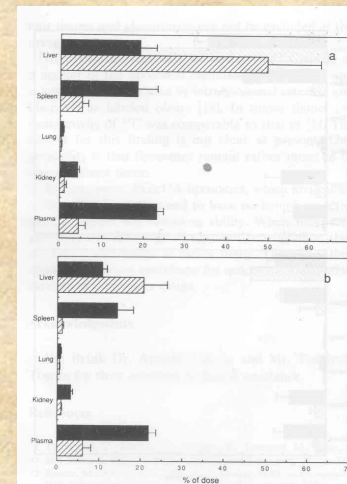


Fig. 2. Biodistribution of liposomes in normal mice at 12 h after intravenous administration. Mice were injected with PGlcUA-liposomes (closed bar) and DPPG-liposomes (hatched bar) as described in Materials and Methods. Data show the percent injected dose per tissue and S.D. The radioactivity of [3 H]inulin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

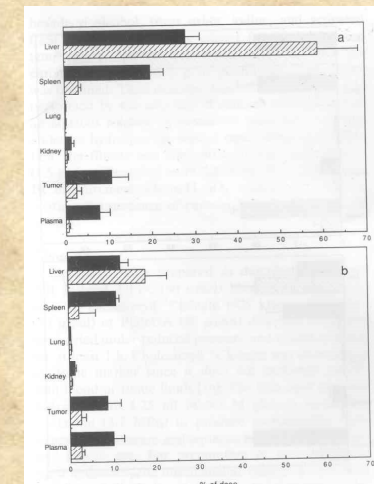


Fig. 3. Biodistribution of liposomes in tumor-bearing mice at 12 h after intravenous administration. Tumor-bearing mice were injected with PGlcUA-liposomes (closed bar) and DPPG-liposomes (hatched bar) as described in Materials and Methods. Data show the percent injected dose per tissue and S.D. The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

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

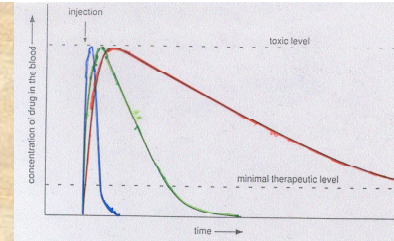
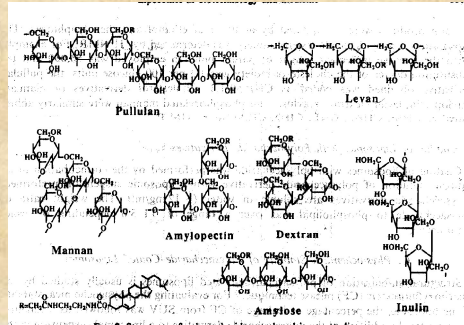
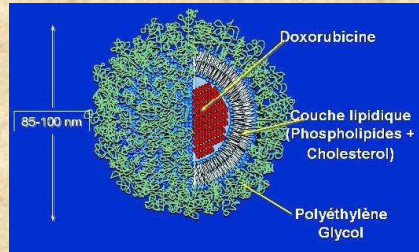


Figure 11. Concentration of a drug injected into the bloodstream depends on the form in which it is delivered. In treating a disease the usual goal is to maintain a therapeutic but nontoxic level of the drug in the blood for as long as possible. A free drug (delivered without a carrier) is usually present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

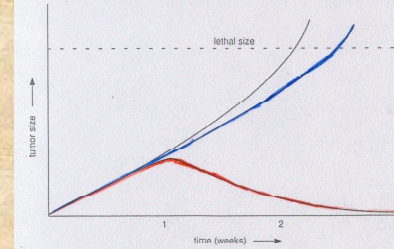
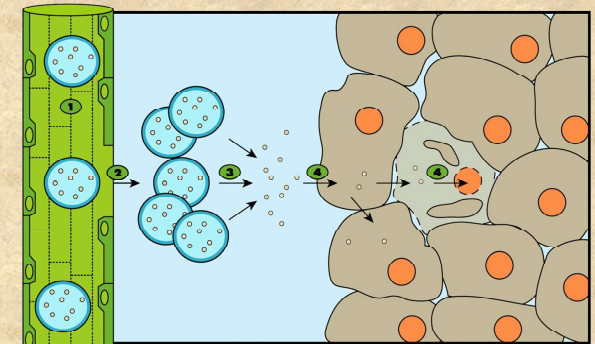
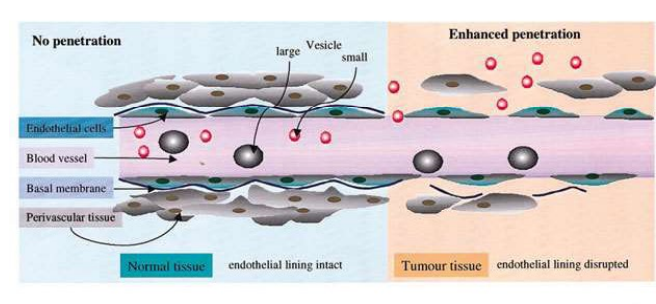
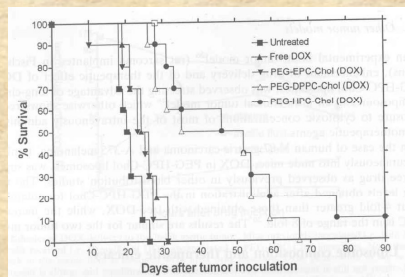
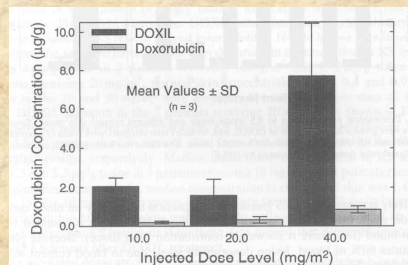
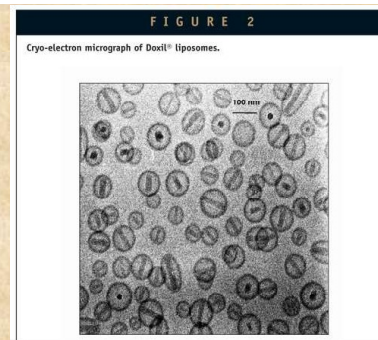
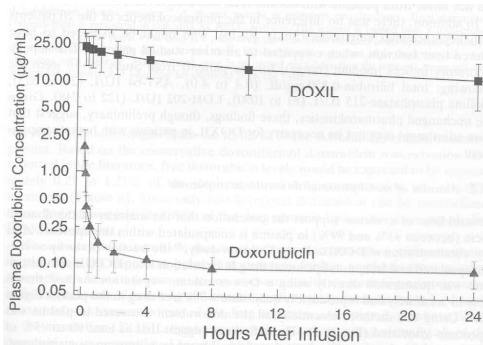
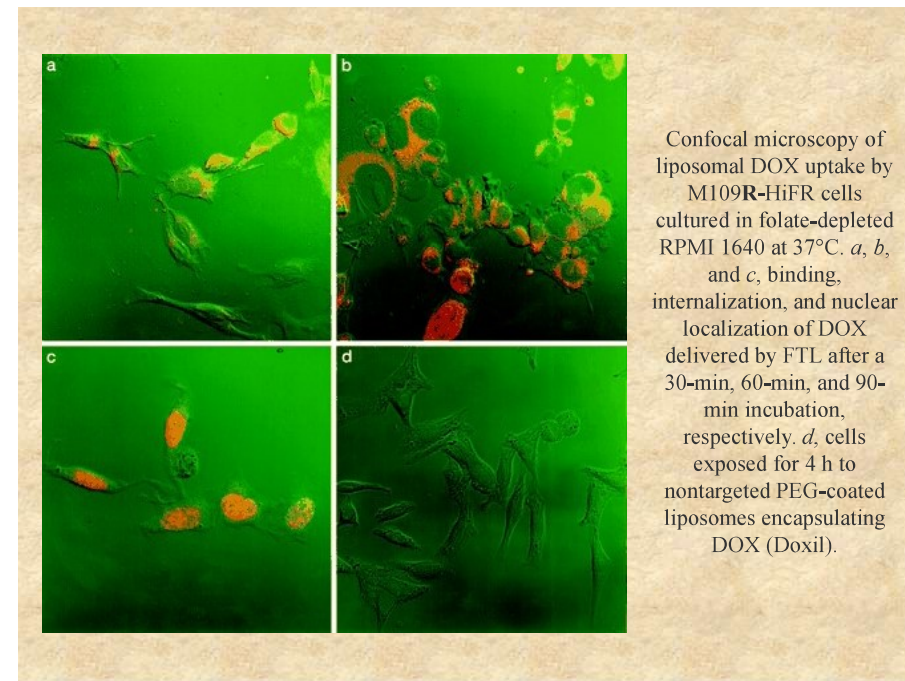
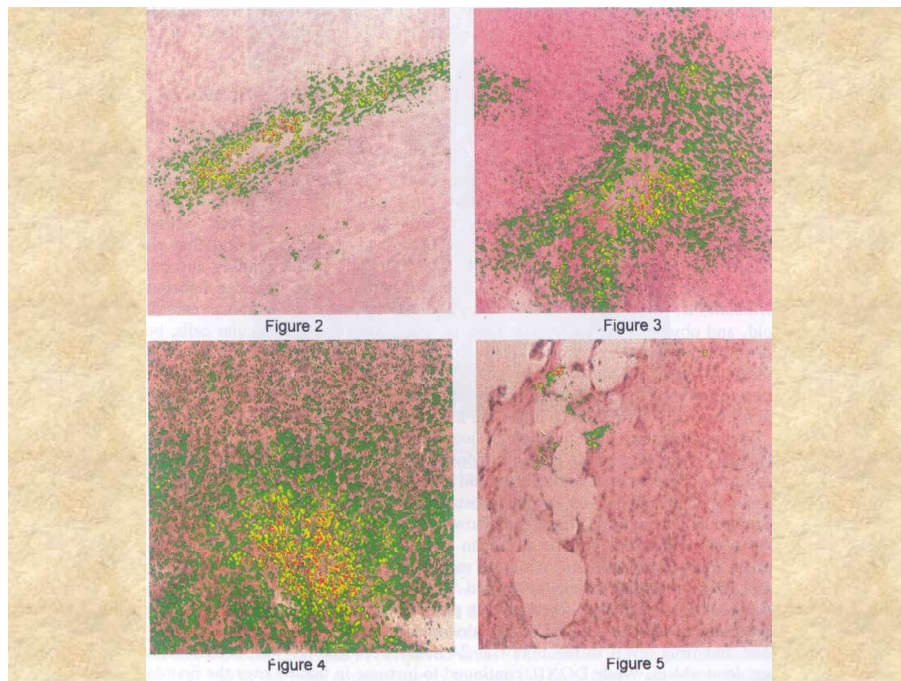


Figure 12. Size of a cancerous tumor in a laboratory animal is reduced with the use of an anti-tumor agent encapsulated within a Stealth liposome (red). In cases where the animal is left untreated (grey) or the drug is administered in its free form (blue) the tumor will continue to grow until it reaches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

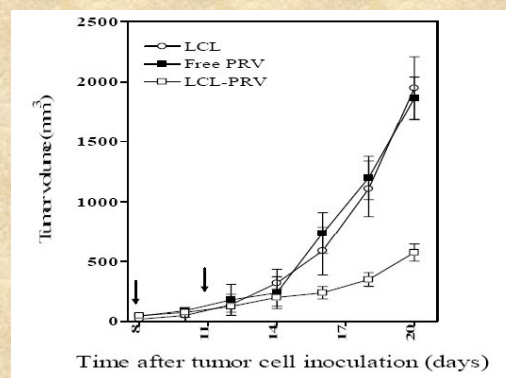




Sztatinok a tumorterápiában

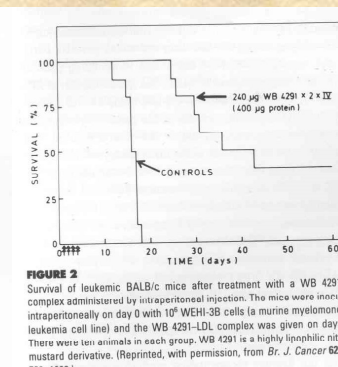
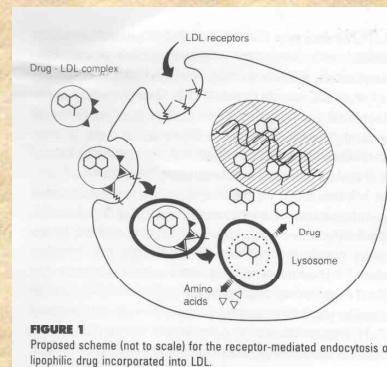
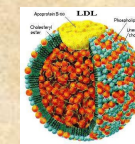
Sokkal nagyobb dózis szükséges, mint a koleszterinszint csökkentéséhez, ezért célszerű liposzómába zárni.
(PRV = pravastatin)

egér melanóma modell

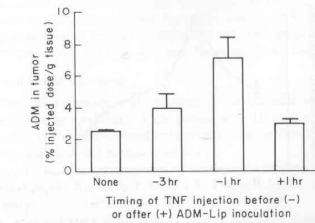
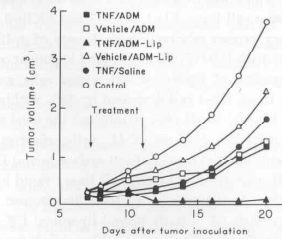
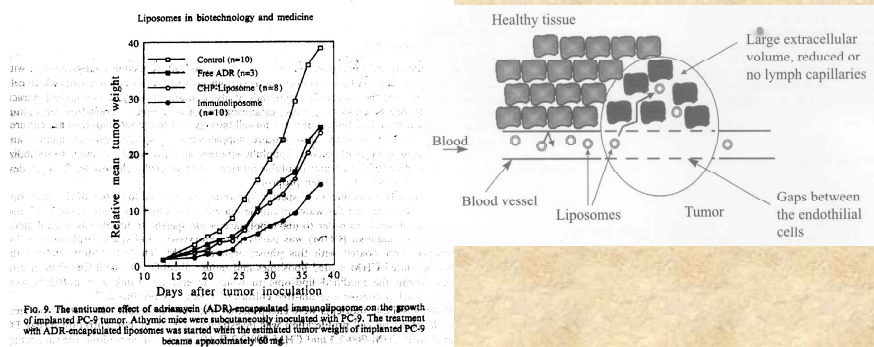


LDL-be zárt citosztatikumok

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

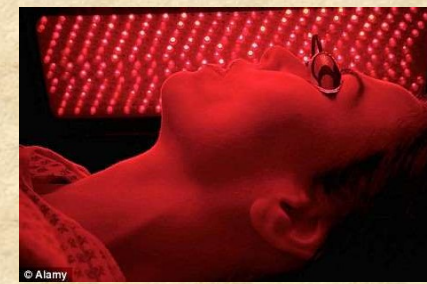
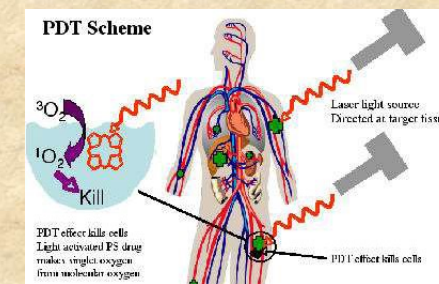
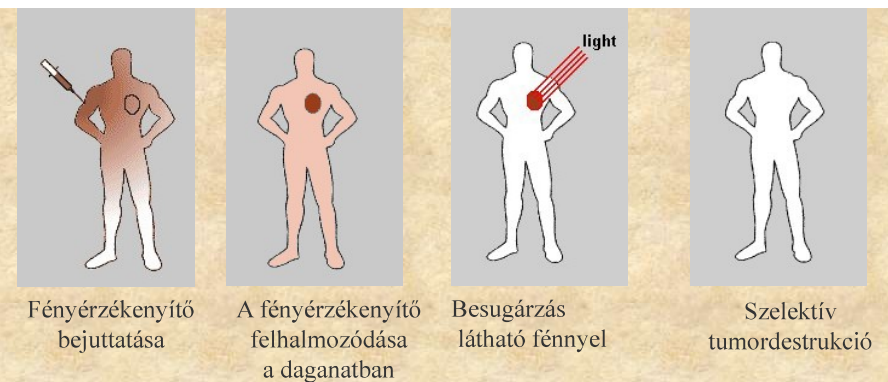


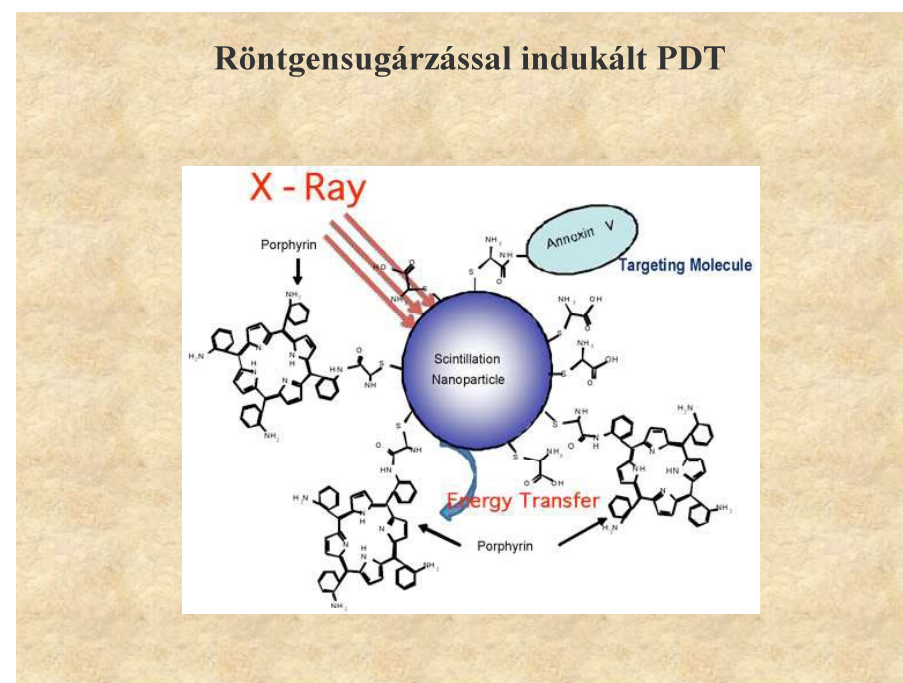
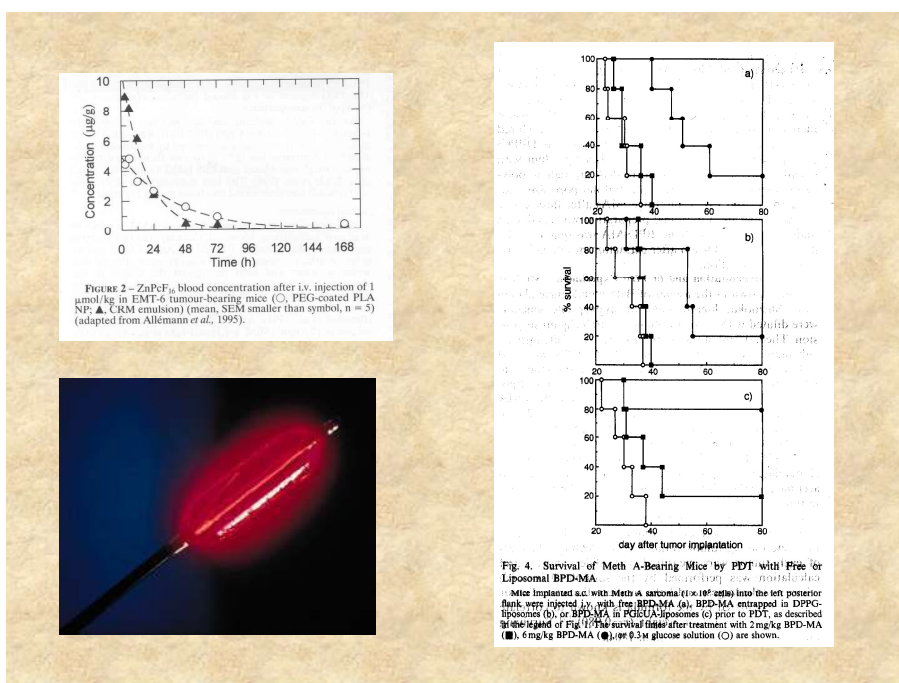
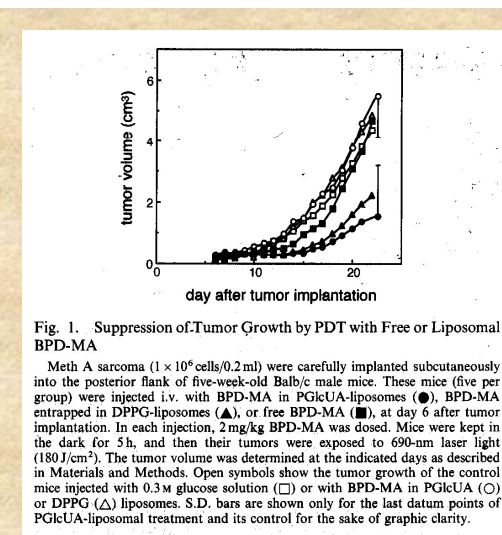
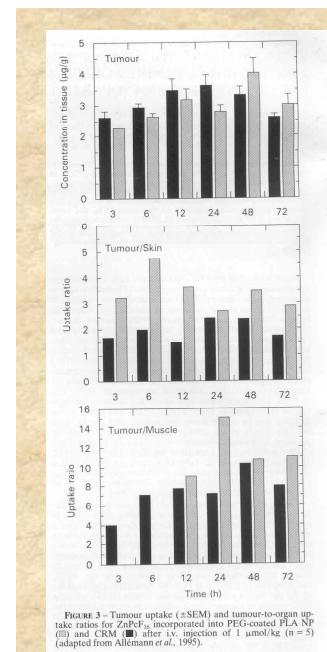
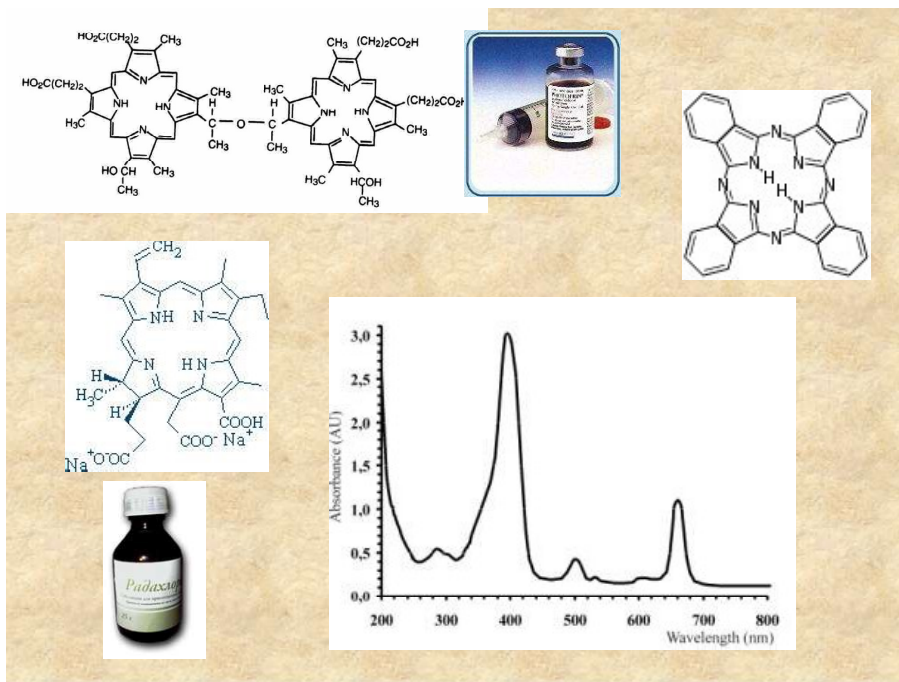
Immunliposzómák: szelektív kötődés a daganatsejthez. Toxin vagy citosztatikum zárható bele. TNF együttes adása segíti a liposzómák átlépését az erekből a tumorba.



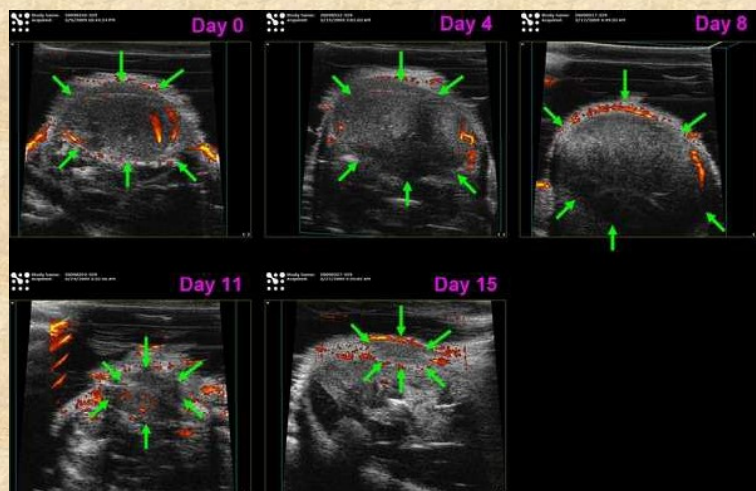
Fotodinámiaiás terápia (PDT)

Fényérzékenyítő anyagot (hematoporfirin, ftalocianin származékok, stb.) juttatnak a tumorsejtekbe. Megvilágítják megfelelő hullámhosszú fényel → 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.





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	-	maligus 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 hisztio plazmózis
Marqibo	vincristin	Talon Therapeutics	-	Philadelphia kromoszóma negatív felnőtt ALL
Lipusu	paclitaxel	Luye Pharma Group	-	petefészekrák, mellrák és nem-kissejtes tüdőrák

Product	Drug	Company	Indication target
Atragen™	Tretinoin	Aronex Pharmaceuticals Inc.	Acute promyelocytic leukemia
Amphotec	Amphotericin B	Sequus Pharmaceuticals Inc.	Fungal infections icshmaniasis
Ambisome™	Amphotericin B	NeXstar Pharmaceuticals Inc.	Serious fungal infections
Amphocil™	Amphotericin B	Sequus Pharmaceuticals Inc.	Serious fungal infections
Abelect™	Amphotericin B	The Liposome Company.	Serious fungal infections
ALEC™	Dry protein free powder of DPPC-PG	Britannia Pharm, UK	Expanding lung diseases in infants
Avian retrovirus vaccine	Killed avian retrovirus	Vineland lab, USA	Chicken pox
DaunoXome™	Daunorubicin citrate	NeXstar Pharmaceuticals Inc., USA	Kaposi sarcoma in AIDS
DepoDur	Morphine	Pacira Pharmaceuticals Inc	Post-surgical pain reliever
Daunoxome	Daunorubicin citrate	Galen Ltd	Kaposi sarcoma in AIDS
Depocyt	Cytarabine	Pacira Pharmaceuticals Inc	Treatment of lymphomatous meningitis
Doxil	Doxorubicin	Sequus Pharmaceuticals Inc.	Kaposi sarcoma in AIDS
Estrasorb	estradiol	Novavax	Menopausal Therapy
Evacet™	Doxorubicin	The liposome company, USA	Metastatic breast cancer
Epaxal -Berna Vaccine	Inactivated hepatitis-A Virions	Swiss serum & vaccine institute, Switzerland	Hepatitis A
Fungizone	Amphotericin B	Bristol-Myers Squibb, Netherland	Serious fungal infections
Mikasome®	Amikacin	NeXstar Pharmaceuticals Inc.	Bacterial infection
Nyotran™	Nystatin	Aronex Pharmaceuticals Inc.	Systemic fungal infections
Topex Br	Terbutaline sulphate	Ozone Pharmaceuticals Ltd.	Asthma
Ventus	Prostaglandin-E1	The liposome company	Systemic inflammatory disease
VincaXome	Vincristine	NeXstar Pharmaceuticals Inc.	Solid Tumors

neighboring tissues. Superparamagnetic liposomes are reported as highly efficient magnetic resonance imaging (MRI) contrast agents. Example, maghemite encapsulated liposomes synthesized from egg PC (phosphatidylcholine) and DSPE-PEG(2000) [127].

New Generation of Liposomes

Liposomal drug delivery has created opportunity to formulate a wide variety of difficult to deliver therapeutic agents. In spite of many products in the market and several others in the

Product Name	API	Approved Year/Area	Dosage Form	Adm. Route	Indication
Doxil Caelyx	Doxorubicin hydrochloride (DOX HCl)	1995, US 1996, EU	Suspension	IV	Ovarian cancer, Kaposi's sarcoma, myeloid melanoma
DaunoXome	Daunorubicin	1996, US	Suspension	IV	Kaposi's sarcoma
AmBisome	Amphotericin B (AmpB)	1997, US	Lyo	IV	Systemic fungal infection
DepoCyt	Cytarabine	1999, US	Suspension	IT	Lymphomatous meningitis
DepoCyt	Cytarabine	2001, EU	Suspension	IT	Lymphomatous meningitis
Myocet	DOX HCl	2000, EU	3 vials	IV	Breast cancer
Visudyne	Verteporfin	2000, US 2000, EU	Lyo	IV	Wet AMD
DepoDur	Morphine	2004, US	Suspension	Epidural	Postoperative pain
Mepact	MTP-PE	2009, EU	Lyo	IV	Osteosarcoma
Exparel	Bupivacaine	2011, US 2020, EU	Suspension	Local infiltration	Post-surgical analgesia
Marqibo	Vincristine Sulfate	2012, US	3 vials	IV	Leukemia
Onivyde	Irinotecan hydrochloride trihydrate	2015, US 2016, EU	Suspension	IV	Pancreatic adenocarcinoma
Vyxeos	Daunorubicin, cytarabine	2017, US 2018, EU	Lyo	IV	Leukemia
Shingrix	Recombinant varicella-zoster virus glycoprotein E	2018, EU	2 vials (powder and suspension)	IM	Against shingles and post-herpetic neuralgia
Arikayce	Amikacin sulfate	2018, US 2020, EU	Suspension	Oral inhalation	Lung disease

4. AIDS

Mind a celluláris (főleg a $CD4^+$ limfociták számának csökkenése miatt) mind a humorális immunválasz csökkent \rightarrow védekezési képtelenség a fertőzésekkel szemben (oportunisták 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 \rightarrow 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- $3'$ -dezoxitimidin (AZT) = zidovudin (ZDV)

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

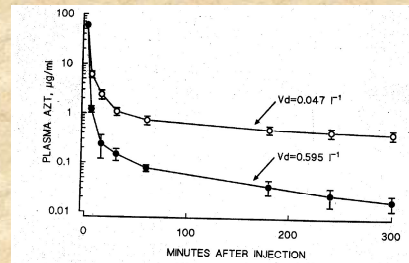
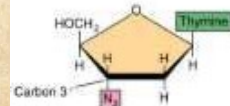


Fig. 7. Plasma AZT levels following the administration of AZT (●) or liposomal AZT (○). Plasma AZT levels (as 3H -methyl-AZT) were determined at 15-300 min following the i.v. administration of a bolus of AZT or liposomal AZT at a dose of 60 μg/mouse (2 mg/kg body weight). Results shown are the mean \pm SD of groups of 5 mice. Volume of distribution (V_d) were calculated using the equation $V_d = A/C$ where $A = 60 \mu g$ 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)	
		DPPC/DMPG	Liposomal AZT DPPC/PS
Liver	0.59 \pm 0.06	3.10 \pm 0.21 ^(b)	2.10 \pm 0.13 ^(bc)
Spleen	0.60 \pm 0.10	2.30 \pm 0.08 ^(b)	2.15 \pm 0.12 ^(b)
Kidney	7.00 \pm 1.45	2.30 \pm 0.41 ^(b)	1.10 \pm 0.08 ^(b)
Lung	0.30 \pm 0.19	1.90 \pm 0.17 ^(b)	4.60 \pm 0.35 ^(bc)
Muscle	0.40 \pm 0.11	0.50 \pm 0.14	0.45 \pm 0.17
Brain	0.15 \pm 0.09	0.18 \pm 0.10	0.16 \pm 0.06
Bone marrow ^(d)	0.02 \pm 0.003	< 0.0001 ^(c)	< 0.0001 ^(c)
Urine	59.9 \pm 4.8	23.8 \pm 3.6 ^(b)	21.9 \pm 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 (0 mg/kg body weight) spiked with 3H -methyl-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 DPPC/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.

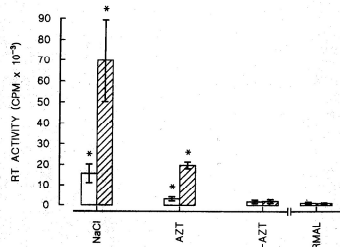


Fig. 8. Effect of treatment with AZT or liposomal AZT on the development of plasma RT activity in LP-BMS-infected C57BL/6 mice.

Groups of 5 LP-BMS-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. Black columns: 2 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 ($\times 10^9$)	RBC ml/blood ($\times 10^9$)
Vehicle control	13.9 \pm 1.6	10.3 \pm 2.1	9.8 \pm 0.7
Liposomes	14.2 \pm 1.1	10.6 \pm 0.9	10.1 \pm 0.7
AZT			
0.08	11.8 \pm 0.9	9.1 \pm 1.7	8.7 \pm 0.6
0.4	9.0 \pm 1.4 ^(a)	7.9 \pm 0.8	7.3 \pm 0.4 ^(a)
2.0	6.6 \pm 1.8 ^(b)	6.1 \pm 0.9 ^(b)	6.3 \pm 0.4 ^(a)
10.0	6.3 \pm 1.3 ^(b)	5.1 \pm 1.6	6.0 \pm 0.6 ^(a)
50.0	5.9 \pm 0.8 ^(b)	5.2 \pm 1.9	5.4 \pm 0.7 ^(a)
Liposomal AZT			
0.08	14.0 \pm 2.1	10.0 \pm 1.3	9.6 \pm 0.8
0.4	13.6 \pm 1.6	10.4 \pm 1.3	9.8 \pm 0.5
2.0	13.3 \pm 1.8	10.6 \pm 0.9	9.7 \pm 0.8
10.0	13.9 \pm 1.2	9.8 \pm 1.3	9.2 \pm 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 3 treatments. The results shown are the mean \pm SD.

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

^(b) Significantly different from control treatment ($P < 0.001$).

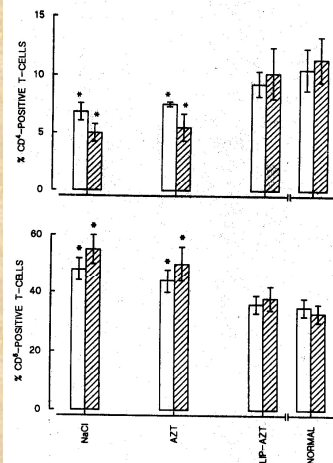


Fig. 9. Effect of treatment with AZT or liposomal AZT on splenic $CD4^+$ and $CD8^+$ T-cell populations.

Groups of 5 LP-BMS-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

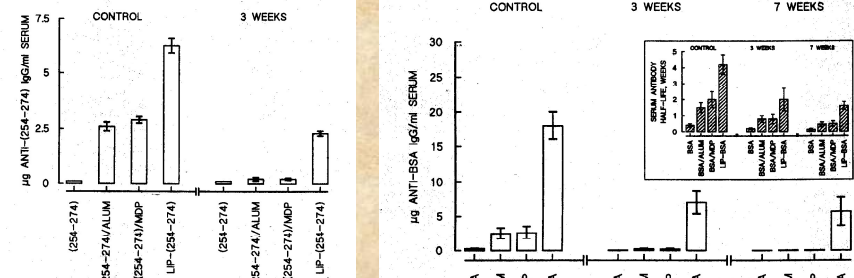


Fig. 4. Immunoadjuvant activity against peptide (254-274) in normal mice and in mice with MAIDS.

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	38–46
rHBsAg	0.20	16	31–33
LV39	0.20	16	74–82
Interleukin-2	Up to 10 ⁶ units	16	00–70
Poliovirus 1-VP2 peptide	0.22	16	74–82
Poliovirus-VP2 peptide	0.22	16	62–68
rHBsAg S peptide	1.00	32	42–40
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).

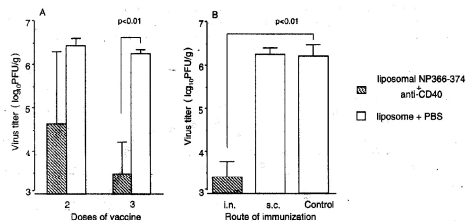


Fig. 1. Protective effects of two or three immunizations with liposomal NP366-374 together with anti-CD40 mAb (A). Three to four mice in each group were immunized intranasally twice or three times at 2-week intervals. Subcutaneous immunization provided no effective immunity against virus replication 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 (H2N2) 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.

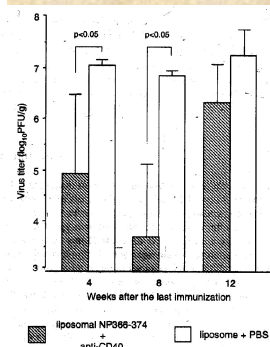


Fig. 2. Duration of the protective effect of immunization with liposomal NP366-374 together with anti-CD40 mAb. Three to four mice in each group were immunized intranasally three times at 2-week intervals. Mice were challenged with A/Aichi/2/68 (H2N2) 4, 8, or 12 weeks after the last immunization. Five days later, lungs of mice were collected to calculate virus titers. Bars and error bars represent means and standard deviations for each group.

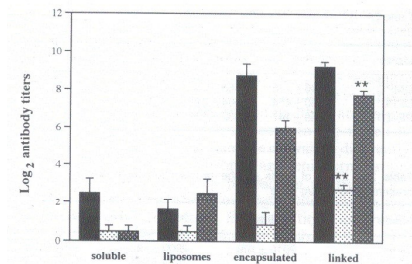


Figure 1 Groups of five mice were injected intraperitoneally with empty liposomes (1 μmol) or with 100 μg of either free, encapsulated or surface-linked cAlb. Blood was collected on the 12th day following immunization and specific anti-cAlb total Ig, IgM and IgG were measured on serum samples. The results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

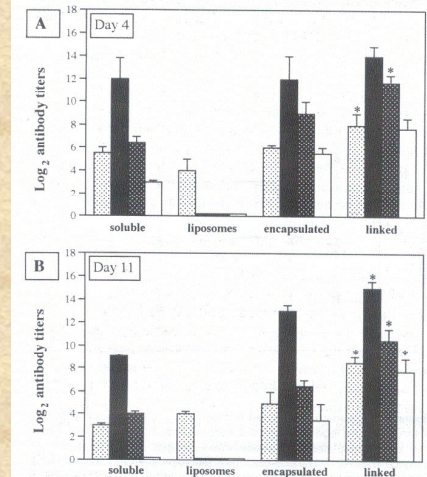
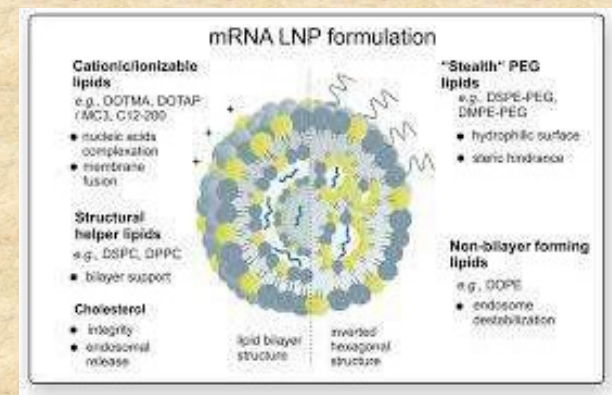


Figure 2 Mice were injected with empty liposomes (1 μmol) or 20 μg of either free, encapsulated or surface-linked cAlb and rechallenged 28 days later with the same antigenic formulations. The production of specific serum anti-cAlb IgM, IgG1, IgG2a and IgG3 was measured on the 4th day (A) or the 11th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

Pfizer-BioNTech, illetve Moderna COVID-19 vakcina

mRNA lipid nanoparticulumban



Lipid Name	Role	Abbreviation or Lab Code	CAS Registry Number
BNT162b2 vaccine (Pfizer/BioNTech)			
(4-hydroxybutyl)azanediyl bis(hexane-6,1-diyl)bis(2-hexyldecanoate)	ionizable cationic lipid	ALC-0315	2036272-55-4
(2-hexyldecanoate), 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide	PEG-lipid	ALC-0159	1849616-42-7
1,2-distearoyl- <i>sn</i> -glycero-3-phosphocholine	helper lipid	DSPC	816-94-4
cholesterol	helper lipid	Chol	57-88-5
mRNA-1273 vaccine (Moderna)			
heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate	ionizable cationic lipid	SM-102	2089251-47-6
1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000	PEG-lipid	PEG2000-DMG	160743-62-4
1,2-distearoyl- <i>sn</i> -glycero-3-phosphocholine	helper lipid	DSPC	816-94-4
cholesterol			

Az ionizálható kationos lipid alacsony pH-n pozitív töltésű, így könnyen képez komplexet az mRNS-sel. A vér pH-ján semleges, így csekély a toxicitása.

A lipid nanopartikulumokat alkotó lipidek

Cationic Lipids

ALC-0315
BNT162b2 (Pfizer / BioNTech)

SM-102
mRNA-1273 (Moderna)

PEG-Lipids

ALC-0159
BNT162b2 (Pfizer / BioNTech)

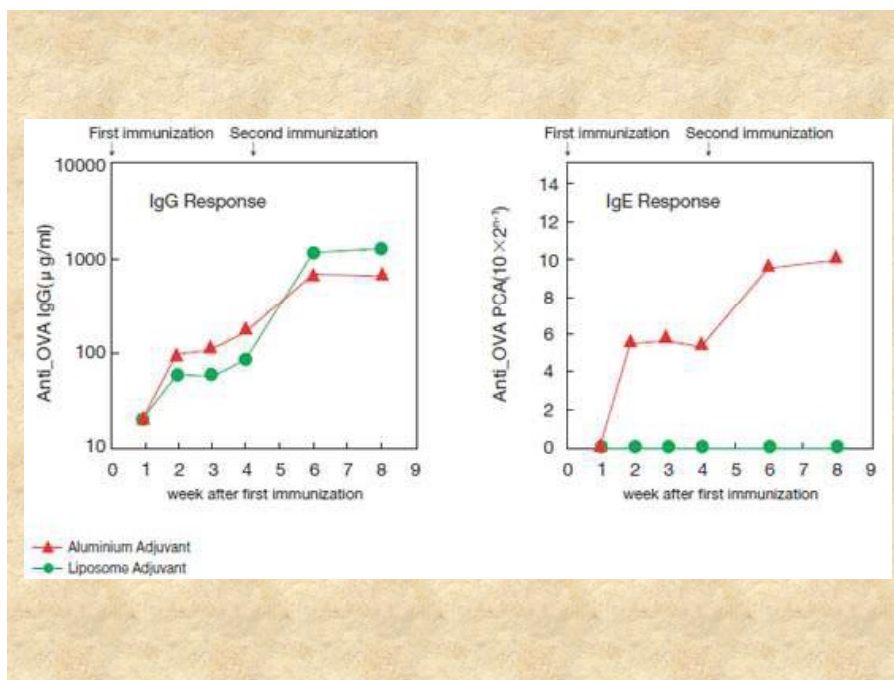
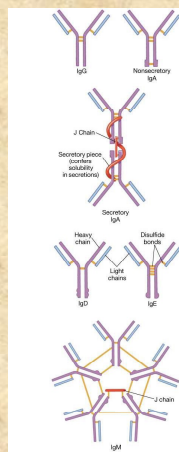
DMG-PEG 2000
mRNA-1273 (Moderna)

DSPC

Cholesterol

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.

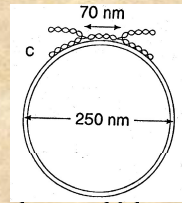
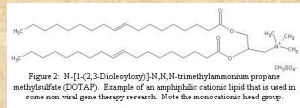


7. Génátvitel

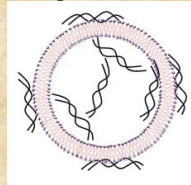
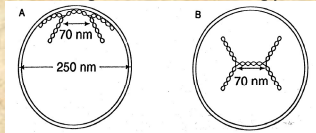
Megvalósítható DNS-darab bezárása, vagy a liposzóma felszínén való megkötése útján (főleg pozitív töltésű liposzómák esetén)

A kötődés lehet:

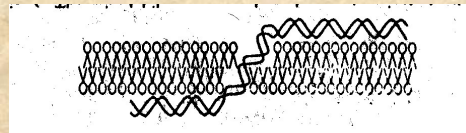
- elektrosztatikus kapcsolódás a külső felszínhez



- a liposzóma üregébe zárva, vagy a belső felszínhez kapcsolódva



- részben belül, részben kívül

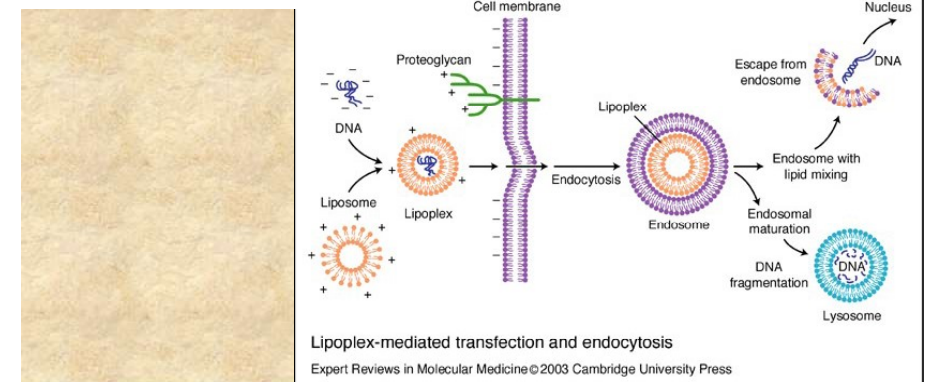
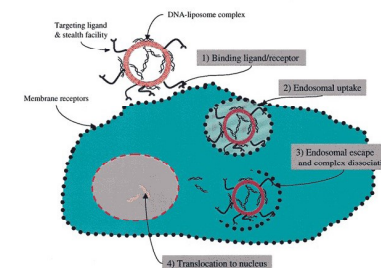
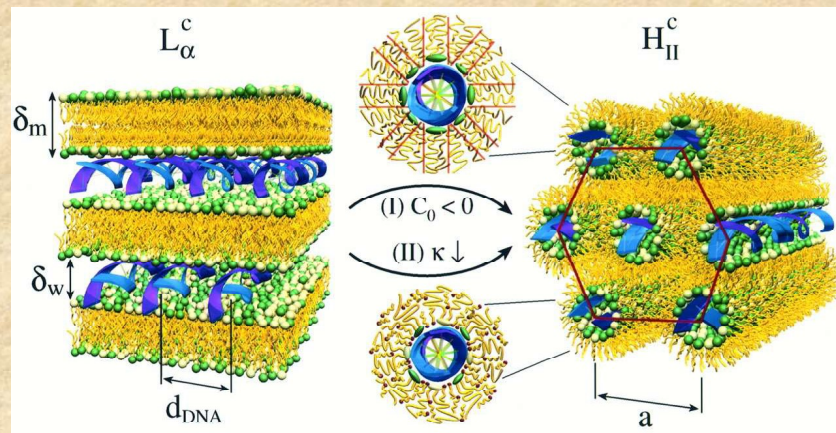
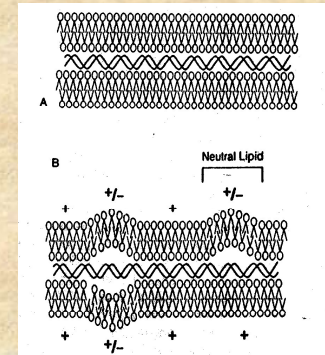
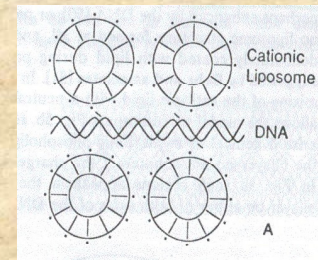


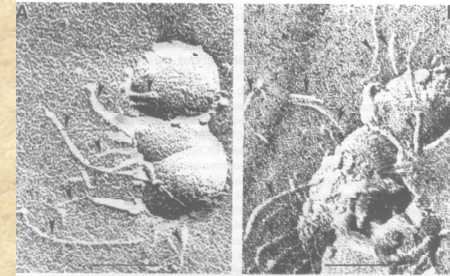
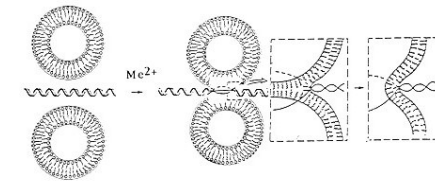
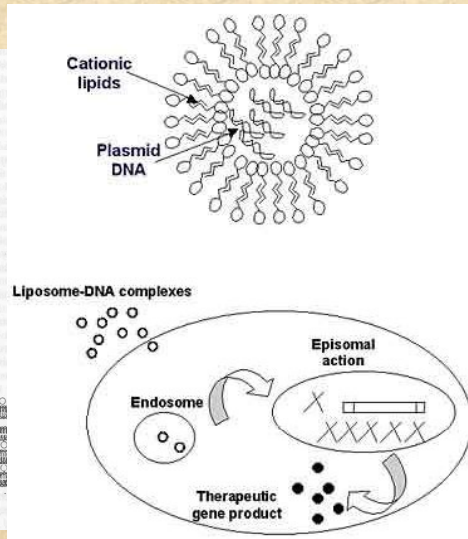
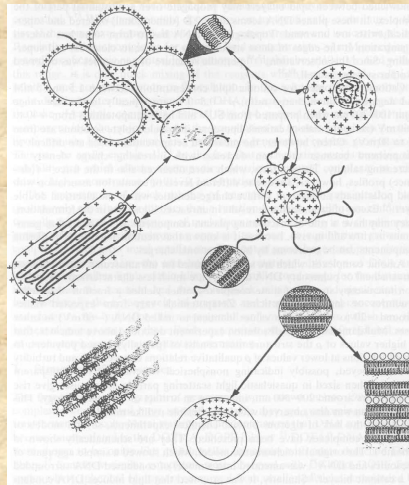
- fúzió során két bilayer közé kerülhet a DNS

A sejtbe juttatáshoz legcélszerűbb a liposzóma és a sejtmembrán fúziója.

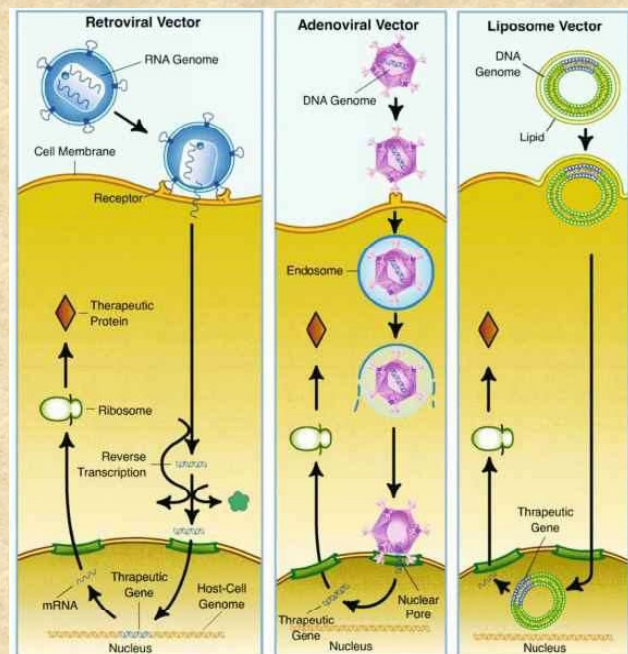
A liposzóma felszíni markereitől függően más-más sejtek vehetik fel, pl. galaktóz – májsejtek; mannóz – fehérvérsejtek

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	+	+	-	-



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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	<ul style="list-style-type: none"> Immunogenicity, presence of contaminants & safety Vector restricted size limitation for recombinant gene
Non viral vectors (liposomes/lipid based systems, polymers & peptides)	<ul style="list-style-type: none"> Favorable, pharmaceutical issue-GMP, stability, cost Plasmid independent structure Low immunogenicity Opportunity for chemical/physical manipulation 	<ul style="list-style-type: none"> low transfection efficiency

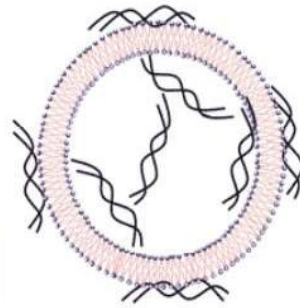
12/12/2015

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