NANOMEDICAMENTS POUR LE TRAITEMENT DES MALADIES INFECTIEUSES

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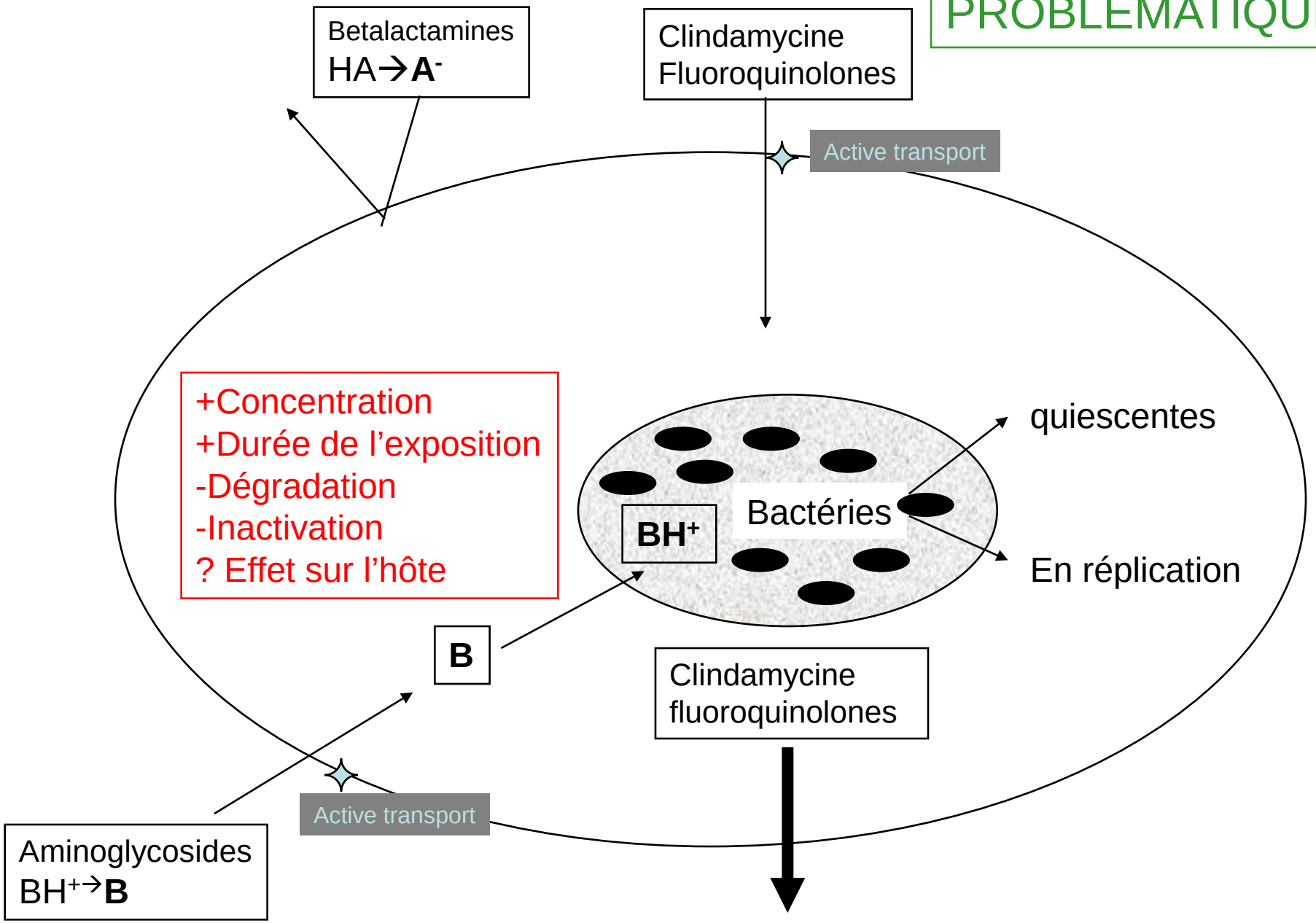
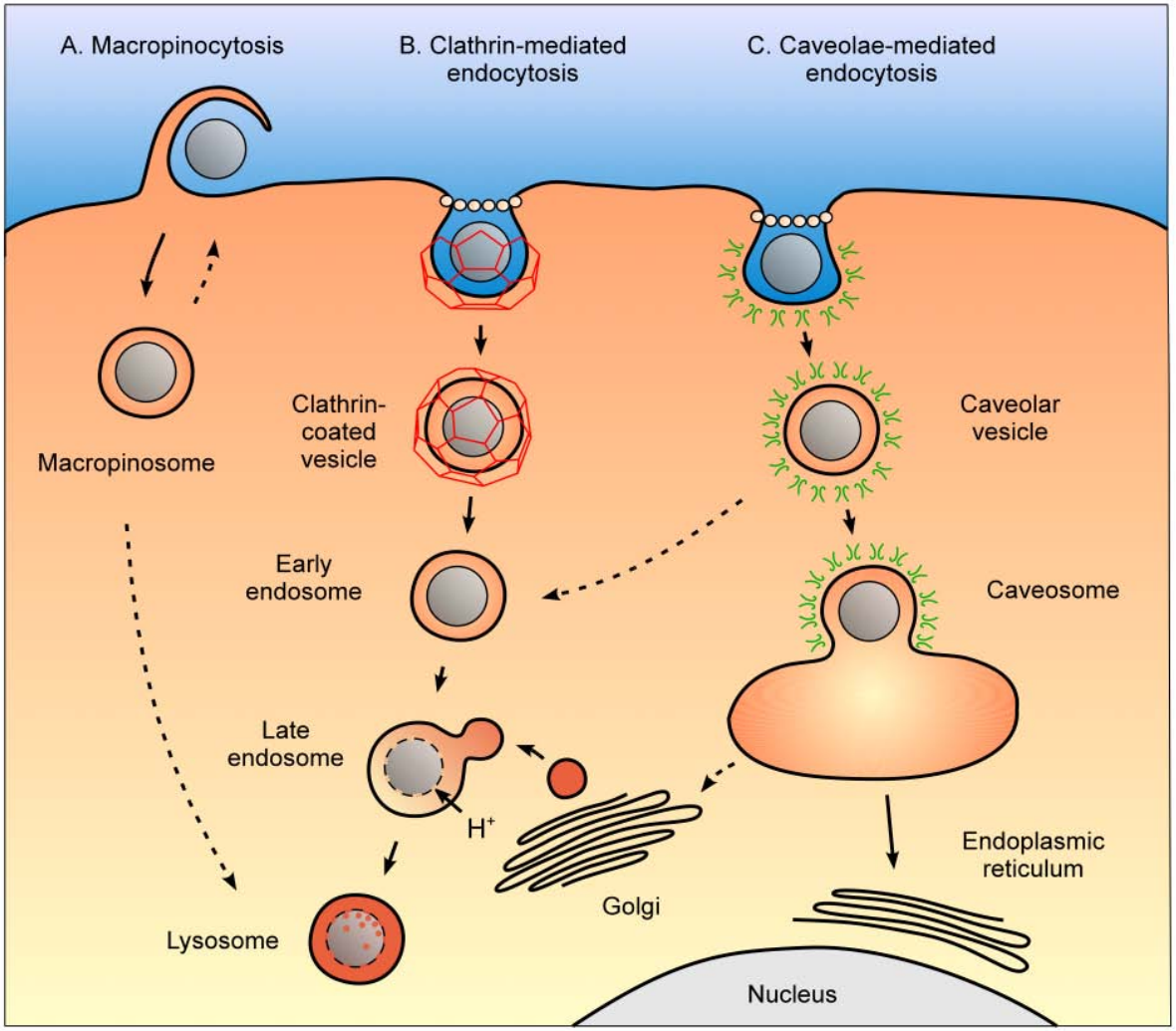
Chaire d'innovation Technologique 2009-2010

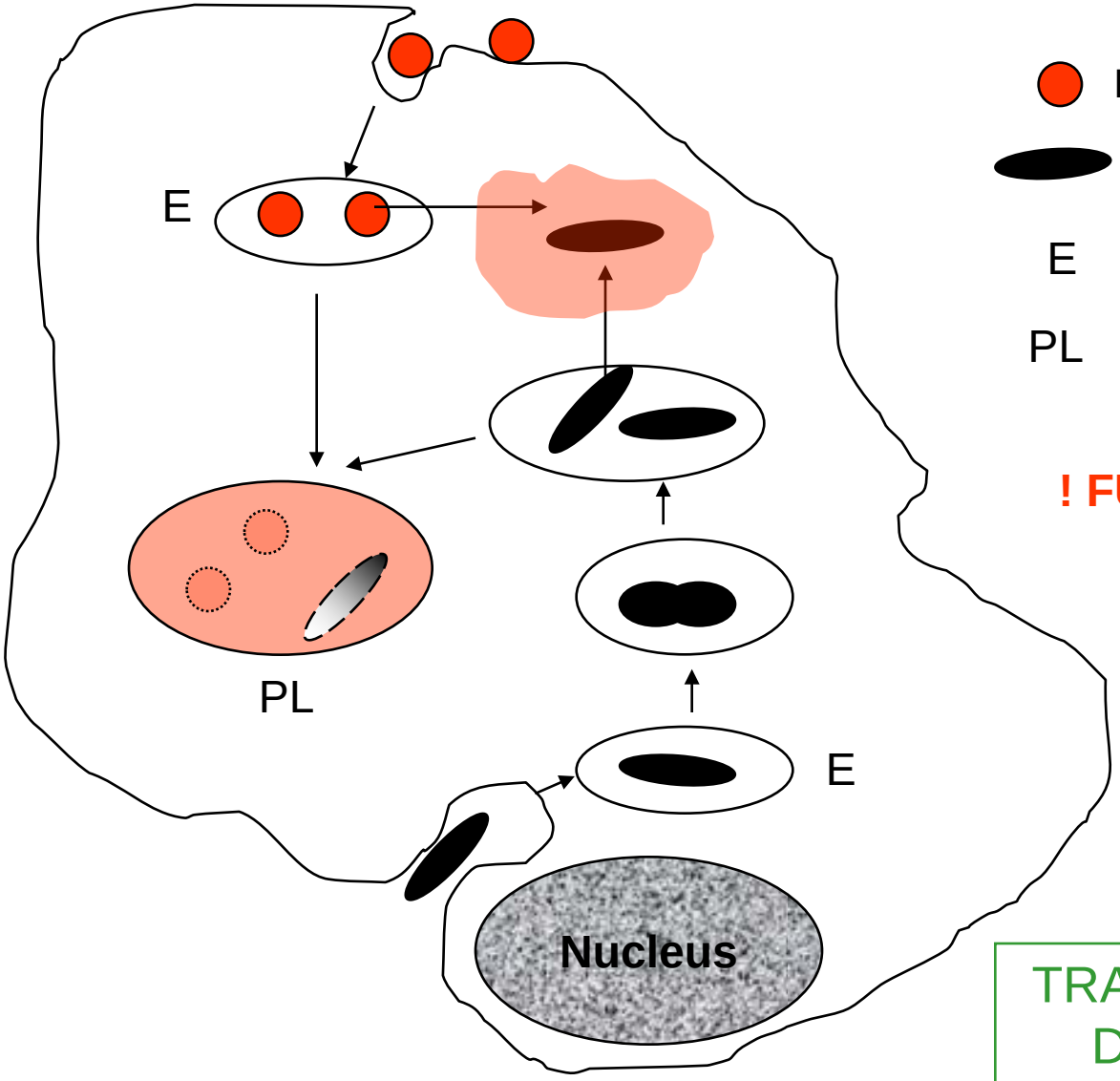
PROBLEMATIQUE



TRAFIC INTRACELLULAIRE DES NANOVECTEURS

H. Hillaireau and P. Couvreur, CMLS, 2009





Liposome or nanoparticle

Intracellular bacterium

E Endosome

PL Phagolysosome

! FUSION PROCESSES ARE NEEDED !

TRAFIC INTRACELLULAIRE DES NANOVECTEURS VERSUS LES MICROORGANISMES

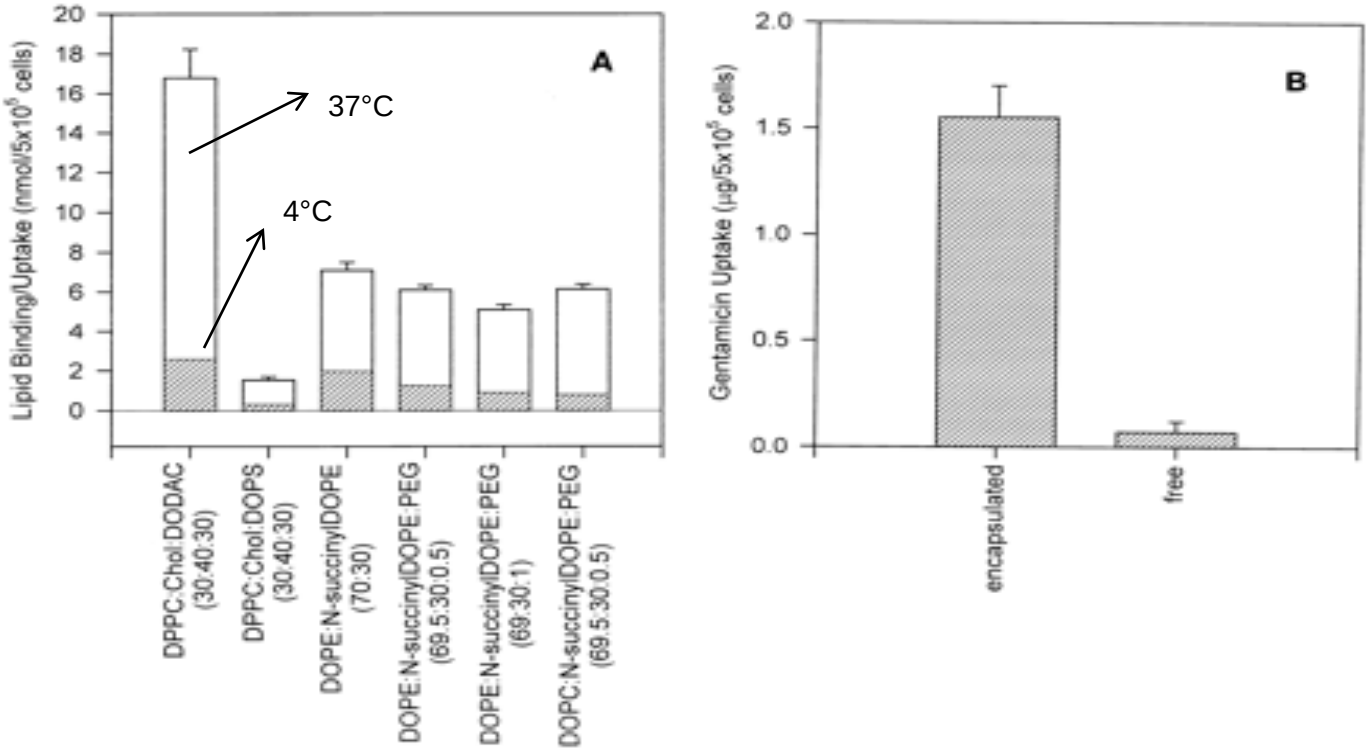
INTRACELLULAR INFECTIONS BY BACTERIA

CELL FEATURES

J774 CELL UPTAKE OF GENTAMICINE LIPOSOMES

P; Lutwyche et al, Antimicrobial Agents and Chemotherapy, 42, 2511-2520 (1998)

Cell membrane is relatively impermeable to gentamicine



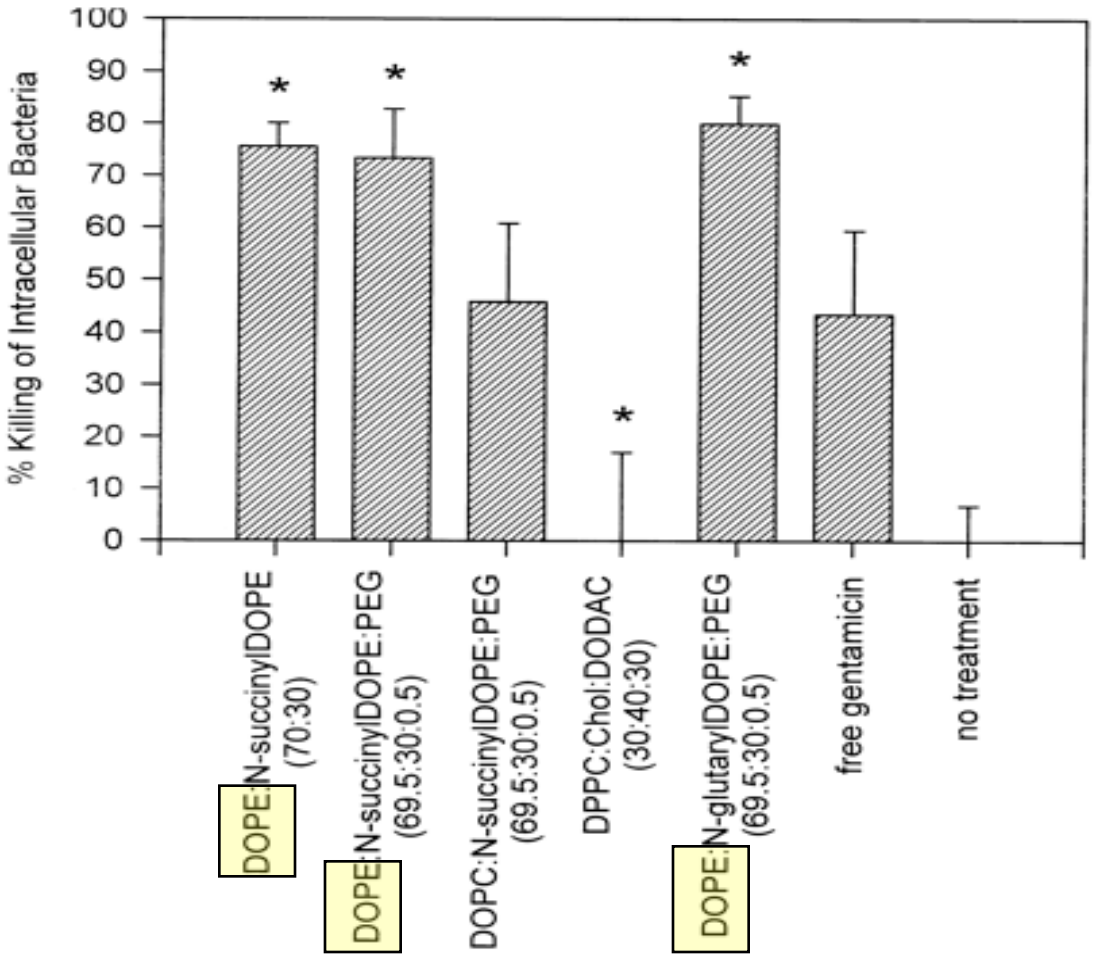
[A] J774 capture of different liposomal formulations

[B] J774 capture of gentamicine

DOPE are pH-sensitive formulations DDAC is a + charged formulation

KILLING INTRACELLULAR S.typhimurium BY GENTAMICINE LIPOSOMES

P; Lutwyche et al, Antimicrobial Agents and Chemotherapy, 42, 2511-2520 (1998)

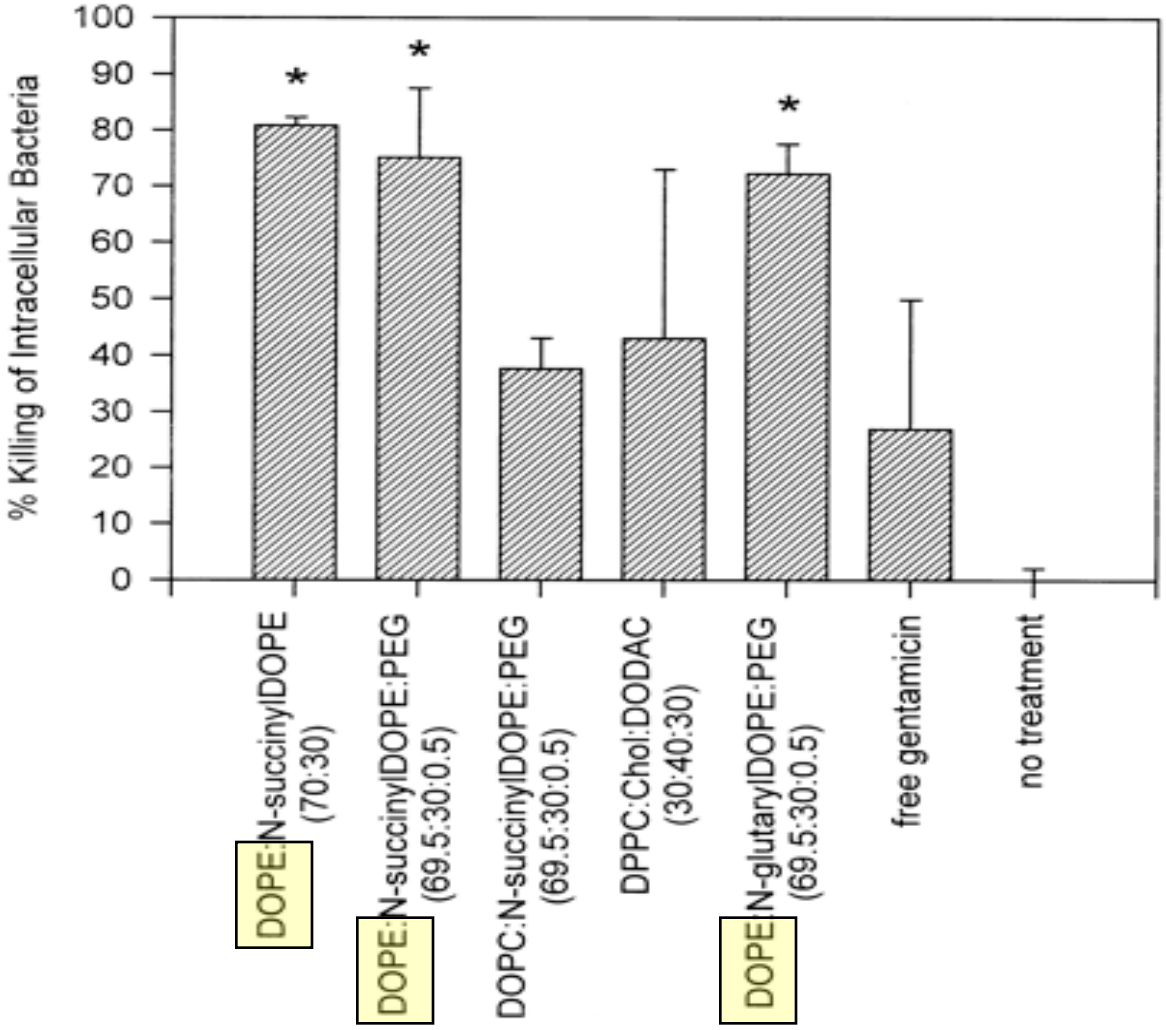


Gentamicine conc.: 150 microgr/ml

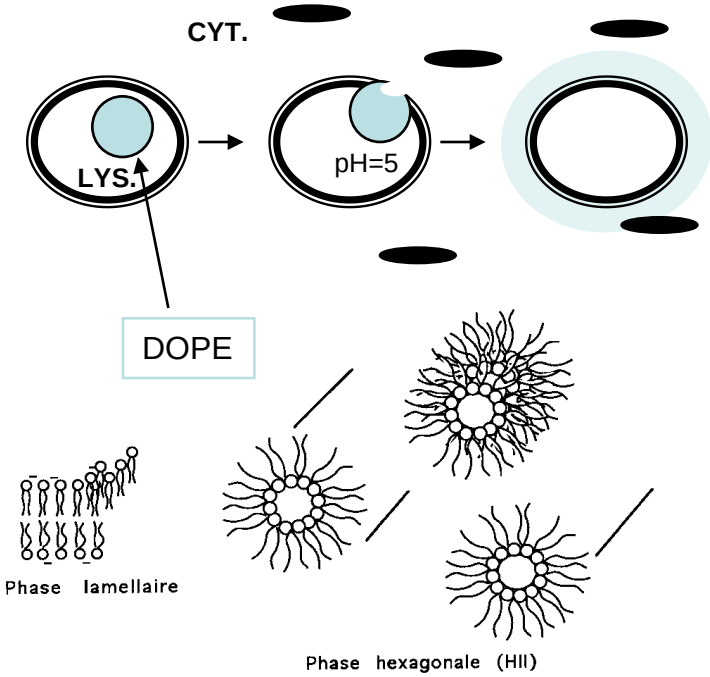
DOPE pH sensitive liposomes are the more efficient

KILLING INTRACELLULAR RECOMBINANT HEMOLYSIN S.typhimurium BY GENTAMICINE LIPOSOMES

P; Lutwyche et al, Antimicrobial Agents and Chemotherapy, 42, 2511-2520 (1998)

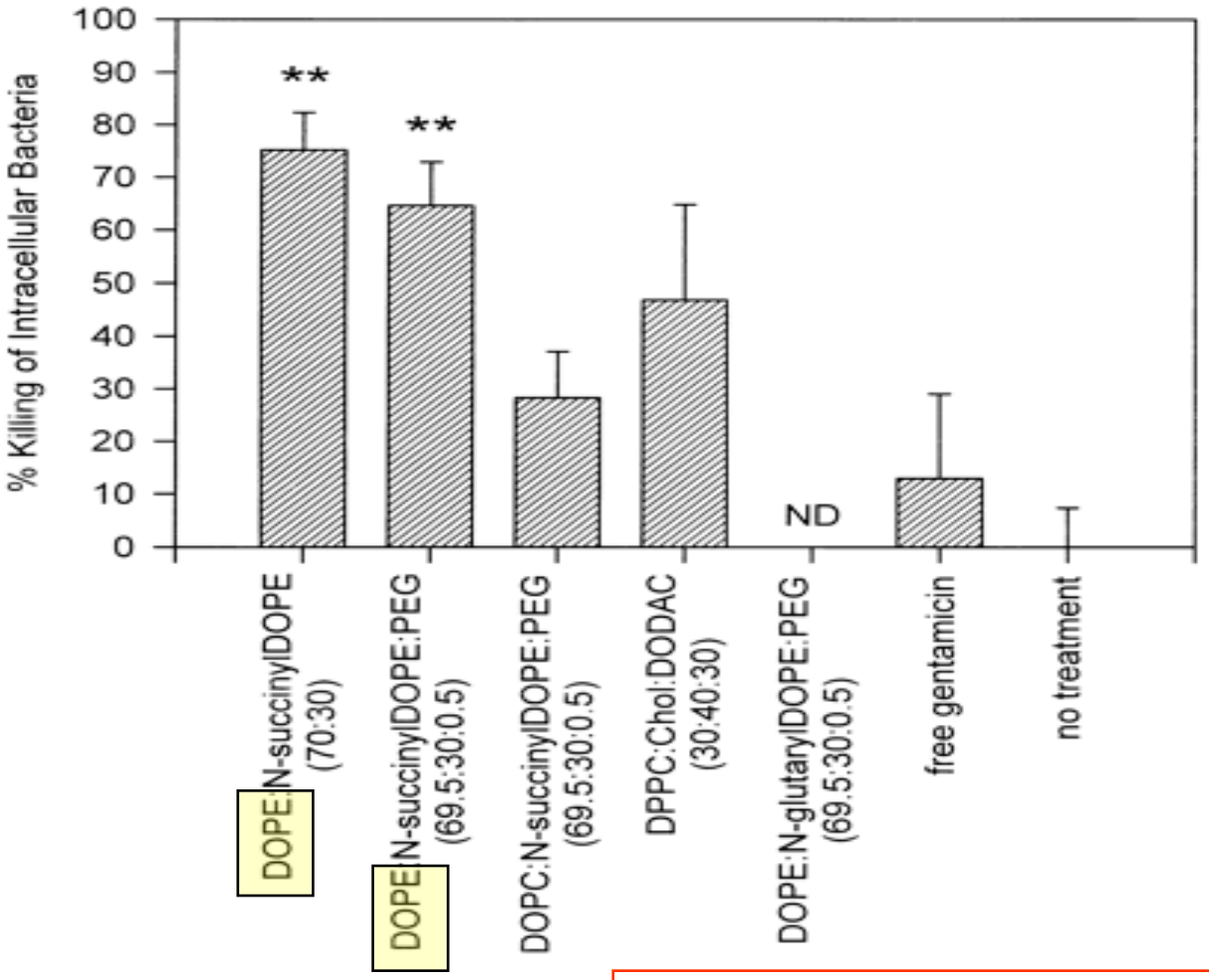


S. Typhimurium wild type → lysosomal localization
S. Typhimurium recombinant hemolysin →cytoplasmic localization (escape from lysosomes)



KILLING INTRACELLULAR L.monocytogenes BY GENTAMICINE LIPOSOMES

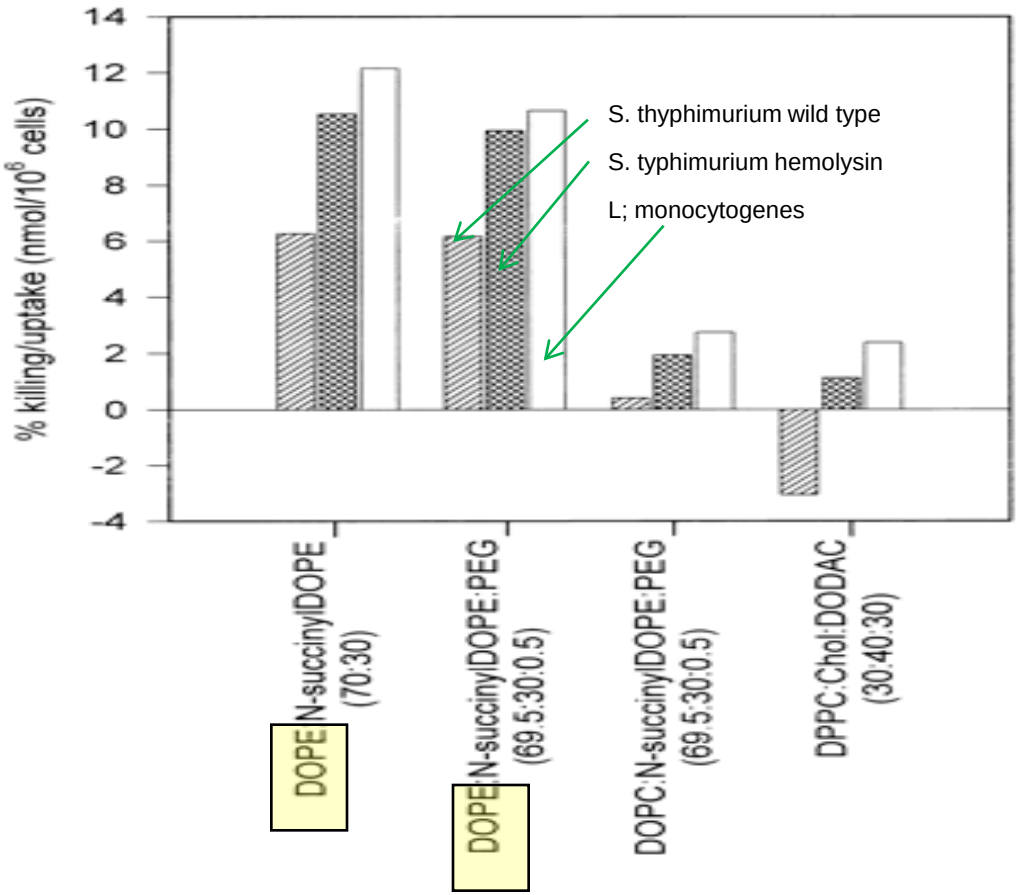
P; Lutwyche et al, Antimicrobial Agents and Chemotherapy, 42, 2511-2520 (1998)



L. Monocytogenes resides in cytosol

COMPARISON OF INTRACELLULAR KILLING BY GENTAMICINE LIPOSOMES OF S. typhimurium WILD TYPE and HEMOLYSIN AND L.monocytogenes

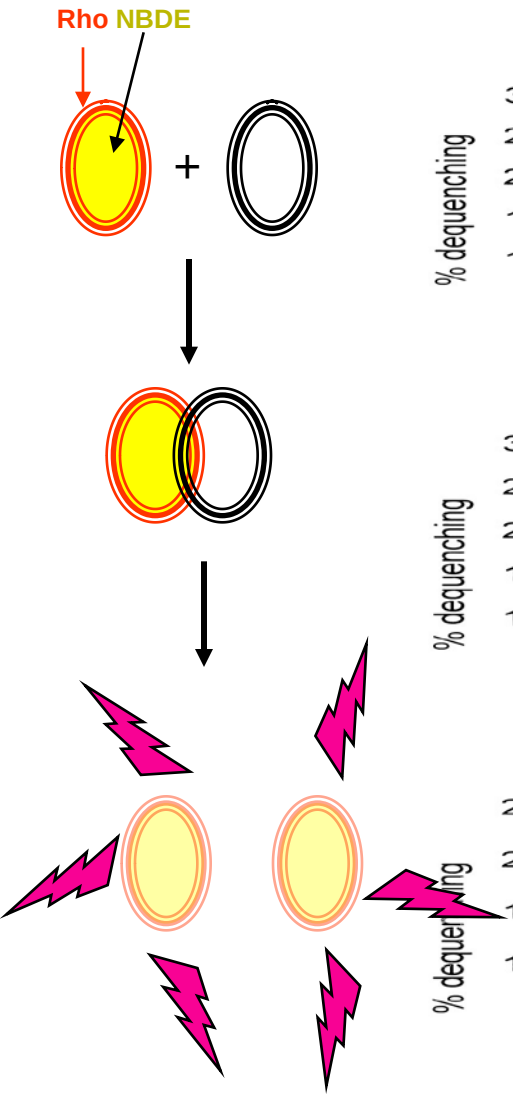
P. Lutwyche et al, Antimicrobial Agents and Chemotherapy, 42, 2511-2520 (1998)

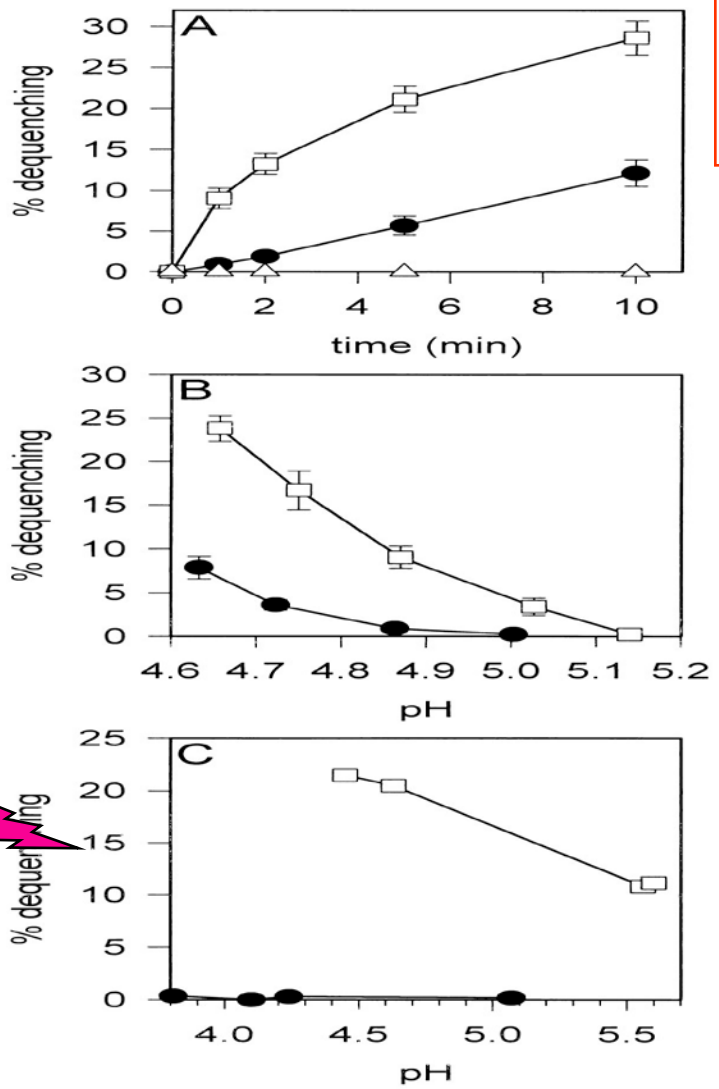


- Corrected killing values were obtained by subtraction of the mean killing observed for free gentamicin from that for the encapsulated gentamicin and dividing by the nanomoles of lipid taken up per 106 cells

LIPID MIXING OF GENTAMICINE LOADED LIPOSOMES

P; Lutwyche et al, Antimicrobial Agents and Chemotherapy, 42, 2511-2520 (1998)





NBDE and Rhodamine are quenching one another and are incorporated in liposomal formulations. Then, in the presence of unlabelled liposomes, dequenching occurs if fusion between liposomes occurs (due to DOPE)

- Lipid mixing activities of gentamicin-containing DOPE-*N*-succinyl-DOPE-PEG (69.5:30:0.5) (open squares), DOPC-*N*-succinyl-DOPE-PEG (69.5:30:0.5) (closed circles), and DPPC-Chol (55:45) (open triangles) were monitored by a resonance energy transfer fluorescence dequenching assay

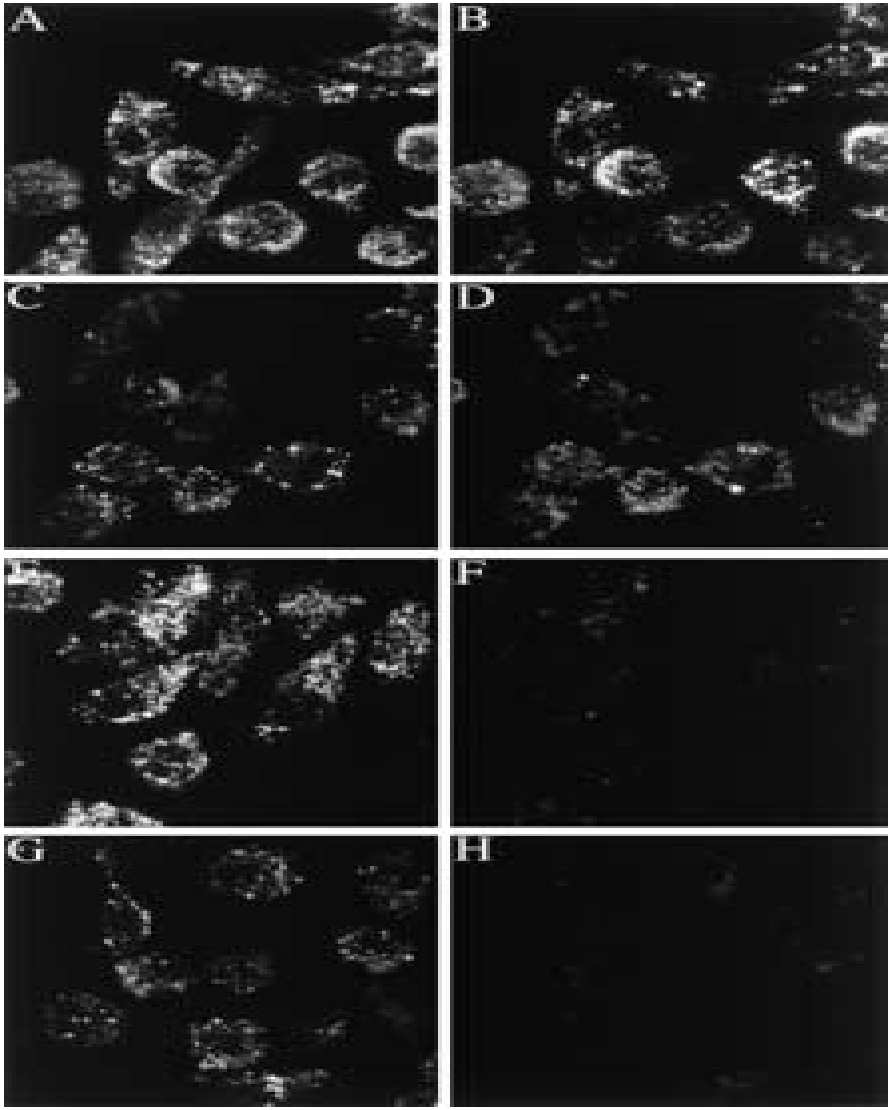
(A) Time course of mixing at pH 4.86 (for the DOPC-containing formulation) and 4.87 (for the DOPE- and DPPC-containing formulations).

(B) pH dependence of lipid mixing after 1 min of incubation.

C) pH dependence of lipid mixing after 1 min in serum-free medium containing 20 mM Ca2+.

INTRACELLULAR CAPTURE OF DIFFERENT LIPOSOMAL FORMULATIONS

P. Lutwyche et al, Antimicrobial Agents and Chemotherapy, 42, 2511-2520 (1998)



- (A) **DOPE-containing formulation** + **Texas red ovalbumin fluorescence showing endocytic pathway** (B) DOPE-containing formulation, 5-sulfofluorescein (SFDA) fluorescence
- (C and D) as for panels A and B, respectively, but in the presence of **bafilomycin, an inhibitor of lysosomal acidification**
- (E) **DOPC-containing formulation** + **Texas red ovalbumin fluorescence**; (F) DOPC-containing formulation, 5-SFDA fluorescence
- (G and H) as for panels E and F, respectively, but in the presence of **bafilomycin**.

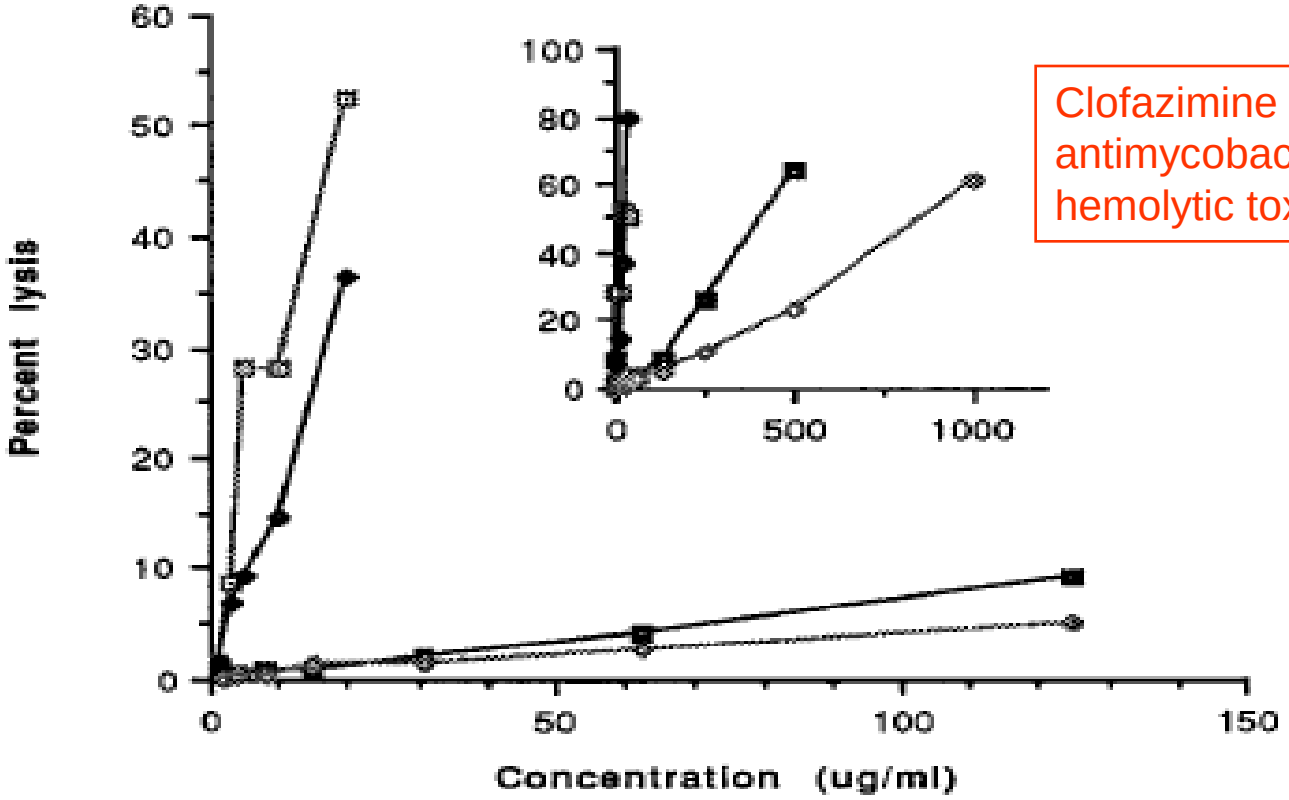
J774 capture of DOPE-lip is >>> DOPC-lip but inhibited by bafilomycin which inhibit acidification of endosomes needed for lipid mixing

INFECTIONS BACTERIENNES INTRACELLULAIRES

VOIE INTRAVEINEUSE

RED BLOOD CELL HEMOLYSIS OF FREE AND LIPOSOMAL CLOFAZIMINE

R. T. Mehta, Antimicrobial Agents and Chemotherapy , 40, 1893-1902 (1996)



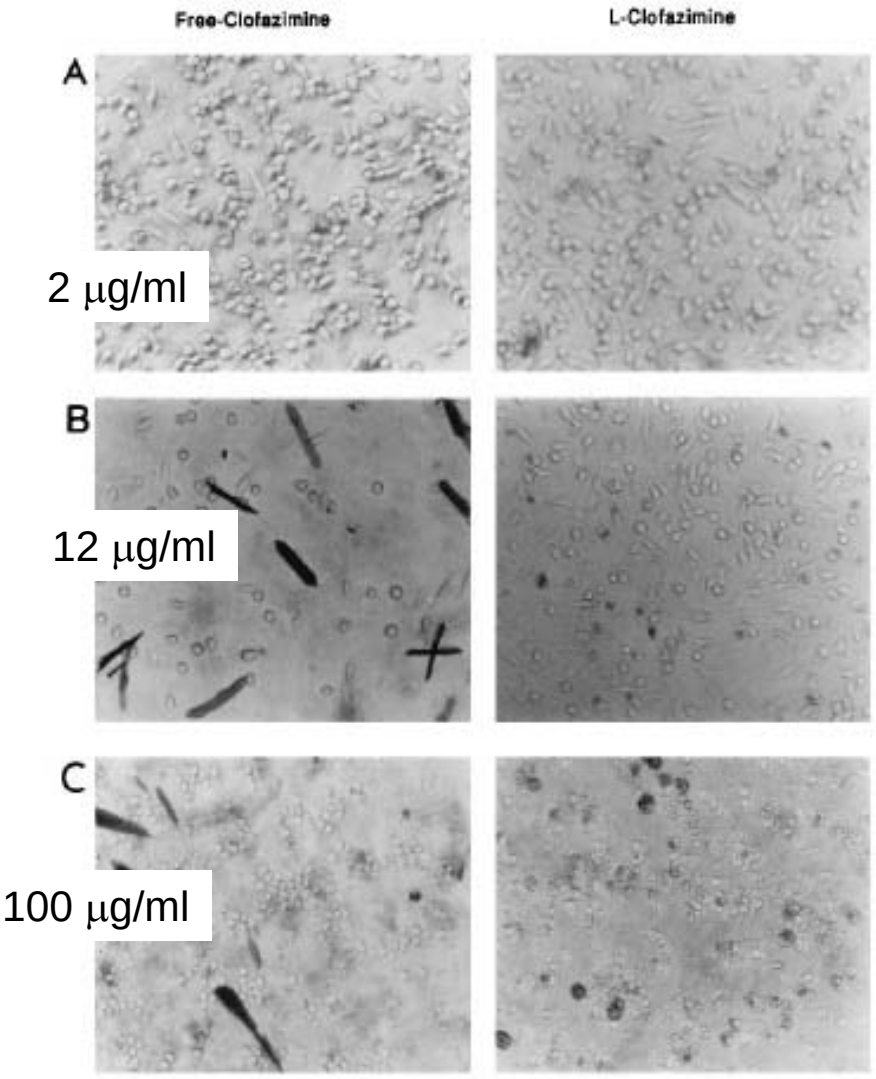
Clofazimine is an antimycobacterial drug with hemolytic toxicity

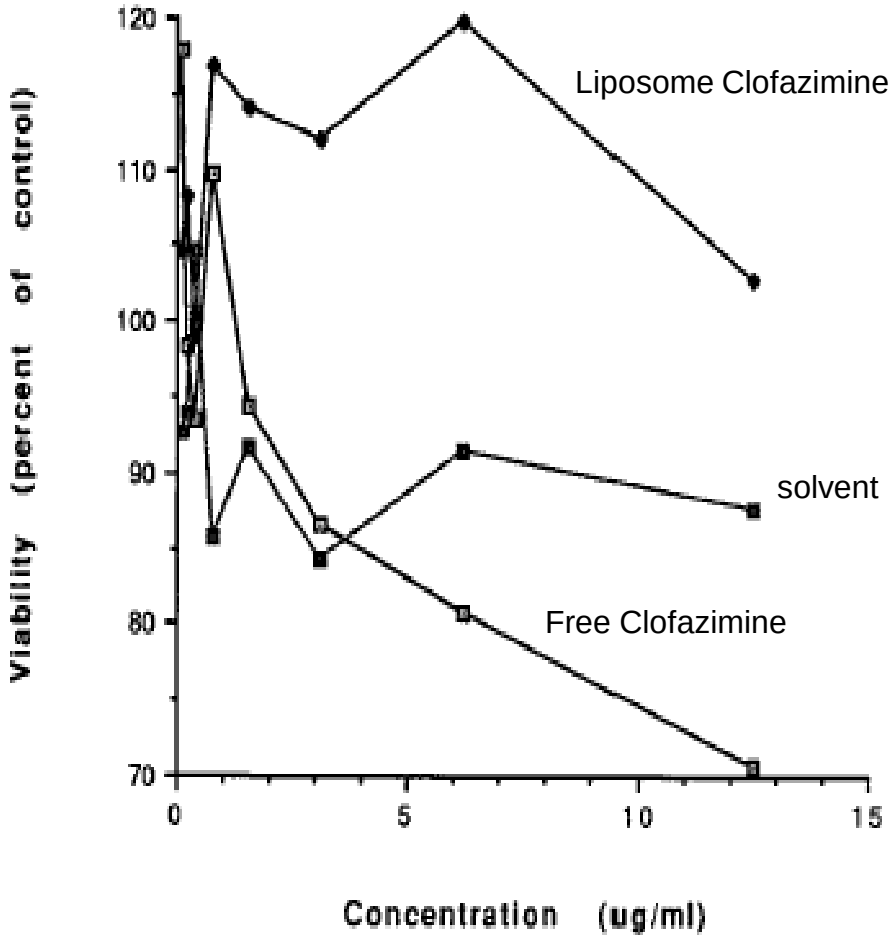
FIG. 1. In vitro toxicities of free and liposomal clofazimine to RBCs. Human RBCs were incubated with various concentrations of free and liposomal drug at 37°C for 45 min, and the hemoglobin that was released was measured at 550 nm. ⊡, free clofazimine (DMSO); ◆, free clofazimine (dimethyl formamide ([DMFA]); ■, liposomal clofazime 1; ◆, liposomal clofazimine 2.

Clofazimine is active against mycobacterium and leprae

MORPHOLOGY OF MACROPHAGES TREATED WITH FREE OR LIPOSOMAL CLOFAZIMINE

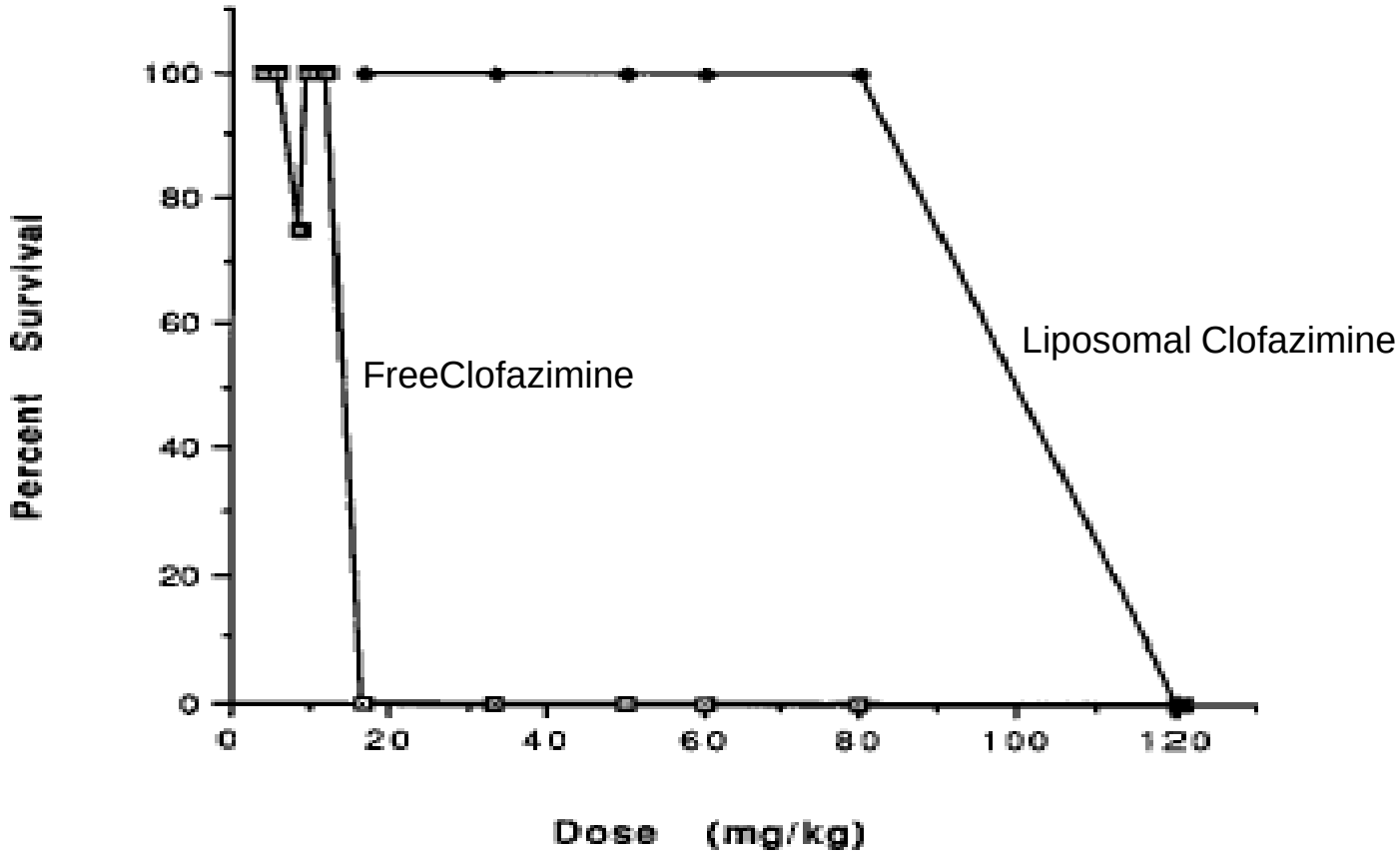
R. T. Mehta, Antimicrobial Agents and Chemotherapy , 40, 1893-1902 (1996)





IN VIVO TOXICITY OF FREE AND LIPOSOMAL CLOFAZIMINE

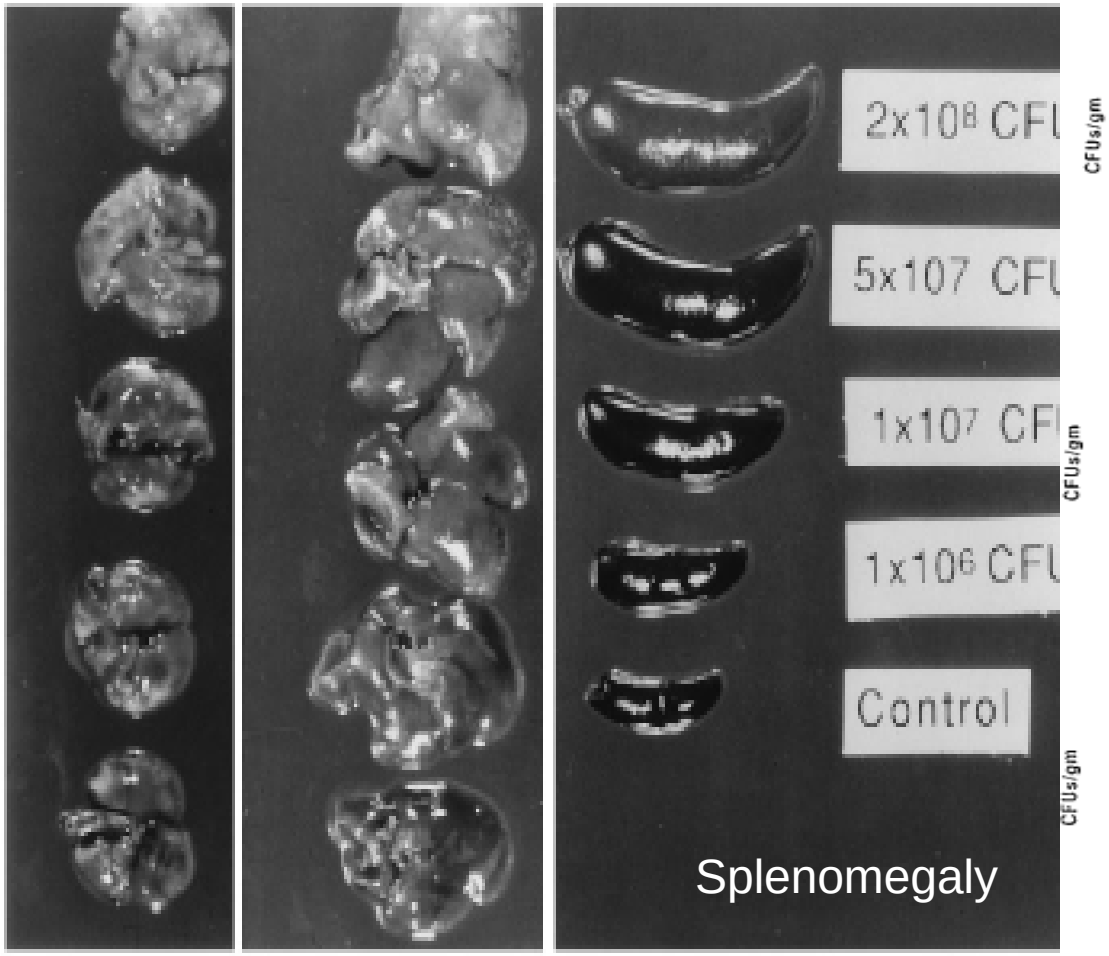
R. T. Mehta, Antimicrobial Agents and Chemotherapy , 40, 1893-1902 (1996)

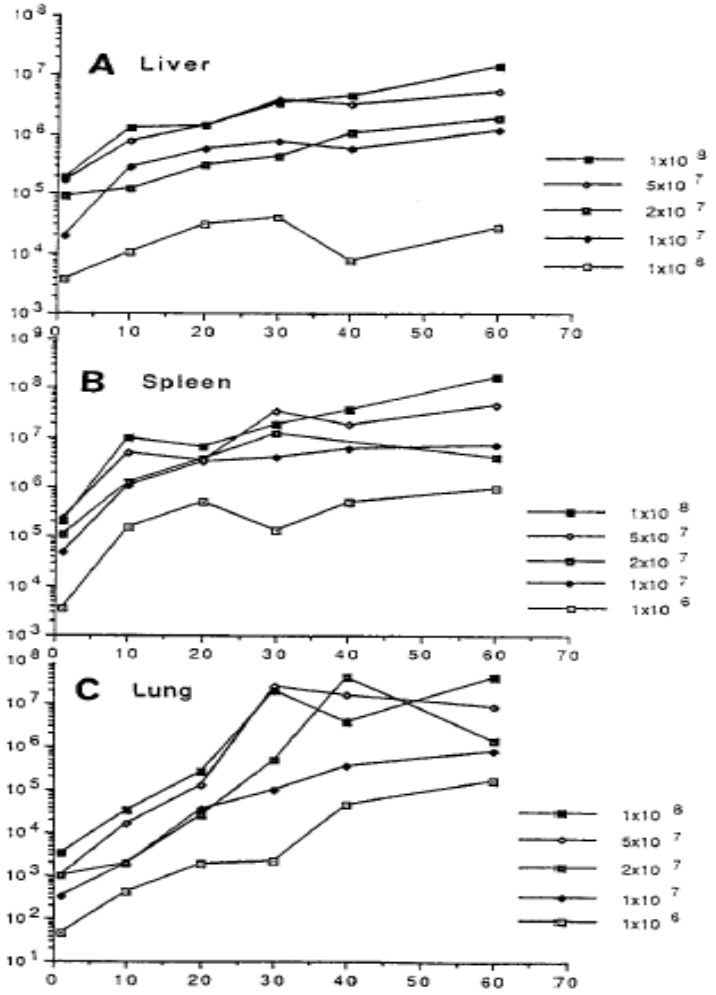


MORPHOLOGY AND BACTERIAL COUNTS OF ORGANS OF MICE INFECTED BY IV INJECTION OF VARIOUS LOADS OF MYCOBACTERIUM AVIUM

R. T. Mehta, Antimicrobial Agents and Chemotherapy , 40, 1893-1902 (1996)

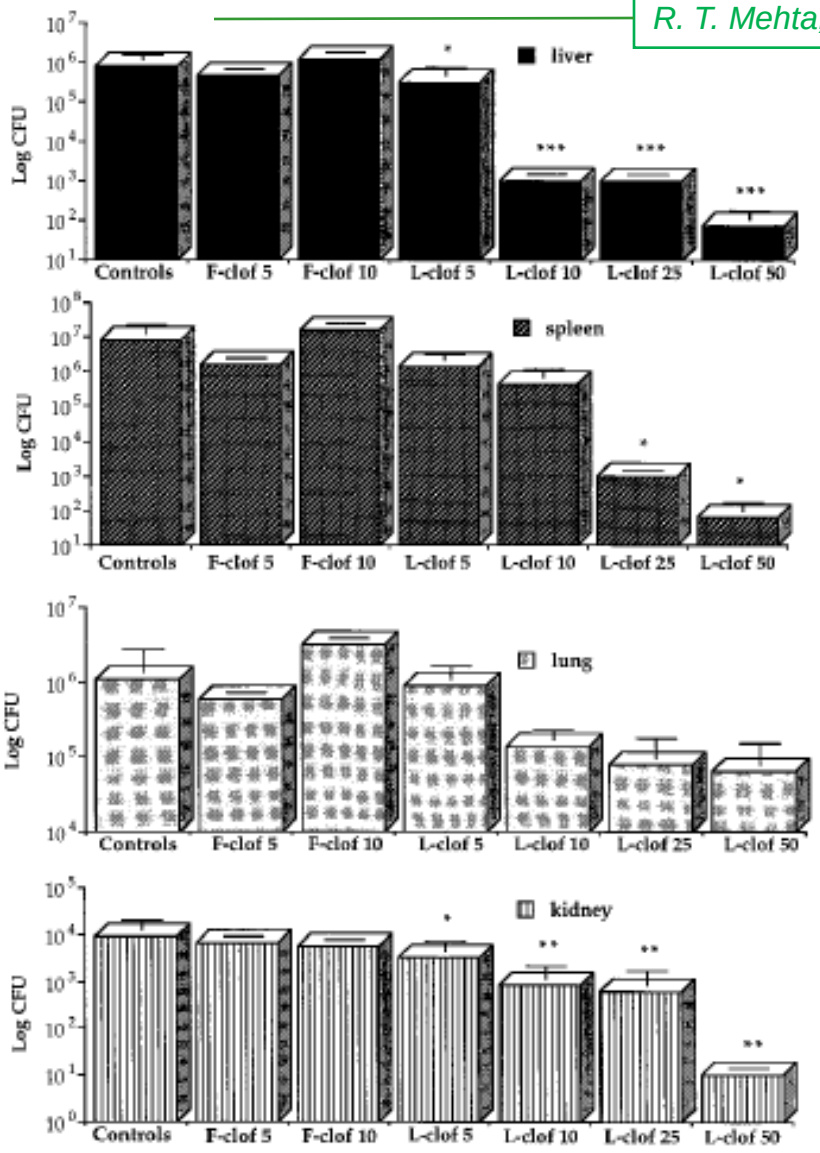
Lungs liver spleen





IN VIVO EFFICACY OF FREE AND LIPOSOMAL CLOFAZIMINE ON MICE INFECTED WITH MYCOBACTERIUM AVIUM

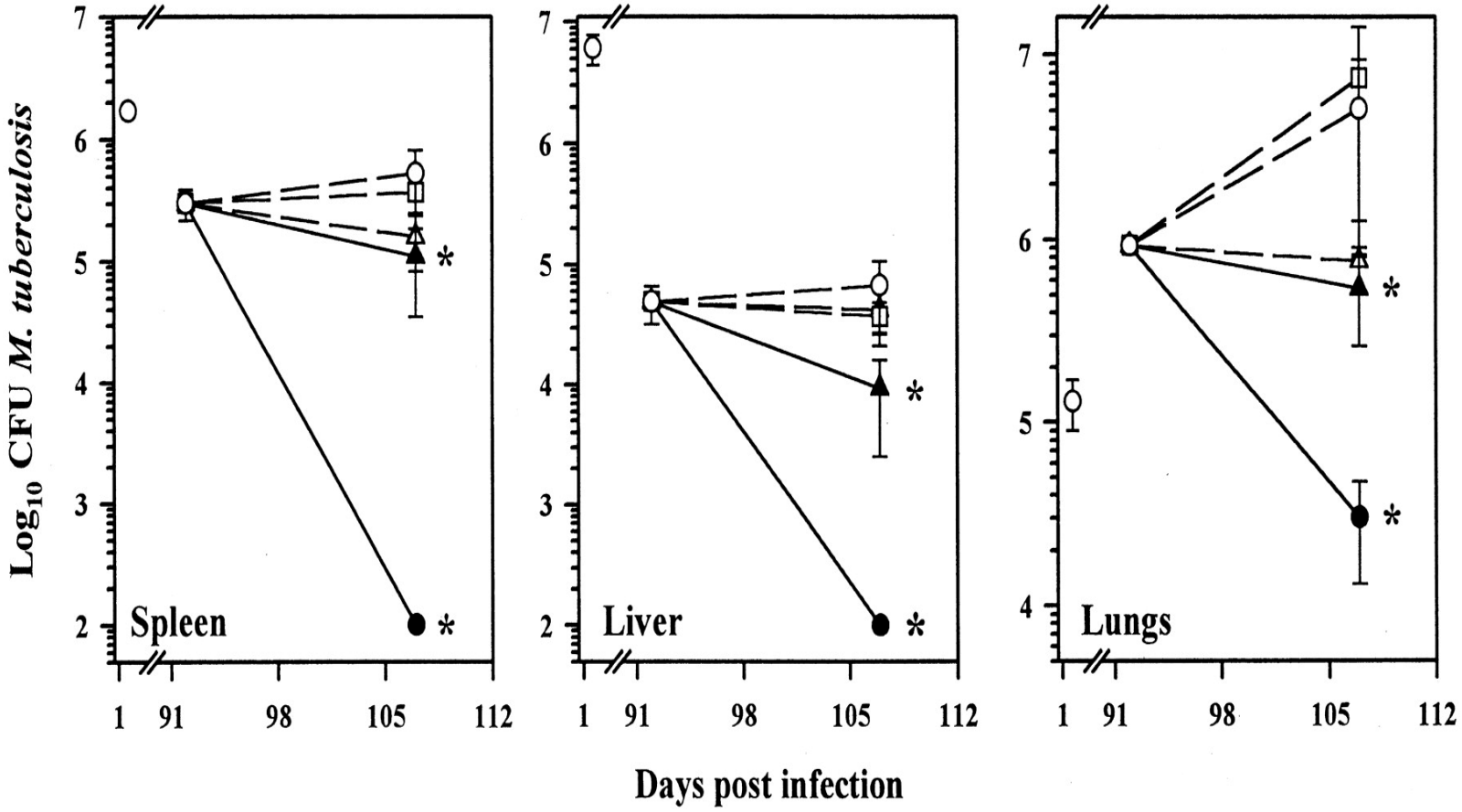
R. T. Mehta, Antimicrobial Agents and Chemotherapy , 40, 1893-1902 (1996)



Treatment started day 28 (iv injection) when infection has been established:
- Free Clofazimine (F-clof) 5 and 10 mg/Kg
- Liposome Clofazimine (L-clof) 5, 10, 25 and 50 mg/Kg

TREATMENT OF MICE INFECTED WITH *M. tuberculosis*

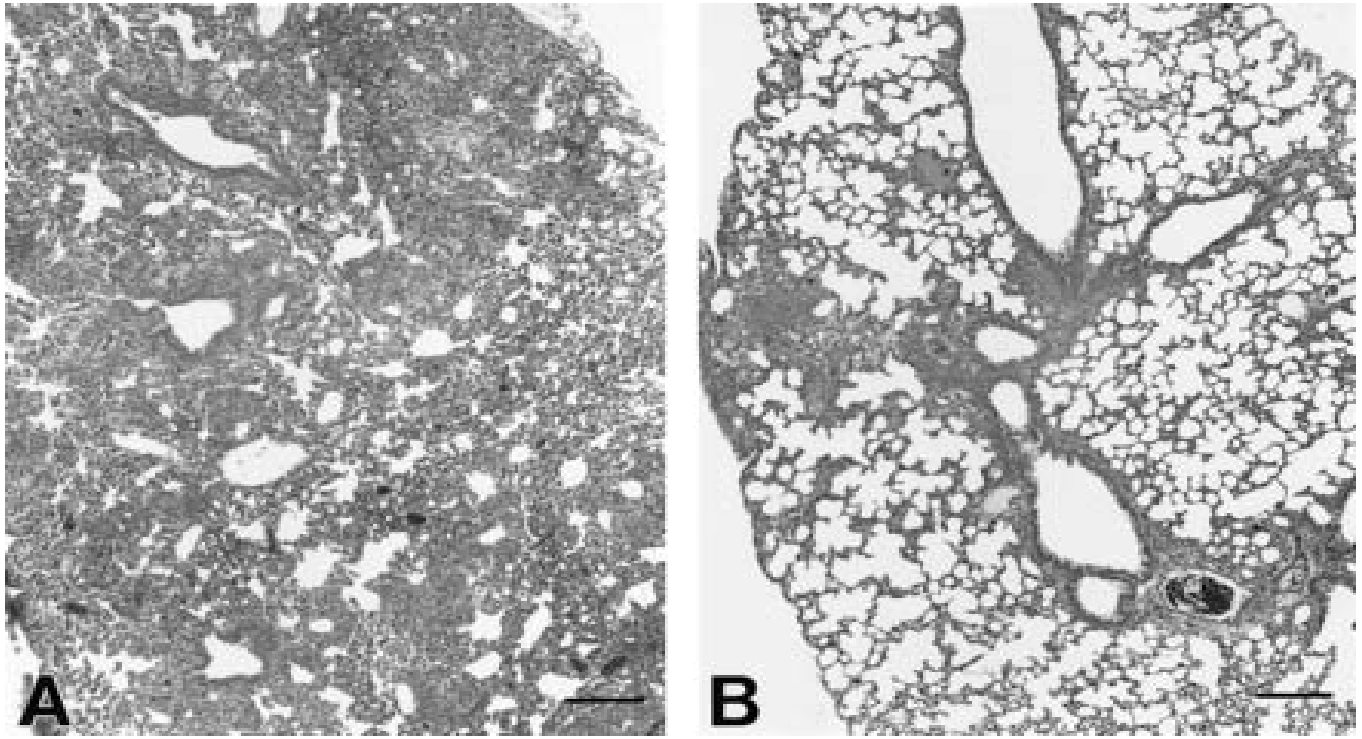
L B Adams et al, Antimicrobial Agents andChemotherapy, 43, 1638-1643 (1999)



left untreated (○) or treated i.v. every 3 to 4 days over a 2-week period (total, five injections) **beginning on day 92 postinfection (established infection)** with L-CLF (●, 50 mg/kg; ▲, 5 mg/kg), F-CLF (△, 5 mg/kg), or empty liposomes (□, lipid content equivalent to 50-mg/kg dose).

EFFECT OF CLF LIPOSOMES ON EARLY GRANULOMA FORMATION

L B Adams et al, Antimicrobial Agents andChemotherapy, 43, 1638-1643 (1999)

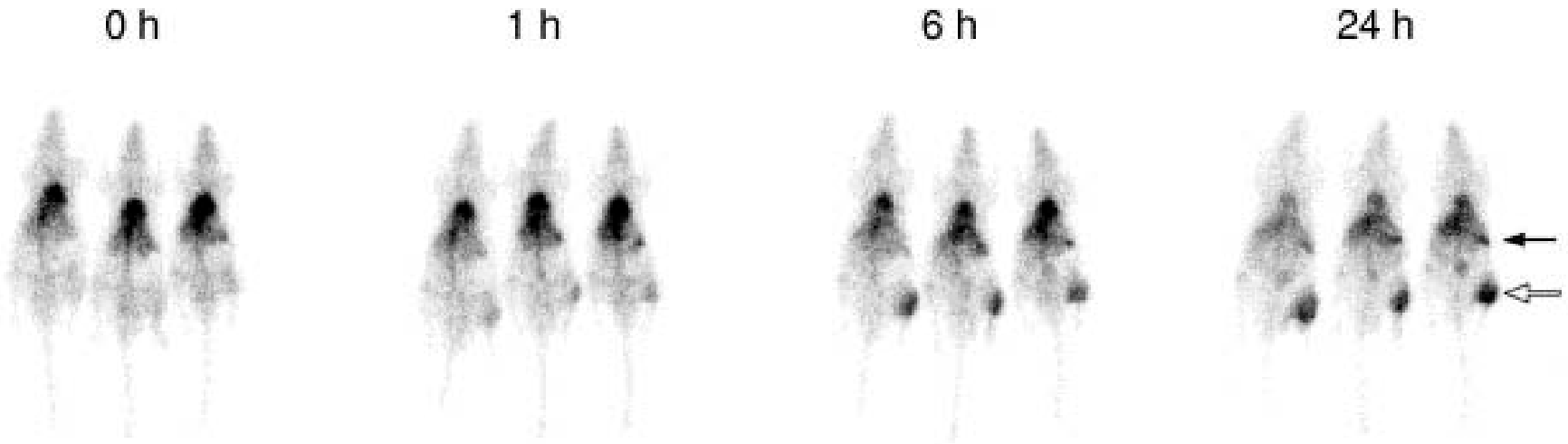


(A) control mice: intense mononuclear cell infiltration into the lung parenchyma. An early granulomatous response with aggregates of epithelioid macrophages interspersed with lymphocytes

(B) Mice treated with L-CLF: perivascular and peribronchiolar cuffing and more localized granuloma formation without extensive involvement of lung parenchyma.

Scintigraphic images of rats with an unilateral *S. aureus* infection

Laverman P. et al, J Control Rel, 75, 347-355 (2001)

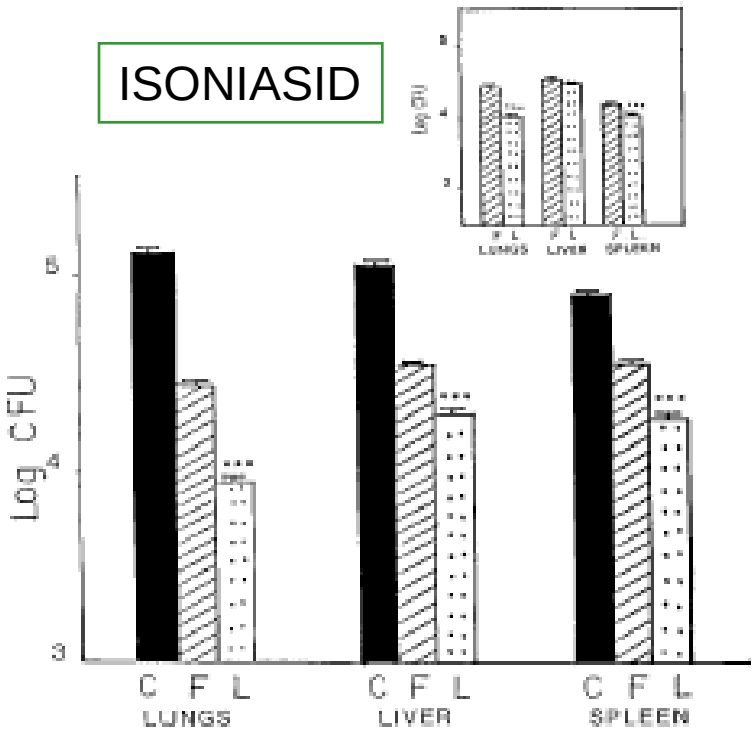


0, 1, 6 and 24 h post injection of 99mTc-labeled PEG-liposomes

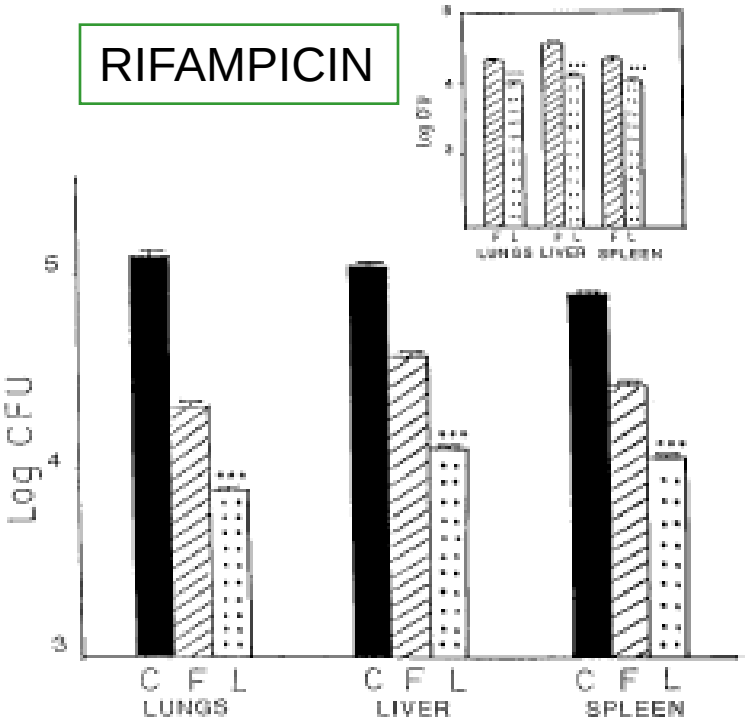
IN VIVO EFFICACY OF ISONIASID AND RIFAMPICIN PEGYLATED LIPOSOMES ON MYCOBACTERIUM TUBERCULOSIS

P. Deol and al, Antimicrobial Agents and Chemotherapy, 41, 1211-1214 (1997)

ISONIASID



RIFAMPICIN



- Untreated controls
- Free drug
- Drug in PEGylated liposomes

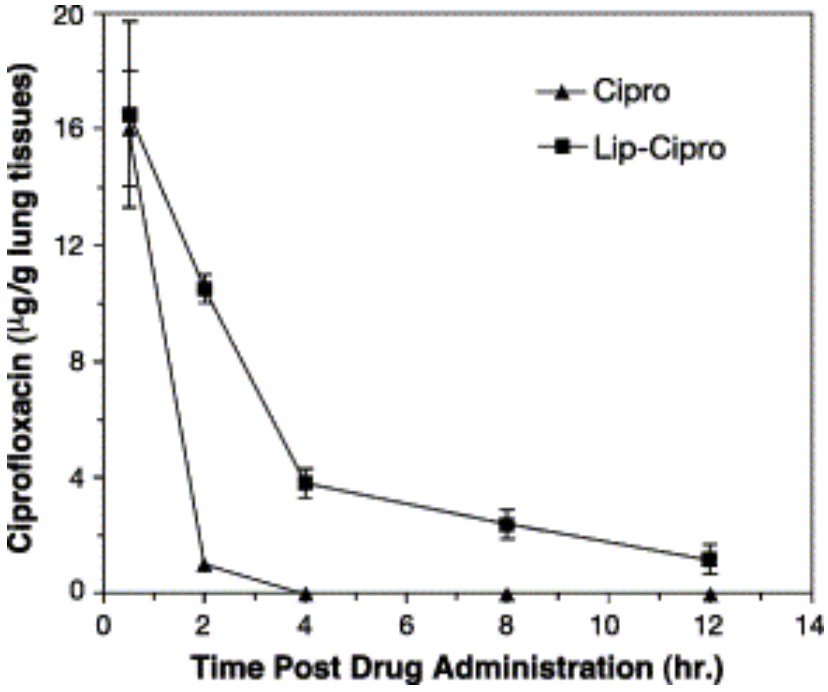
IV administration of 12 mg/Kg
Insert 12 mg/Kg free drug and 4 mg/Kg in liposomes

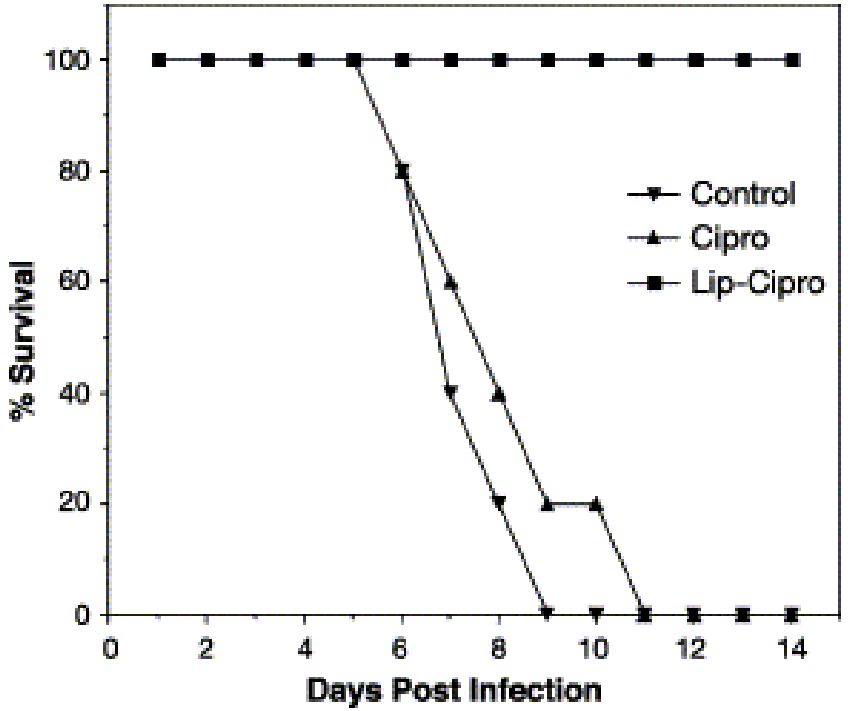
INFECTIONS BACTERIENNES INTRACELLULAIRES

VOIE PULMONAIRE

CIPROFLOXACIN SUV LIPOSOMES BY AEROSOL INHALATION AGAINST PULMONARY INFECTION BY *Francisella tularensis*

P. Wong et al., J Controlled Release, 92, 265-273 (2003)

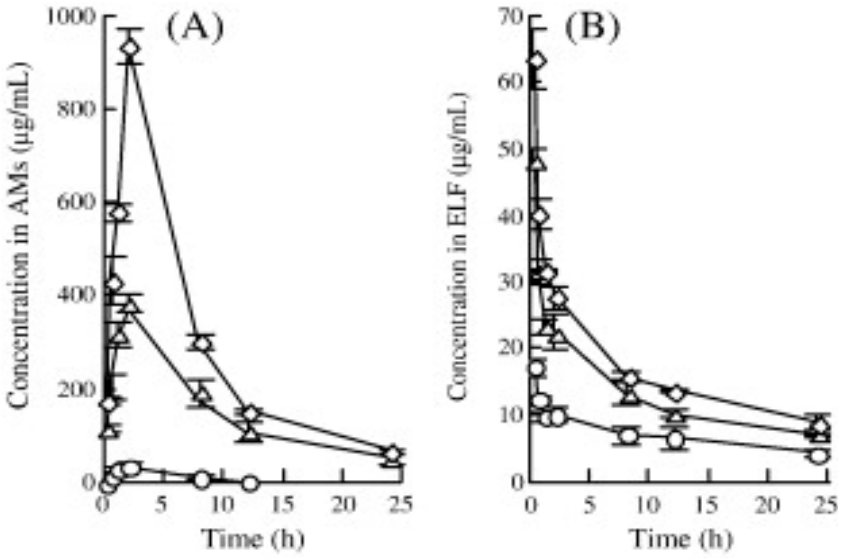


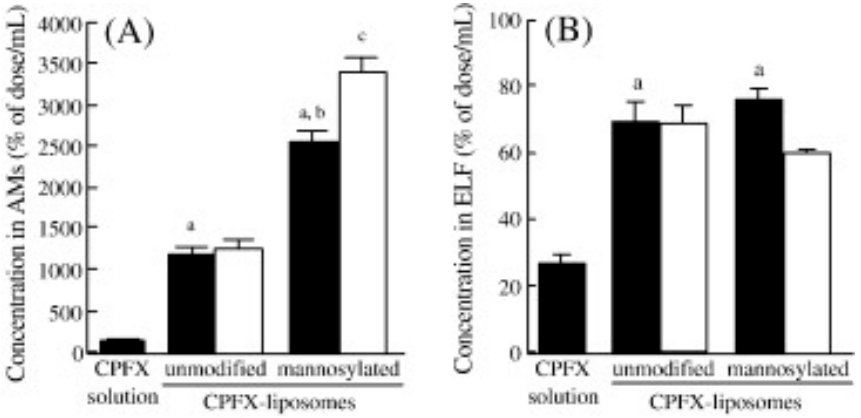


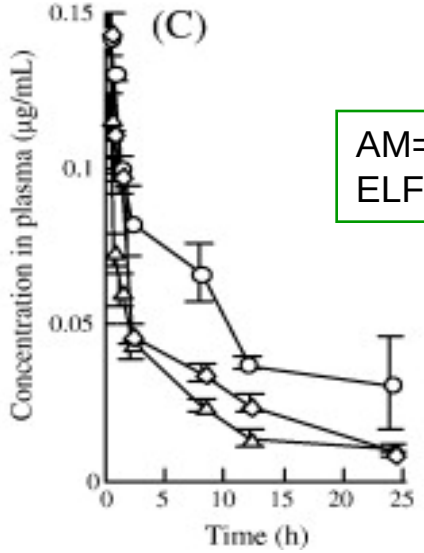
F. tularensis (Tularemia) involves the RES and leads to bacterial growth in lungs, liver and spleen

CIPROFLOXACIN TARGETING TO ALVEOLAR MACROPHAGES BY PULMONARY ADMINISTRATION OF MANNOSYLATED LIPOSOMES

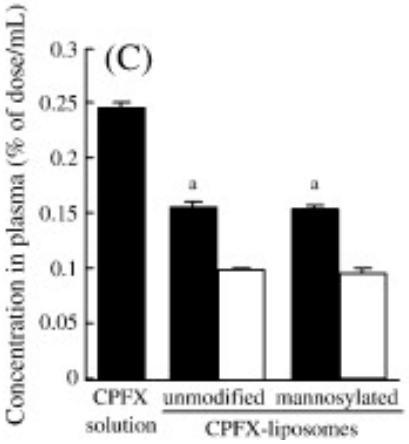
Chono et al, J Control. Rel., 127, 50-58 (2008)







AM= Alveolar macrophages
ELF= Epithelial lining fluid



Ciproflox Conc. (HPLC)

[3H] Cholesterolether

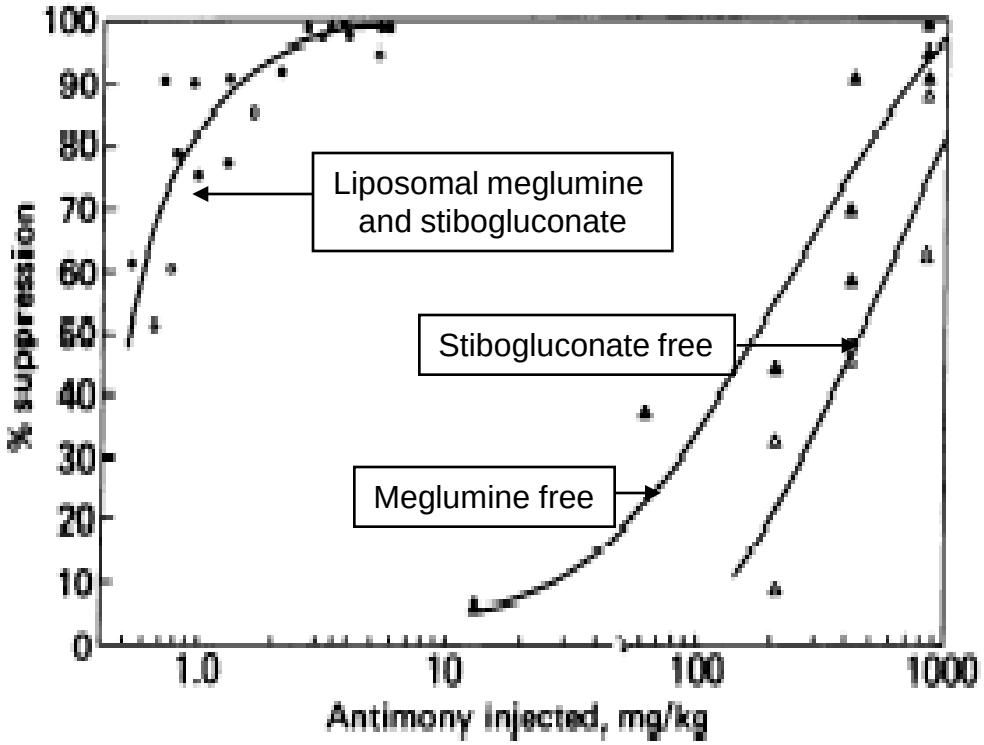
INTRACELLULAR INFECTIONS BY PARASITES

LEISHMANIA DONOVANI (Leishmaniasis)

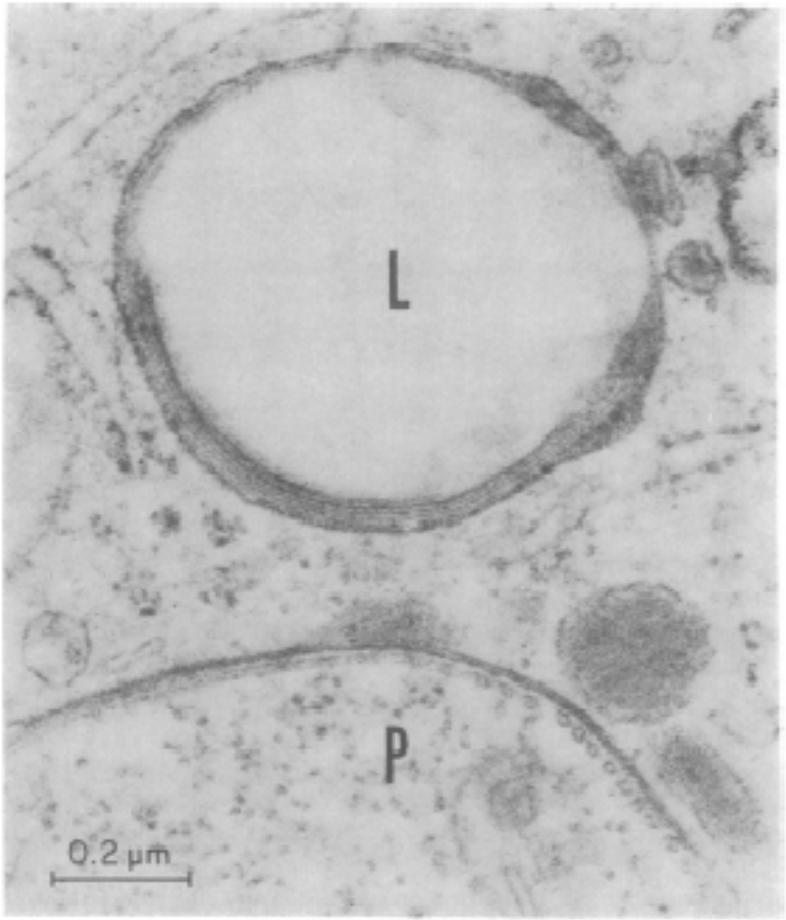
PLASMODIUM (Malaria)

VISCERAL LEISHMANIASIS: SUPERIOR EFFICACY OF LIPOSOME ENCAPSULATED ANTIMONIAL DRUGS

Carl R Alving et al, Proc Natl Acad Sci USA, 75, 2959-2963 (1978)



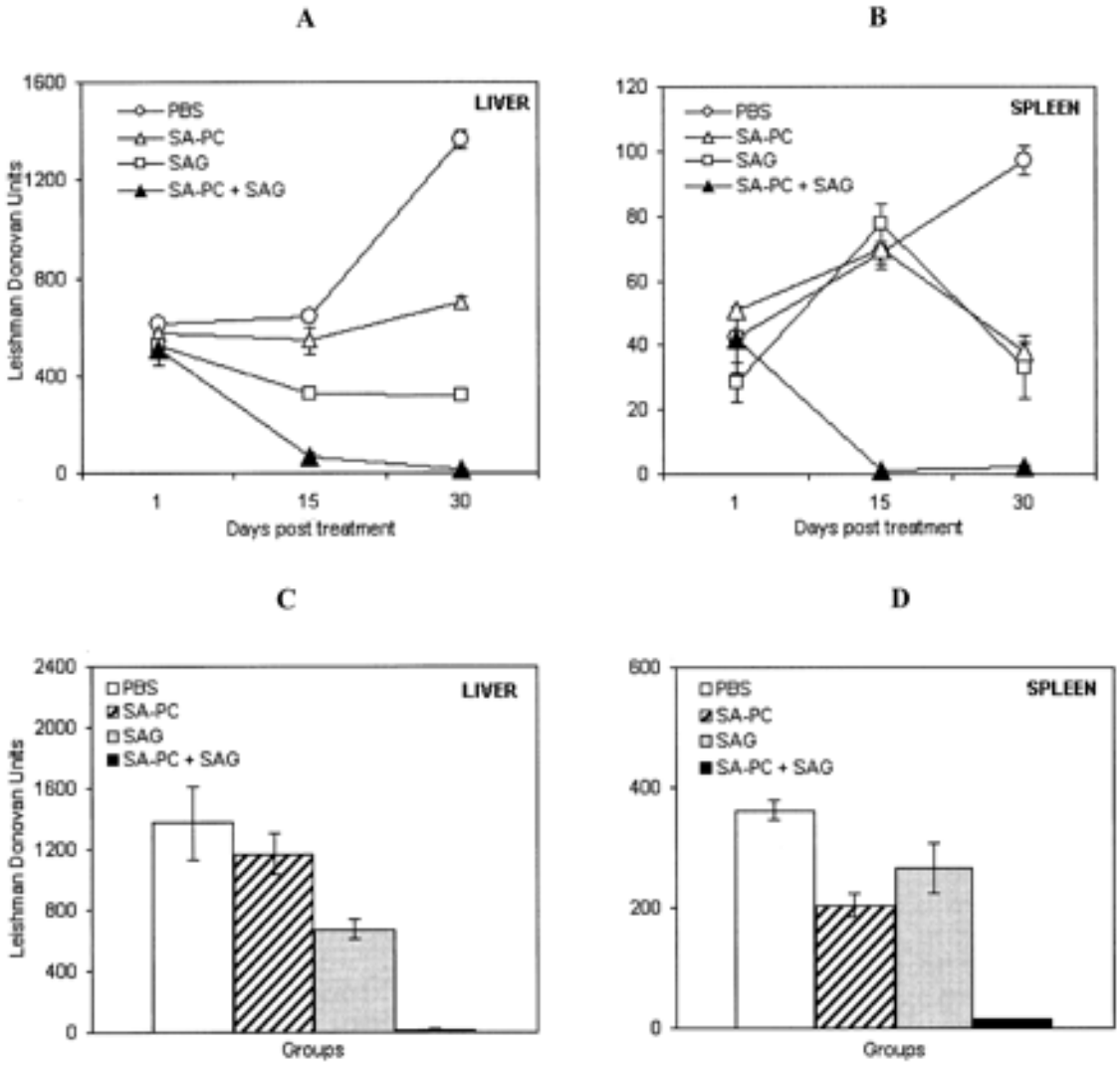
Treatment 17 days after infection of hamsters by *L. Donovani*



Hamster liver showing lamellar structure of DMPC/Chol/DCP liposome adjascent to intracellular parasite (inj. 35 days after infection)

TREATMENT OF MICE INFECTED WITH L. DONOVANI BY SODIUM ANTIMONY GLUCONATE (SAG) ENCAPSULATED STEARYLAMINE-PC LIPOSOMES

Swati Pal and al, Antimicrobial Agents and Chemotherapy, 48, 3591-3593 (2004)



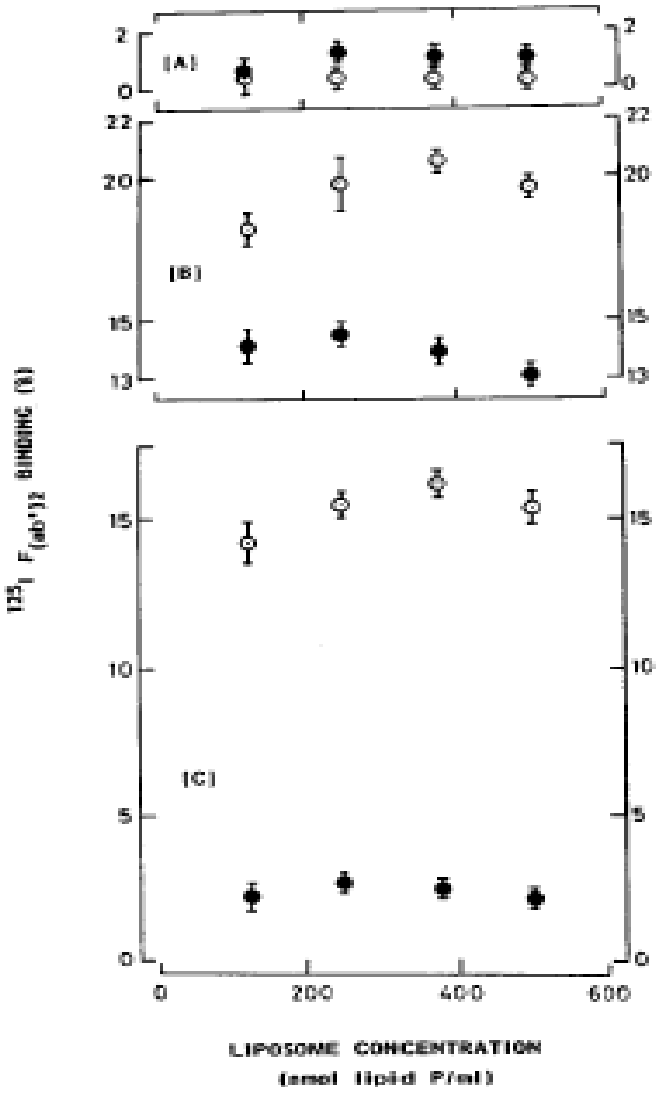
(A and B) Mice were sacrificed on days 1, 15, and 30 posttreatment. Levels of parasite burden in liver (A) and spleen (B) are expressed in Leishman Donovan units.

! Single dose of (16 mg/Kg) SAG resulted in complete elimination of parasite !

(C and D) In the second set, efficacy of the combination therapy for treatment of heavy parasite infection was assessed **(12 weeks).** Liver (C) and spleen (D) parasite loads after 1 month of treatment are shown in Leishman Donovan units.

BINDING OF mAb-LIPOSOMES TO PLASMODIUM BERGHEI INFECTED ERYTHROCYTES

M. Owais et al., Antimicrobial Agents and Chemotherapy, 39, 180-184 (1995)



[A] **Non relevant antibody mIg-Lip**

[B] relevant antibody mAb **D2-Lip** binds to **both infected and non infected** erythrocytes

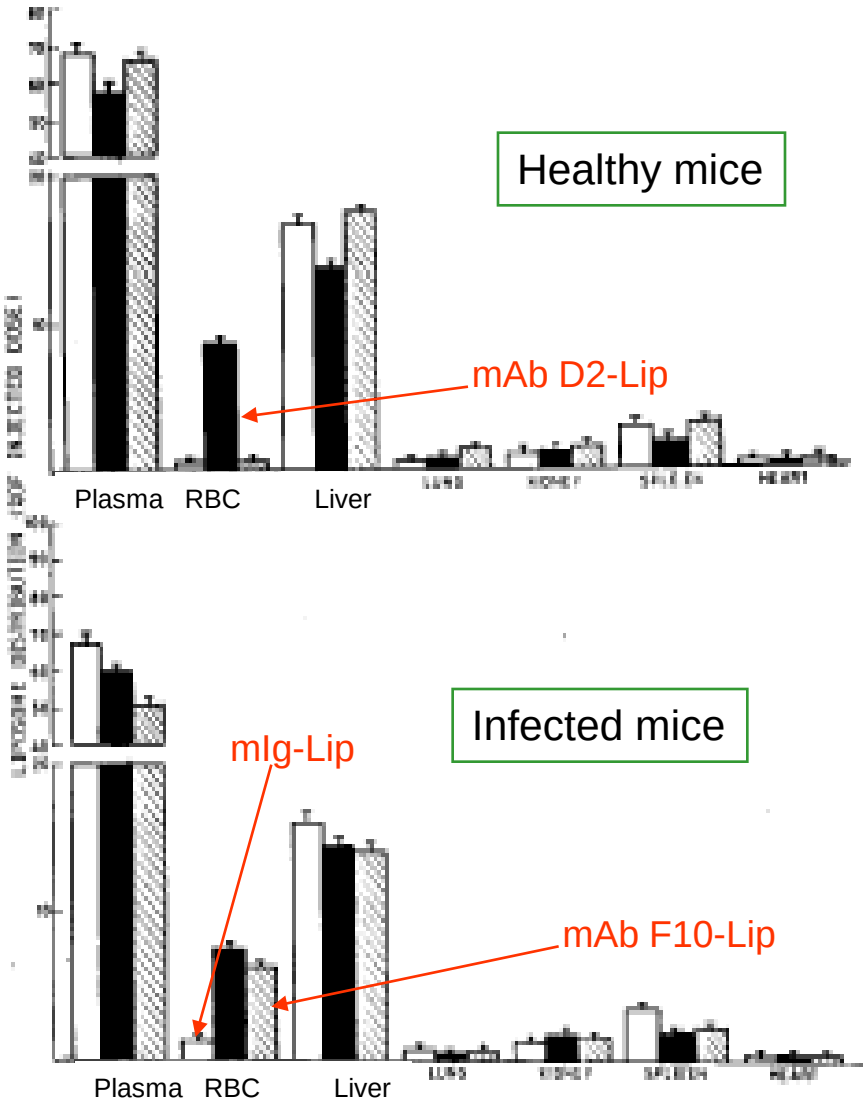
[C] relevant antibody **mAb F10-Lip** binds only to **infected erythrocytes**

- o P. Berghei infected erythrocytes
- ● Uninfected erythrocytes

Monoclonal antibodies were obtained from mice immunized with membranes of P.berghei infected erythrocytes

TISSUE DISTRIBUTION OF mAb-LIPOSOMES AFTER IV INJECTION TO INFECTED MICE

M. Owais et al., Antimicrobial Agents and Chemotherapy, 39, 180-184 (1995)



IN VIVO EFFICACY OF CHLOROQUINE IMMUNOLIPOSOMES AGAINST CHLOROQUINE-RESISTANT *P.berghei* INFECTION

M. Owais et al., Antimicrobial Agents and Chemotherapy, 39, 180-184 (1995)

TREATMENT (iv 5 mg/ kg days 4 and 6)	% PARASITEMIA ON FOLLOWING DAYS POST-TREATMENT		
	6	8	10
Saline	1.57 ± 0.38	4.33 ± 0.90	**All dead**
Free Chloroquine	1.70 ± 0.34	4.20 ± 1.80	**All dead**
PAb-lip-Chloroquine	1.36 ± 0.39	3.40 ± 0.75	**6.80 ± 1.20**
MAbD2-lip-Chloroquine	1.20 ± 0.39	3.23 ± 0.73	**4.56 ± 1.39**
MAbF10-lip-Chloroquine	1.12 ± 0.20	1.58 ± 0.26	**2.58 ± 1.10**

Overcoming chloroquine resistance by MabF10-lip may result to reduced efflux of chloroquine from the resistant parasites

SYSTEMIC MYCOSIS

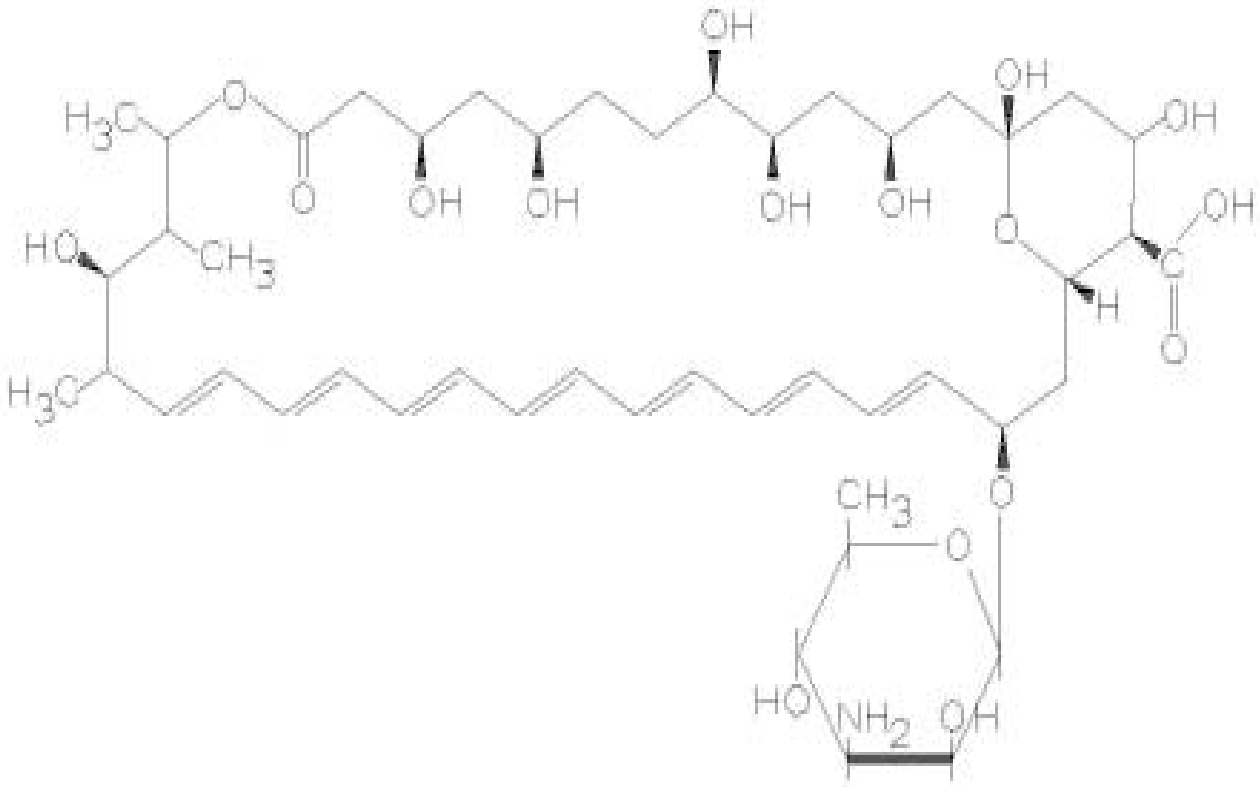
Candidoses

Aspergilloses

Cryptococcoses

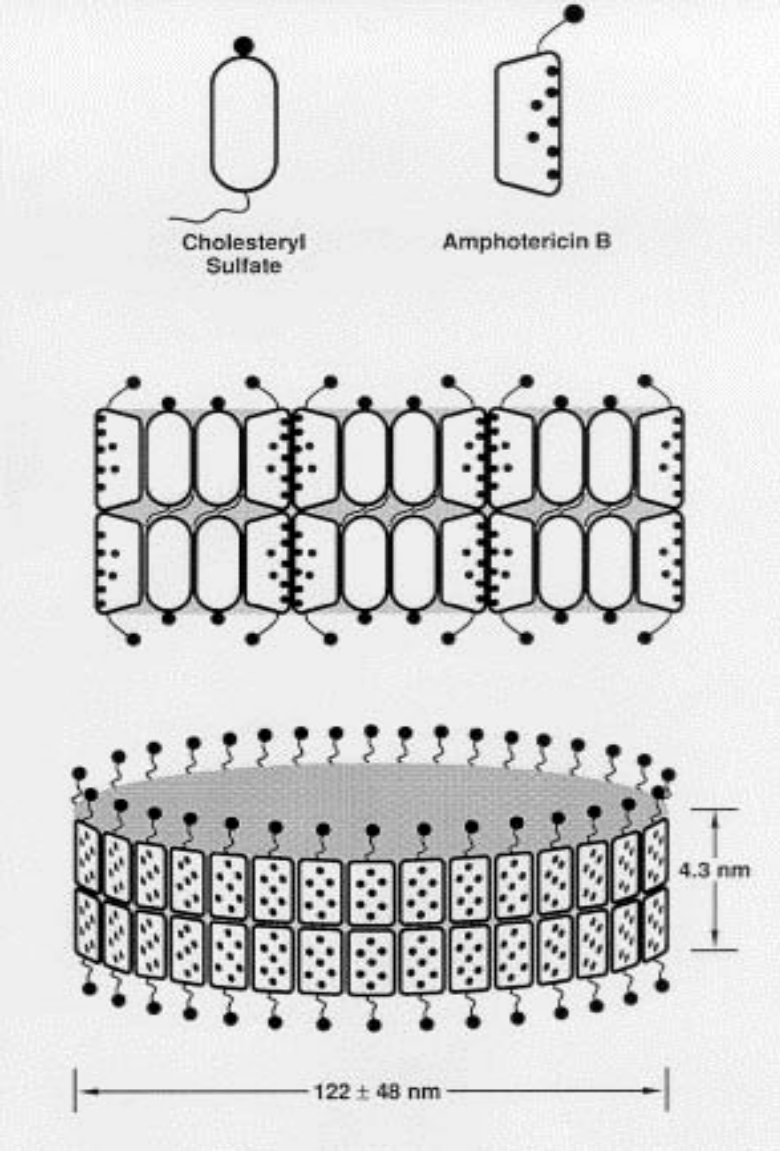
CHEMICAL STRUCTURE OF AMPHOTHERICIN B

Luke S.S. Guo, Advanced Drug Delivery Reviews 47,149-163, (2001)



PROPOSED MODEL STRUCTURE OF ABCD

Luke S.S. Guo, Advanced Drug Delivery Reviews 47,149-163, (200



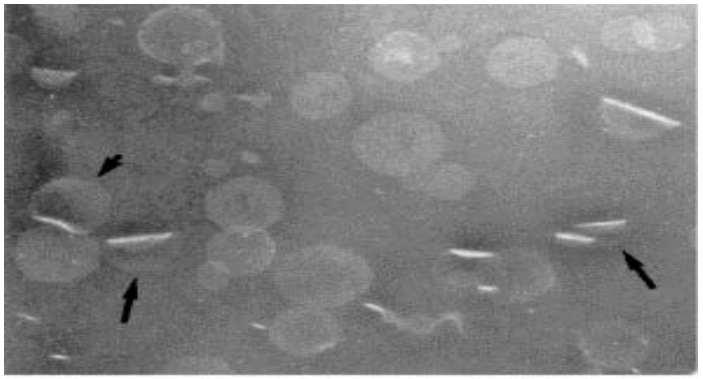
ABCD or AMPHOCIL is a colloidal complex of Amphothericin B with Cholesterylsulfate

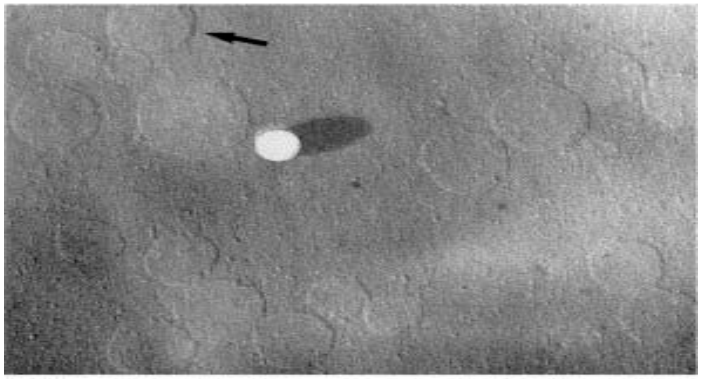
Top: Cartoon molecules of SCS and AMB.

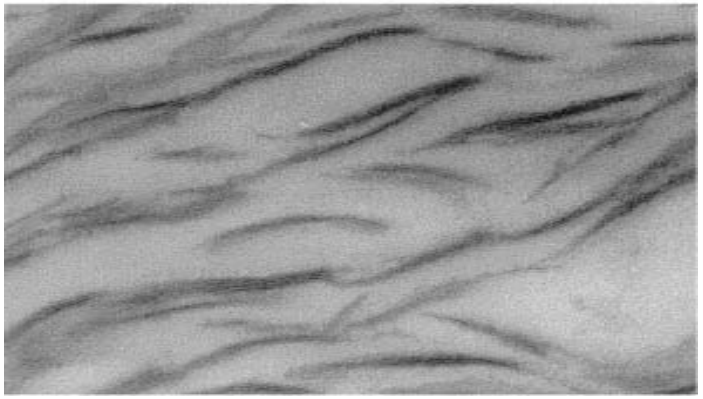
Middle: cross-section view of ABCD shows a bilayer of SCS with AMB interspersed within it.

Bottom: side view of a discoidal complex. AMB forms a shield at the disc edges and is oriented so that the seven hydroxyl groups (black dots) along its side are exposed to the polar aqueous environment.

ELECTRON MICROSCOPY OF ABCD







Luke S.S. Guo, Advanced Drug Delivery Reviews 47,149-163, (2001)

- (Top) Negative staining
- (Middle) Platinum-shadowing a shadowed polystyrene bead (seen as a white spherical particle) of 109 nm diameter
- (Bottom) Thin-sectioning: parallel layers were revealed, delineated by dark bands.

PHARMACOKINETICS AND BIODISTRIBUTION OF CONVENTIONAL AMPHOTHERICIN B (CAB) AND ABCD IN RATS

Luke S.S. Guo, Advanced Drug Delivery Reviews 47,149-163, (2001)

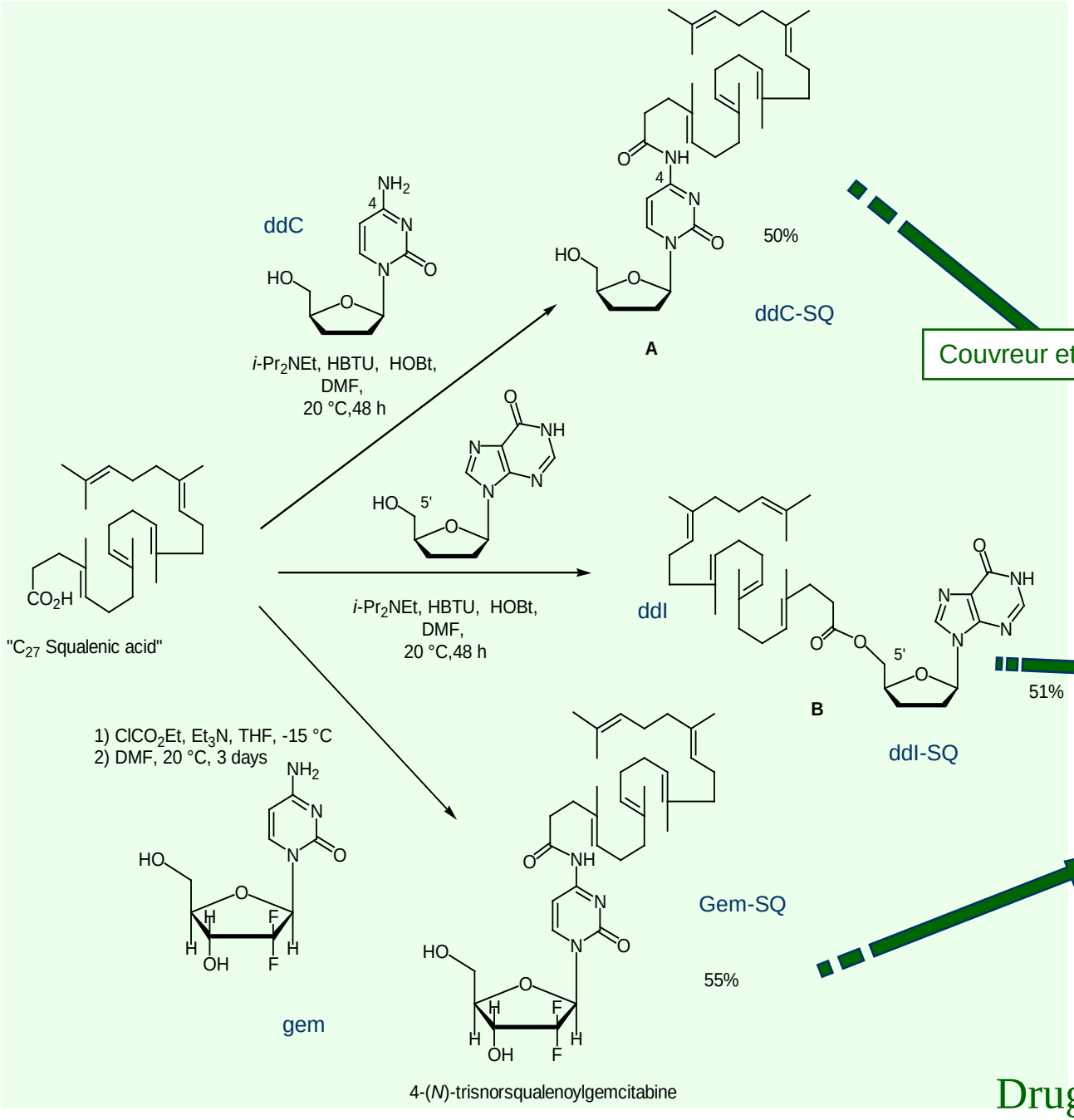
Tissue	Ratio of tissue concentration (ABCD/CAB)	
	Rats (daily dose 1 mg/kg)	Dogs (daily dose 0.6 mg/kg)
Liver	5.2	1.69
Kidney	0.3	0.15
Spleen	0.4	1.09
Lung	0.3	0.02
Heart	0.3	0.01
Bone marrow	nd[a]	2.78
Brain	0.4	0.07
Skeletal muscle	0.6	nd

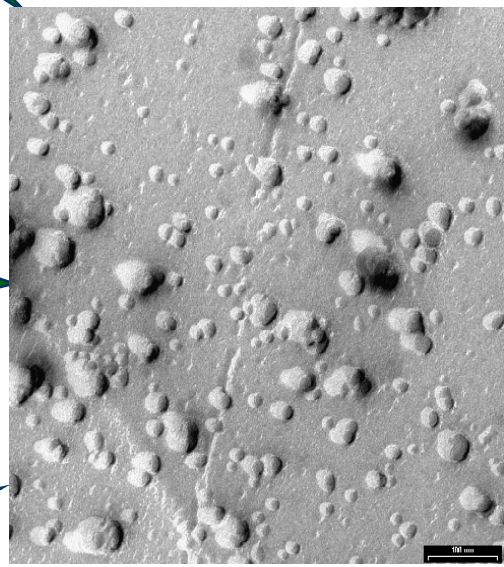
Drug and dose	C_{max} at 1 h (ng/ml)	$AUC_{0-\infty}$ (ng/h/ml)	CL (l/h/kg)	MRT (h)	$t_{1/2}$ (h)
CAB					
1 mg/kg	275±46	3986	0.26	16.59	10.4
ABCD					
1 mg/kg	102±11	4421	0.23	40.38	27.1
5 mg/kg	170±15	11620	0.43	46.57	41.12

VIRAL DISEASES

THE CONCEPT OF *SQUALENIZATION*

Couvreur et al., Nano Letters, **6**, 2544-2548 (2006)





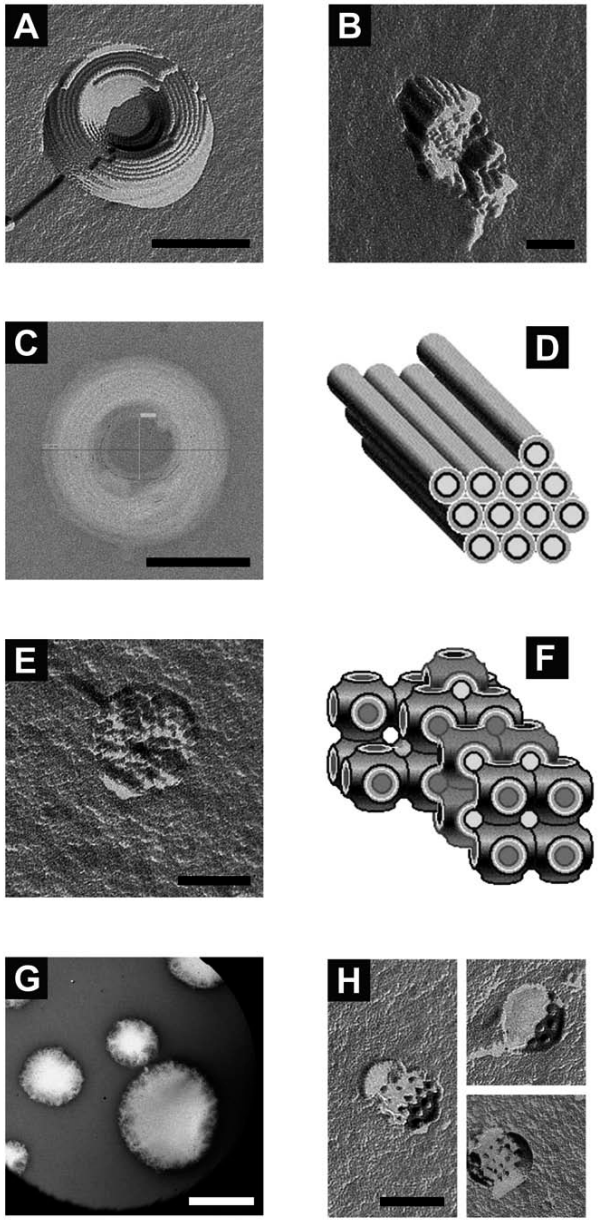
Nanoparticles
100-150 nm

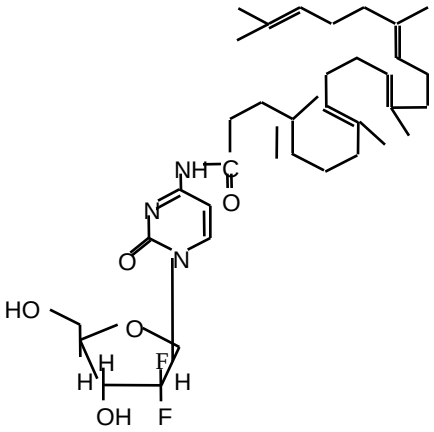
Drug loading: almost 50% !

Mais aussi AZT, ARA-C, Thymidine…

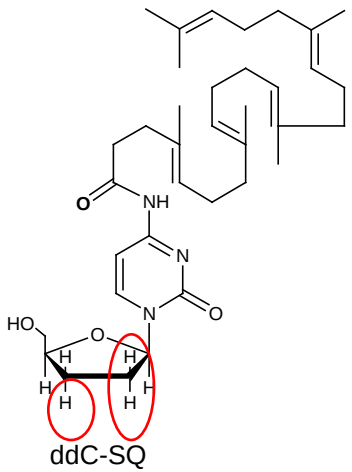
STRUCTURE OF SQUALENOYLATED ddC

Aoun et al., Advanced Funct Mater, in press, 2008

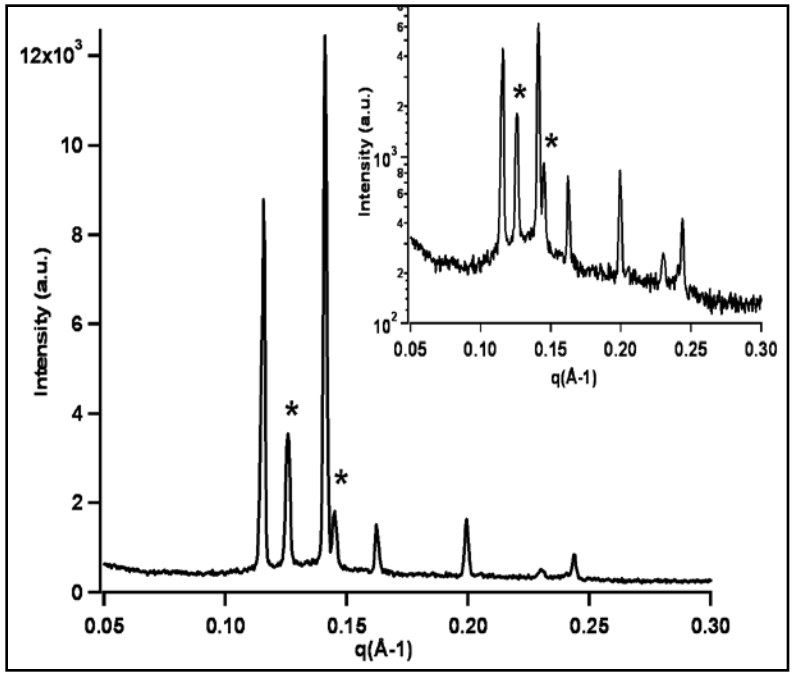




Squalenoylgemcitabine



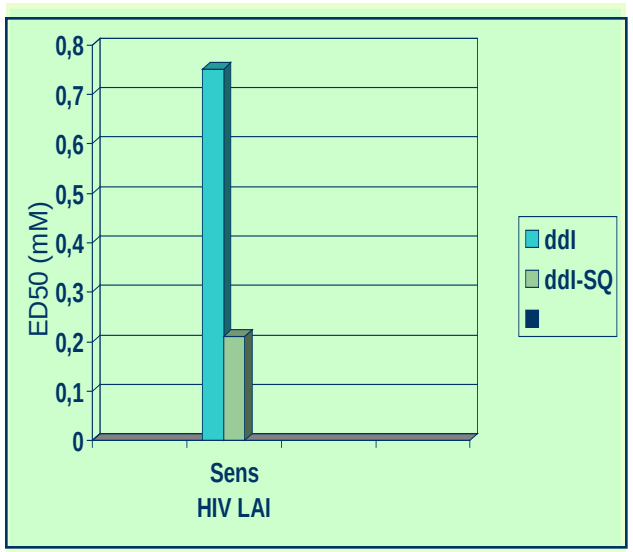
ddC-SQ



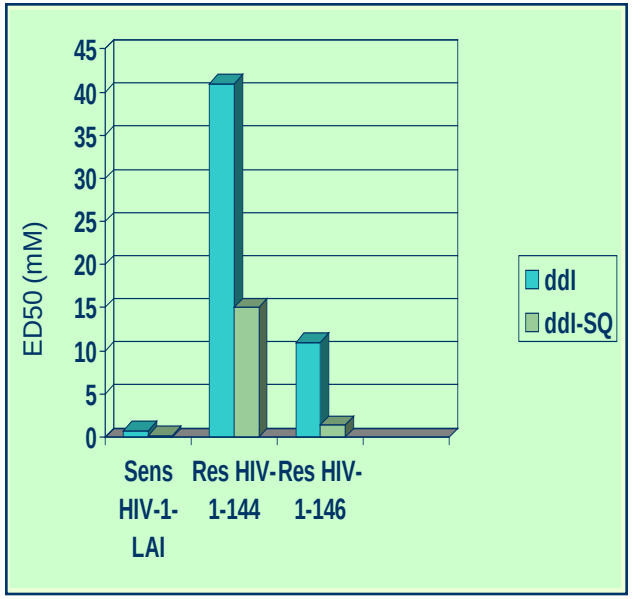
ANTI-HIV ACTIVITY OF ddI-SQUALENE ON HIV INFECTED LYMPHOCYTES

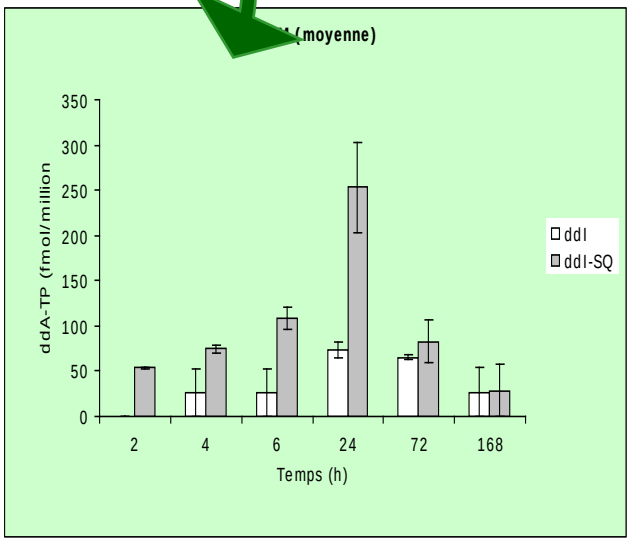
Couvreur et al., Nano Letters, **6**, 2544-2548 (2006)

sensitive



resistant



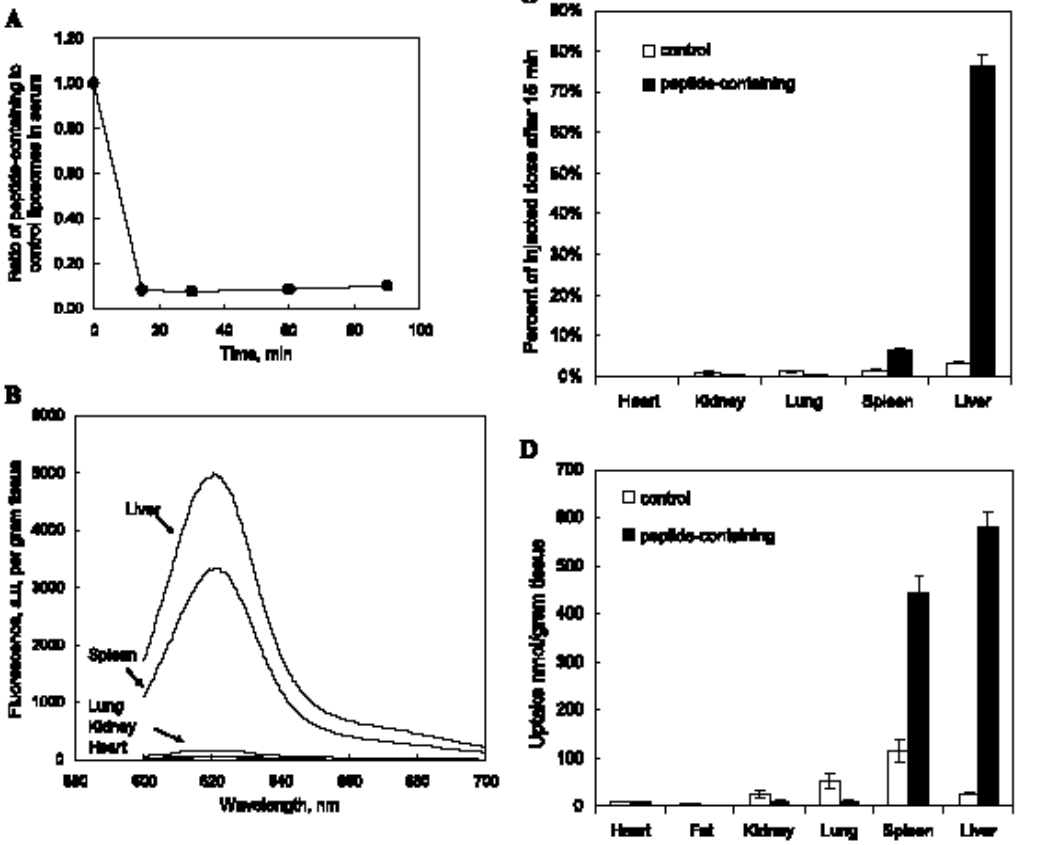


Intracellular concentration of ddA-PPP

A BIOMIMETIC APPROACH: USING PARASITE TOOLS TO TARGET SPECIFIC CELLULAR COMPARTMENTS

EFFECTIVE TARGETING OF LIPOSOMES TO HEPATOCYTES BY INCORPORATION OF Plasmodium AMINO SEQUENCE

K. J. Longmuir et al, Pharmaceutical Research? 23, 759-769 (2006)



THE BIOMIMETIC APPROACH:
The19-amino acid sequence of circumsporozoite protein (CSP) binds to the highly sulfated heparan sulfate proteoglycans found in the liver located on the basolateral site of the hepatocytes and Disse space

Formulation

Dierucoylphosphatidylcholine 82%
Dierucoylphosphatidylcholine-PEG5000 10%
Dierucoylphosphatidylcholine-PEG-Peptide 4%
Dierucoylphosphatidylcholine-Bodipy-TR-X 4%
(red fluorescence)

Fluorescence in organs

Control liposomes

Peptide containing liposomes

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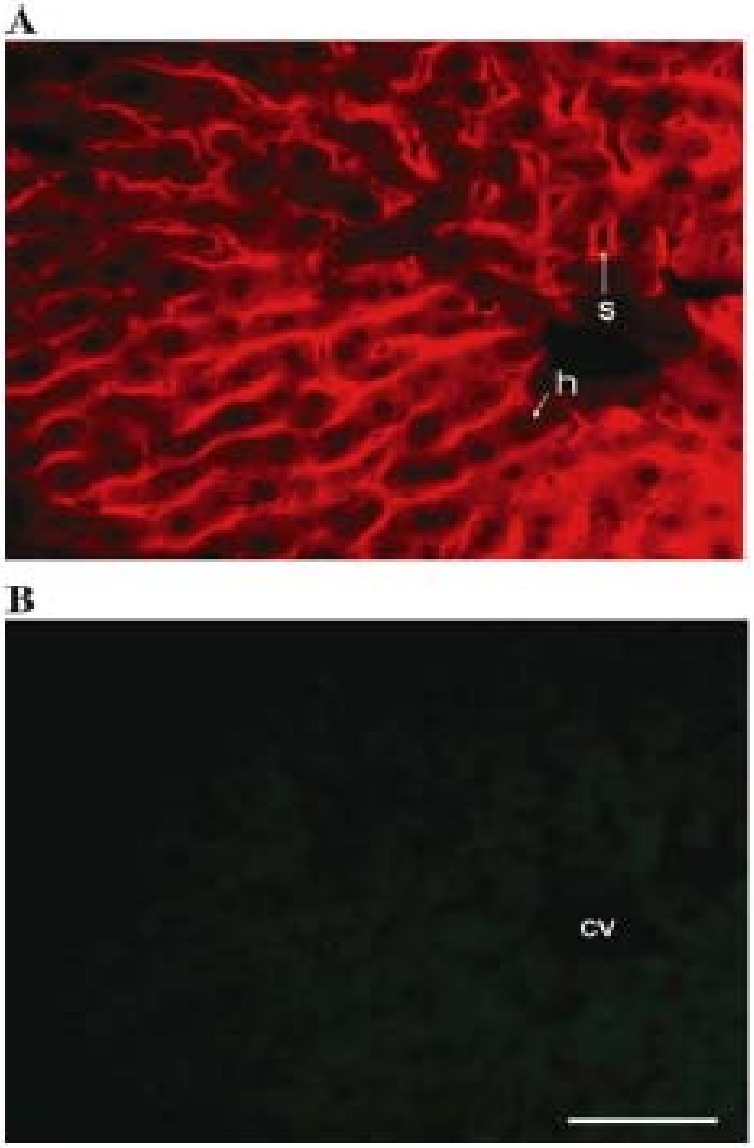
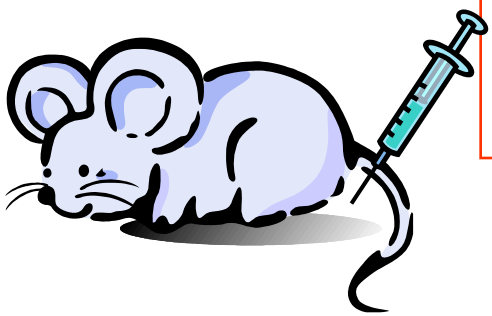


[A] Liver section showing the red fluorescence of Liposomes with liver-targeting peptide plus body-TR-X (red).
concentration in hepatocytes (except nucleus) + predominantly labelled regions adjascent to sinusoidal capillaries



peptide-liposomes labelled with body-TR-X (red) + **control liposomes with Body-FL (green)**

[B] Same section under fluorescein optics suitable for green illumination of Body-FL from control liposomes

EFFECTIVE TARGETING OF LIPOSOMES TO HEPATOCYTES BY INCORPORATION OF Plasmodium AMINO SEQUENCE

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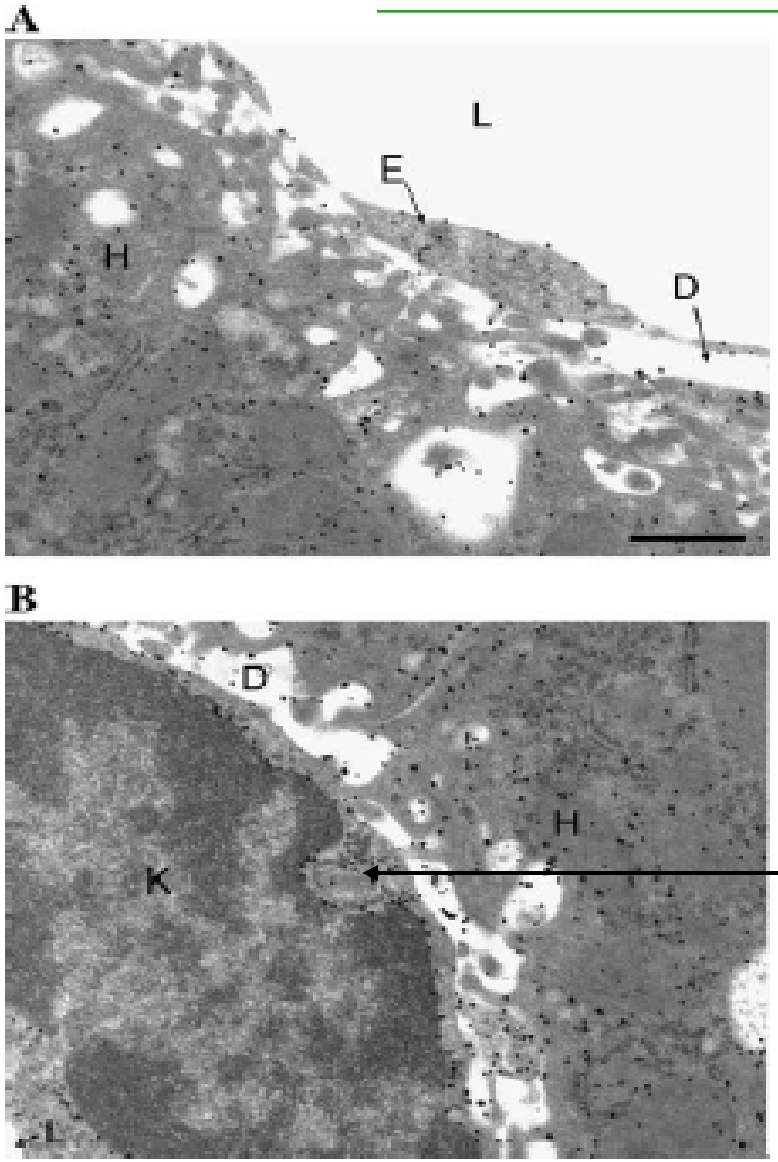
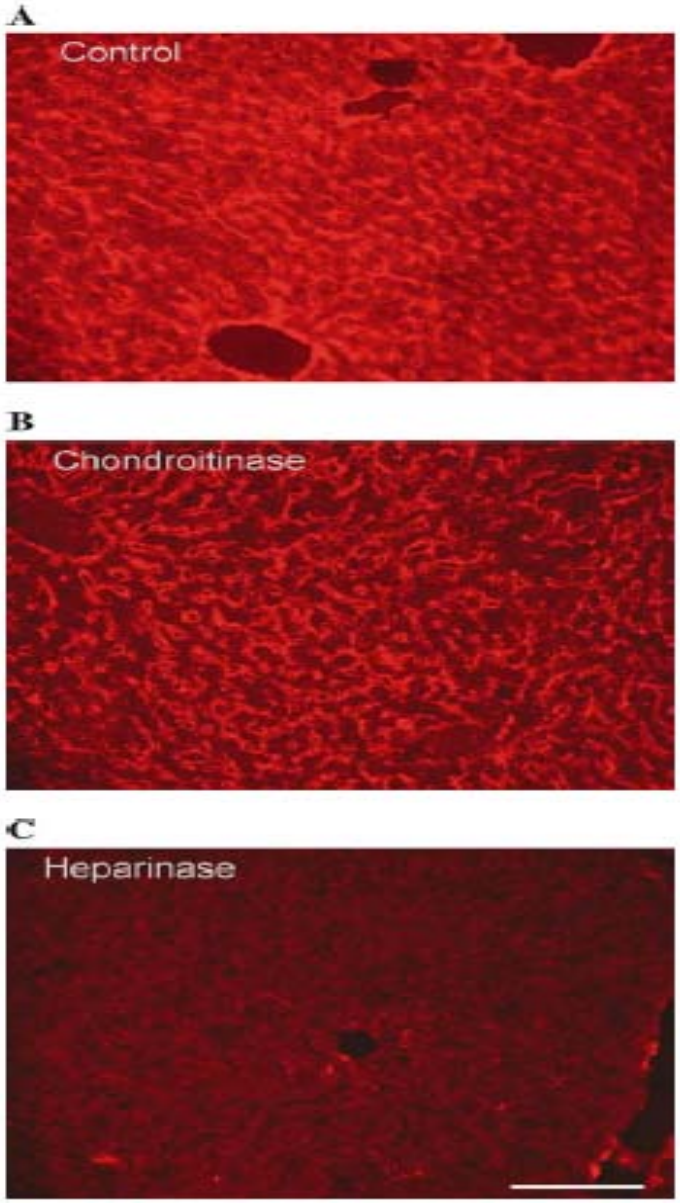


Gold labelled peptide-liposomes overlying cytoplasm of an endothelial cell (E), hepatocyte (H) and Kupfer cell (K)
Lumen of the capillary (L) and Disse space (D)

Just as Plasmodium, liposomes are located in cell cytoplasm and not into the nucleus

LIPOSOME BINDING TO ENZYME TREATED SLICES

K. J. Longmuir et al, Pharmaceutical Research, 23, 759-769 (2006)



Control looks like in vivo treated mice

Chondoitinase treated slices has no influence on liposome binding

Heparinase treated slices eliminated liposomes binding
→ Specificity for heparan sulfate proteoglycans

LES NANOMEDICAMENTS ANTIMICROBIENS: UN CHEVAL DE TROIE POUR LES MICROORGANISMES A LOCALISATION INTRACELLULAIRE