Description

The invention relates to synthetic membrane vesicles (liposomes) as described in the preamble of claim 1, and particularly to vesicles exhibiting on their surface fusion peptide molecules and other cell-specific proteins. The fusion peptide molecule may be in the form of a synthetic or purified peptide or as a part of a spike glycoprotein molecule of an enveloped virus, e.g. hemagglutinin, such as of influenza, parainfluenza or Semliki Forest virus. The cell-specific protein may be an IgG antibody, e.g. the CD4 antibody.

When a drug is given to a subject it must usually pass from the site of administration into the plasma compartment, and therefore the route of administration may have an important effect on the pharmacokinetic profile of the drug in the circulation. Thus, oral administration of the drug, while convenient, results in a slow onset of drug action and is somewhat unreliable in terms of achieving optimum plasma drug levels. By contrast, intravenous injection results in the prompt and exact establishment of circulating levels, at the cost of some pain and inconvenience to the patient. However, even if the drug is injected directly into the systemic circulation, the relationship between administered dose, drug levels and duration of the target site is by no means simple. These parameters are determined by a complex and often competing network of pathways leading either to accumulation of active drug molecules at the target site, or to the inactivation and excretion of the drug. These pathways involve biotransformation in the liver and in other tissues, excretion via the kidney or the bile, binding of drugs to fixed or circulating cells or macromolecules, and the passive or mediated passage of the drug across membrane barriers (Creasy, W.A. (1979): Drug Disposition in Humans, Oxford Univeristy Press, New York).

In recent years, there has been a good deal of interest in the prospect of influencing the distribution and metabolism of drugs in beneficial ways by using various sorts of carrier or drug delivery systems. These systems are designed to control one or more of the following parameters (Juliano, R.R. (1975), Can. J. Physiol. Pharmaco 56, 683-690):

a) the rate of input of the drug into a particular body compartment;

b) the distribution and localization of the drug in the body;

c) the persistence or rate of metabolism of the drug.

A major improvement in controlled drug delivery systems was the development of liposomes which were first described by Bangkam et al. (Bangkam, A.D., Standish, M.M. and Watkins, J.C. (1965), J. Mol. Biol. 13,238-252). Today, the literature claims an extravagant variety of benefits to be gained by delivering particular drugs in liposomes. These can be loosely grouped under the following headings:

1. Liposomes may cross biomembranes and may facilitate the transport of drugs through normally impermeable barriers. In particular, liposomes facilitate the intracellular penetration of encapsulated compounds.
2. Liposomes may be designed to interact with specific tissues, improving drug selectivity and reducing toxicity.
3. Drug pharmacokinetics may be beneficially modified by liposomes, through modulation of drug release, distribution and removal from the systemic circulation.
4. Chemically and metabolically labile drugs may be protected by liposomes from deactivation.

Drugs of potential therapeutic interest may be sequestered in this way, the encapsulated compounds exhibiting modified properties, at least in vitro, when compared with the unmodified substances. Unfortunately, early hopes of a revolutionary new approach to chemotherapy have not been completely realised by the experimental facts (Fildes, F.J.T. (1981), Liposomes. From physical structure to therapeutic applications. Knight (ed.), Elsevier/North-Holland Biomedical Press).

A better result using liposomes as vectors could be achieved by the targetting of liposomes with specific proteins. If substances encapsulated in liposomes were to be delivered more successfully to selected organs or tissues, these targetting techniques had to be devised in order to bypass the accumulation of liposomes at undesirable sites (thus reducing toxicity) and to optimize the delivery to specific cells (thus enhancing the desired effect).

Several investigations have utilized the coating of liposomes with aggregated immunoglobulins in order to optimize delivery to phagocytic cells (Ismail, G., Boxer, L.A. and Bachner, R.L. (1979), Pediatr. Res., 13. 769-773/Finkelstein, M.C., Kuhn, S.H., Schieren, H., Weissmann, G. and Haffstein, S. (1980): Liposomes and Immunobiology (Tom, B.H. and Six, H.R. eds.) 255-270. Elsevier/North-Holland Publishing Co., Amsterdam).

A further improvement was described by Gregoriadis and Neerunjun (1975), Biochem. Biophys. Res. Commun., 65, 537 - 544, whereby the targetting of liposomes was enhanced by associating cell-specific IgG antibodies. The uptake of liposomes was augmented 3- to 25-fold when different cell strains were presented with liposomes associated with IgG immunoglobulins raised against the particular cell strain. However, the technique employed showed that the liposomes targetted in this way failed to fuse with the

cell membrane and therefore an efficient delivery of drugs into the cells was prevented. (Leserman, L., Weinstein, J.N., Blumenthal, R., Sharrow, S.O. and Texy, W.D. (1979), J. Immunol. 122, 585 - 591).

A major improvement in the production of drug delivery systems was the targetting of liposomes with viral proteins: liposomal membranes have been reconstituted with proteins such as the hemagglutinin from influenza virus (Almeida, J.D., Brand, C.M., Edwards, D.C. and Heath, T.D. (1975), Lancet 2, 899 -901). The efficiency and specificity of early viral interaction with host cells (adsorption, penetration) may be conferred upon liposomal carriers by incorporating the appropriate viral proteins into the liposomal membranes. Indeed, the incorporation of Sendai virus spike proteins into liposomal bilayers produced at least a 100-fold enhancement in the uptake by mouse L cells of diphteria toxin fragment A as compared with fragment A-containing liposomes without spikes (Uehida, T., Kim, J., Yamaizumi, M., Miyahe, Y. and Okada, Y. (1979). Cell. Biol. 80, 10 - 20).

The main drawbacks of the above methods lie in the lack of fully active influenza hemagglutinin fusion peptides. Influenza A viruses penetrate their host cells by membrane fusion. After binding to the cell surface, virus particles are internalized and transported to endosomes and lysosomes. The acidic environment in these organelles activates fusion between the viral and host cell membranes. The advantage of vesicle-cell fusion at low pH lies in the fact that the content of the vesicles is released after internalizing into the cytoplasma of the cells (pH in the endosome is around 5.2). The discovery of this low-pH-induced fusion of influenza virus glycoproteins led to many attempts to develop effective drug delivery systems with influenza hemagglutinin targetted liposomes.

Kawasaki, K. et al. (Biochemica et Biophysica Acta, (1983), 733, 286 - 290, used reconstituted vesicles of influenza hemagglutinin glycoproteins in egg yolk phophatidylcholine / spin-labeled phosphatidylcholine / cholesterol (molar ratio 1.6 : 0.4 : 1). Preparations at appropriate protein to lipid ratios (1 : 44 and 1 : 105 mol/mol) contained vesicles with a diameter of 100 - 300 nm and a high densitiy of spikes on the surface, but have several drawbacks:

1) Due to the high cholesterol content and a residue of detergent they show reduced endocytosis by phagocytic cells. Kawasaki et al themselves report only a 66% activity; but according to a more recently developed, improved test method, the activity is only 1% (!) after 7 minutes (see Example 10 below). This might be due to the fact that Triton X-100 used by Kawasaki et al partially reacts with hemagglutinin and is not completely removed by dialysis.

2) To study the role of the viral membrane components in the fusion reaction in detail, it is necessary to be able to manipulate these components. For this purpose a method is required for the isolation and reconstitution of the viral spike proteins, producing reconstituted virosomes with full biological fusion activity. Despite the efforts of Kawasaki et al., reconstitution of influenza virus envelopes, displaying full biological fusion activity, has not been reported.

3) The attachment of the targetted liposomes containing pharmaceutical active drugs to specific cells could not be demonstrated.

Most of the other methods that have been employed to reconstitute viral envelopes are based on solubilization of the viral membrane with a detergent and, after sedimentation of the internal viral proteins and genetic material, removal of the detergent from the supernatant. Detergents with a high critical micelle concentration, such as octylglucoside, may be removed effectively by dialysis. Reconstitution employing octylglucoside has been reported for Semliki Forest virus (SFV) (Helenius, A., Sarvas, M. and Simons, K. (1981), Eur. J. Biochem. 116, 27 - 35); vesicular stomatitis virus (VSV) (Eidelman, O., Schlegel, R., Tralka, T.S. and Blumenthal, R. (1984), J. Biol. Chem. 259, 4622 - 4628); influenza virus (Huang, R.T.C., Wahu, K., Klenk, H.D. and Rou, R. (1980) Virology, 104, 294 - 302); and Sendai virus (Harmsen, M.D., Wilschut, J., Scherphof, G., Hulstaert, C. and Hoekstra, D. (1985), Eur. J. Biochem., 149, 591 - 599). However, properly reconstituted viral envelopes were not produced in all cases. For example, virosomes formed from SFV had a protein to lipid ratio deviating from that of the viral membrane, and virosomes produced from VSV did not exhibit biological fusion activity.

Another method, described in the literature, is the detachment of influenza viral spikes from the virus particles with bromelain. The hemagglutinin recovered in such a way was attached to liposomes (Doms, R., Helenius, A. and White, J. (1985), J. biol. Chem., 260 (5) 2973 - 2981). The main drawback of this method is the limited biological activity of such vesicles due to the cleavage by bromelain.

Thus, the technical problem underlying the present invention is to provide vesicles which transport desired drugs or pharmaceutically active substances to specific cells, such as macrophages, T4-helpercells, brain cells or other cells of specific organs, which then are fully attached to these cells, internalized by phagocytosis or endocytosis so as to - immediately after endocytosis - deliver the desired drug into the cytoplasma of the desired cell.

The solution to the above technical problem is achieved by providing the vesicles which are characterized in claim 1. Specific embodiments of the invention and improved vesicles are described in subclaims 2 to 9. A process for the preparation of such vesicles is described in claims 10 and 11. Uses of such vesicles are described in claims 12 and 13.

Accordingly, vesicles are provided which contain a liposome ideal for endocytosis and a biologically fully active cell-specific marker, preferably a viral hemagglutinin glycoprotein or a derivative thereof, or a synthetic fusion peptide being capable of inducing the immediate fusion of said vesicles after endocytosis by the desired cells.

In another embodiment of the invention, a suitable crosslinker which adsorbs to the specific liposome is used in the mixture together with a specific antibody, directed to the responsible receptor of the desired cell for inducing the endocytosis mechanism, which is bound to the crosslinker in such a manner that it is still fully biologically active.

The essential feature of these drug delivery vesicles is that they carry on their surface said fully active viral glycoproteins or a derivative thereof and biologically active, specific antibodies being capable of attaching to the desired cells, of being internalized by phagocytosis or endocytosis by these cells, inducing the immediate fusion of said vesicles with internal cytoplasmic membranes and releasing the virosome's content into the cytoplasma of these cells. Due to the fully active fusion peptides of the present invention, the drugs are released immediately after phagocytosis so as to avoid an undesired long stay in the endocytosomes which would give rise to unspecific degradation of the pharmaceutical substances contained in the viral hemagglutinin vesicles of the present invention. At pH 5, the influenza fusion peptides on the surface of the vesicles are activated in the same way as is the case with live influenza virus. The content of the vesicles is released into the cytoplasma, as is the case with influenza virus and the released nucleoprotein.

The term "liposome" refers to medium sized bilamellar phospholipids prepared by controlled detergent removal. The size of the vesicle initially formed upon detergent removal depends on the detergent and phospholipid used and, in some cases, on the method and rate of detergent removal.

The present invention also relates to a method of preparing vesicles which are specially suited for phagocytosis. It comprises the following steps:

1) Dissolution of one or two phospholipids in a non-ionic detergent;
2) vesicle formation through detergent removal with polystyrene beaded micro-carriers (mesh size - wet - 20-50;
3) a defined mechanical movement is performed during detergent removal;
4) the desired diameter of vesicles (50 - 100 nm) is achieved by ultrasonification.

In still another embodiment, the present invention refers to vesicles where the phospholipid comprises 70-95% phosphatidylcholine and 5-30% by weight of another phospholipid, such as phosphatidylethanolamine. The cholesterol content is preferably less than 1%.

The term "fusion peptide" refers to viral spike glycoproteins containing the fusion peptide. In one embodiment, the present invention refers to the complete hemagglutinin trimer of viral surface spikes or to one monomer or to one or both cleaved subunits, the glycopeptides HA1 and HA2, containing the functional fusion peptide. In another embodiment, the present invention refers to the fusion peptide itself, isolated or synthetically produced. In a particularly preferred embodiment of the present invention, these polypeptides, containing the fusion peptide, refer to influenza hemagglutinins, especially the one of the A-H_1N_1 subtype.

The term "crosslinker" refers to an organic heterofunctional molecule capable of linking to the surface of vesicles prepared according to this invention and capable of binding polypeptides. In a preferred embodiment of the present invention, this molecule is an organic, bifunctional molecule containing a carboxylic group and a thiol group, particularly a sulfosuccinimidyl-(S-)derivate, such as S-4-(p-maleimidophenyl)butyrate, S-acetate, S-2-(m-azido-o-nitrobenzamido)ethyl-1,3' -dithiopropionate, S-6-(4'-azido-2'-nitrophenylamino)hexanoate S-(4-azidophenyldithio)propionate, S-2(p-azidosalicylamido)ethyl-1,3'-dithiopropionate, S-2-(biotinamido)-ethyl-1,3'-dithiopropionate, S-6-(biotinamido) hexanoate, S-3-(4-hydroxyphenyl)propionate, S-(4-iodoacetyl)aminobenzoate, S-4-(N-maleimidomethyl)cyclohexane-1-carboxylate, S-2,2,5,5-tetramethylpyrroline-1-oxyl-HCl.

The term "cell-specific" protein or marker refers to a protein capable of linking to the crosslinker at the vesicle's surface and linking to the receptor of cells inducing the endocytosis mechanism. In a preferred embodiment of the present invention, this molecule refers to a cell receptor specific antibody, particularly to a monoclonal antibody.

The examples and figures illustrate the invention:

Example 1:

Preparation of synthetic membrane vesicles of phosphatidylcholine with fully functionally active viral fusion peptides in a hemagglutinin trimer from influenza virus and containing dextran sulfate as antiviral drug.

Fig.1 shows the principle of the procedure; a circle designates a liquid solution or suspension; a square designates a solid pellet or precipitate. In general:
A fusion buffer solution FB containing 2700 mg solids and a hemagglutinin suspension HA containing 46 mg HA and 31.1 µmol viral phospholipids VPL were mixed and subjected to ultracentrifugation UC1. The resulting pellet was dissolved in solution III containing fusion buffer FB and octaethylene glycol monododecyl ether OEG as a mild detergent. Another ultracentrifugation UC2 yielded a supernatant IV containing FB, HA, VPL and OEG, to which was added the antiviral drug dextran sulfate DS to make up a solution V. In the meantime, a phosphatidylcholine (PC) solution VI was vacuum dried in a rotating round-bottom flask producing a thin PC layer on the inner surface of the flask. Solution V was added to this layer and dissolved it to a solution VII which was treated three times with polystyrene beaded microcarriers PBM to remove the OEG and to result in a solution VIII, in which - by ultrasonification US - vesicles VES of the desired size were developped; the resulting suspension IX was diluted with NaCl solution X to give the drug suspension DR.

In detail:

A fusion buffer solution (I) was prepared containing 7.9 mg NaCl/ml, 4.4 mg/ml trisodiumcitrat dihydrate, 2.1 mg/ml 2-morpholinoethane sulfonic acid monohydrate (MES), and 1.2 mg/ml N-hydroxyethyl-piperazine-N'-2-ethane sulfonic acid in H_2O (pH adjusted with 1-N NaOH to 7.3).

Influenza virus (strain A/ Shanghai / 16/ 89 (H3N2)) was grown in the allantoic cavity of hen eggs, and was purified twice by ultracentrifugation in a sucrose gradient, as described by Skehel and Schild 1971 (Virology 44, 396 - 408). The purified influenza virion suspension (II) contained 266 µg of influenza virus hemagglutinin per ml and a total of 31.1 µmol of viral phospholipids, which were determined as follows:
Viral phospholipids were extracted according to Folch et al. (Folch, J., Lees, M. and Sloane, S.G.H. (1957); J. Biol. Chem. 226, 497-509). For analysis of phospholipids, the lower organic phase was evaporated and the residue either subjected to phosphate determination (Chen, P.S., Toribara, T.Y. and Warner, H. (1956); Anal. Chem. 28, 1756-1758), or dissolved in a small volume of chloroform-methanol for subsequent TLC analysis of the phospholipids. Silica gel plates (Merck) were used and were developed in the solvent system chloroform-methanol-acetic acid-water (25:15:4:2; v/v/v/v). The individual phospholipids were visualized by exposure to iodine and were identified on the basis of their RF values (comparison with reference samples). Protein was determined by a modified Lowry procedure (Peterson, G.L. (1977); Anal. Biochem. 83, 346-356).

A total of 173 ml of solution (II) was mixed with the same volume of said fusion buffer solution (I). This influenza virus dilution was pelletted by ultracentrifugation at 100,000 x g for 10 minutes.

15.3 ml of a detergent solution containing 54 mg/ml (=100 µmol/ml) of octaethylene glycol monododecyl ether (OEG) in said fusion buffer solution was added to the influenza virus pellet. This corresponds to 18 µg = 33.3 nmol OEG per µg hemagglutinin. After 10 minutes, the pellet was completely dissolved. The solution was subjected to a 1 hour ultracentrifugation at 100,000 x g. 3000 mg of the dried antiviral drug dextran sulfate (Lüscher, M. and Glück, R., Antiviral Research 14, 39 - 50) were added to the remaining supernatant IV containing the solubilized influenza hemagglutinin trimer plus the non-essential neuraminidase residue and other constituents, making up solution V.

Phosphatidylcholine (SIGMA) was dissolved in a 2 : 1 mixture of chloroform and methanol to a concentration of 10 mg/ml. 28 ml of this solution VI were carefully evaporated by applying a vacuum in a rotary evaporator, forming a thin phospholipid layer of 280 mg on the inner surface of a round-bottom flask.

The solution V was now added to the phospholipid layer in the round-bottom flask. After shaking for at least 15 minutes and complete dissolution of the phosphatidylcholine, the resulting solution VII was transferred to a glass container together with 4.8 g of polystyrene beaded microcarriers PBM having a mesh size (wet) of 25 - 50.

The container was then shaken in such a way that the content moved twice per second from one end to the other. One hour later, the suspension was aspirated with a thin pipette and transferred to a new container with 2.4 g of polystyrene beaded microcarriers of the same size. The column was shaken for 30 minutes. This procedure was repeated twice. After the last step, the resulting solution VIII was removed from the beads, fixed in a waterbath and treated with an ultrasonification apparatus (Bransonic, Branson Europe BV, frequency 50 kHz ±10%). 10 seconds of ultrasonic shocks repeated twice, after intervals of 10 seconds each, yielded medium sized vesicles with a diameter of about 50 - 100 nm. The final solution IX

was then diluted 1:100 with physiological NaCl solution X.

Example 2:

Preparation of synthetic membrane vesicles containing an antiviral antibody drug adsorbed with hemagglutinin trimers of influenza virus and CD4 monoclonal antibodies.

The vesicles were prepared according to Example 1 with the following modification: instead of 10 mg, only 9 mg of phosphatidylcholine, and 1 mg of phosphatidylethanolamine (kephaline) per ml were dissolved in a 2:1 mixture of chloroform and methanol.

After the preparation of hemagglutinin vesicles according to Example 1, 20 mg of sulfosuccinimidyl-4-(N-maleimidophenyl)butyrate (SMPB) (as a crosslinker) in 2 ml of water were added to the solution. After 1 hour at room temperature under gentle shaking, the vesicles were pelletted by ultracentrifugation during 10 min at 100,000 x g.

8 ml of Anti-Leu 3A (Becton & Dickinson) were added to the pellet. The resuspended material was carefully shaken for a few seconds every 5 minutes during one hour at room temperature. Finally, the material was pelletted again (to remove non-bonded antibodies) by ultracentrifugation at 100,000 x g for 10 minutes. The pellet was resuspended in 1,500 ml of physiological NaCl solution.

Example 3:

Example 2 was repeated with the following modifications:
Instead of dextran sulfate, 1000 mg each of imidazolcarboxamide and hydroxy-urea (pharmaceuticals efficient against melanomas as described by Brunner and Nagel, Springer Verlag, 2nd edition, Internistische Krebstherapie, page 93 (1979)) were added to the solution IV (see Example 1).

After adding the crosslinker and further processing as in Example 2, 1 mg of a monoclonal antibody (either R 24 as described by Houghton, A.N. et al. (1985), Proc. Nat. Ac. Sc. Vol. 82, p. 1242; or 0.5 mg L55 + 0.5 mg L72 as decribed by Iric, R.S. et al., Lancet (1989), p. 786-787) were added to the vesicle solution VES to result in the following total composition for 1000 human doses:
46 mg hemagglutinin
250 mg phosphatidylcholine
30 mg phosphatidylethanolamine (kephaline)
20 mg crosslinker (Sulfo - SMPB)
1 mg monoclonal antibody
1000 mg imidazol-carboxamide
1000 mg hydroxy-urea

These pharmaceuticals so far conventionally had to be applied in about 5-fold quantitative dosage, i.e. the indicated quantities are for 200 human doses only.

Example 4:

Example 2 was repeated with the following modifications:
Instead of dextran sulfate, 1000 mg each of at least one of the following pharmaceuticals: adriplastin, endoxan, fluoro-uracil (as described by Brunner and Nagel, Internistische Krebstherapie; Springer-Verlag, 2nd edition, page 309, 1979) and colchicine (SIGMA) were added to the solution IV (see Example 1).

After adding the crosslinker and further processing as in Example 2, 1 mg of a monoclonal antibody JDB1 (as described by Strelkauskas, A.J., Cancer-Immunol. Immunother. Vol. 23, p.31, 1986) was added to the vesicle solution VES to result in the following total composition for 1000 human doses of a pharmaceutical against mamma-carcinomas.
46 mg hemagglutinin
250 mg phosphatidylcholine
30 mg phosphatidylethanolamine
20 mg crosslinker (Sulfo - SMPB)
1 mg monoclonal antibody
1000 mg adriplastin
1000 mg endoxan
1000 mg fluoro-uracil

Example 5:

Examples 1 and 2 were repeated with the following modifications:
Instead of influenza hemagglutinin trimers, the monomers including the fusion peptide were used. The hemagglutinin-2-monomer containing the fusion peptide was obtained by cleavage of the S-H bridges by chemical reduction with D4-dithiothreitol (DTT, Sigma) and subsequent separation from the hemagglutinin-1 peptide by gel chromatography (Sephadex G 50) at pH 6. The purified hemagglutinin-2-monomer suspension contained 180 µg of monomers including the fusion peptide.

Example 6:

Example 2 was repeated with the following modifications:
Instead of influenza hemagglutinin trimers, the crude fusion peptide was used. The influenza fusion peptide used for preparation of synthetic membrane vesicles was obtained by chemical synthesis. Any one of the aminoacid sequences listed in Fig.2 may be used. The arrangement of at least one, preferably three, cystein groups at one end of the respective sequence has been found useful for the fusion activity.

The solution with one of these synthetic fusion peptides contained 4 µg per ml.

The vesicles containing one of the above fusion peptides have been prepared as follows:
9 mg of phosphatidylcholine and 1 mg of phosphatidylethanolamine per ml were dissolved in a 2:1 mixture of chloroform and methanol. 28 ml of this solution were carefully evaporated by applying a vacuum in a rotary evaporator forming a thin phospholipid layer on the inner surface of a round-bottom flask.

3 g of dextran sulfate were dissolved in 15.3 ml of a detergent solution containing 100 µmol of octaethylene glycol monododecyl ether per 1 ml of fusion buffer; the solution was then added to the phospholipid layer in the round-bottom flask. After shaking for at least 15 minutes and complete dissolution of the phospholipid layer, the solution was transferred to a glass container together with 4.8 g of polystyrene beaded microcarriers having a mesh size (wet) of 25-50.

The container was then shaken in such a way that the content moved twice per second from one end to the other. One hour later, the suspension was aspirated with a thin pipette and transferred to a new container with 2.4 g of polystyrene beaded microcarriers of the same size. The container was shaken for 30 minutes. This procedure was repeated twice. After the last step, the resulting solution was removed from the beads, fixed in a waterbath and treated with an ultrasonification apparatus (Bransonic, Branson Europe BV, frequency 50 kHz ±10%). 10 seconds of ultrasonic shocks repeated twice, after intervals of 10 seconds each, yielded medium size vesicles with a diameter of about 50-100 nm.

2 ml of water containing 20 mg of sulfo-SMPB (Pierce) were added to the above suspension. After 1 hour at room temperature under gentle shaking, the vesicles were pelletted by 15 minutes of ultracentrifugation at 100,000 x g.

2 ml of the solution containing the synthetic fusion peptide were added to the pellet. The resuspended material was carefully shaken for a few seconds every 5 minutes during 1 hour at room temperature. Finally, the material was pelletted again (to remove non-bonded fusion peptides) by ultracentrifugation at 100,000 x g for 10 minutes. The pellet was resuspended in 200 ml of physiological NaCl solution.

Example 7:

Example 6 was repeated with the following modifications:
2 ml of a solution containing 2 µg of a synthetic fusion peptide and 2 ml containing 100 µg of Anti-Leu 3A were added to the pellet containing the vesicles. The resuspended material was carefully shaken for a few seconds every 5 minutes during one hour at room temperature. Finally, the material was pelletted again (to remove non-bonded fusion and cell specific peptides) by ultracentrifugation at 100,000 x g for 10 minutes. The pellet was resuspended in 200 ml of physiological NaCl solution.

Example 8:

Vesicles according to Examples 1 through 4 were prepared with the fusion peptide or hemagglutinin from rhabdoviruses, parainfluenzaviruses or togaviruses.

a) The rhabdovirus rabies was produced in human diploid cells. The harvests (supernatants) containing 107 rabies viruses per ml were purified and concentrated by sucrose density ultracentrifugation. The purified rabies virus suspension contained 210 µg of rabies virus hemagglutinin per ml and was further processed according to Example 1.

b) The parainfluenza virus type III was grown in the allantoic cavity of hen eggs and was purified twice by ultracentrifugation in a sucrose gradient as in Example 1. The purified parainfluenza virion suspension contained 245 µg of parainfluenza virus hemagglutinin per ml and was further processed according to Example 1.

c) The togavirus rubella was produced in human diploid cells and purified according a). The purified rubella virion suspension contained 205 µg of rubella virus hemagglutinin per ml and was further processed according to Example 1.

Example 9:

Antiviral drugs were prepared according to Example 1. hu-PBL-SCID mice were treated for 14 days with ribonuclease dimer for 10 days alternatively with a phosphate buffer solution (blank test) only, with dextran sulfate or ribonuclease dimer (prepared according to Example 1 of PCT/US90/00141) at three dose levels, with dextran sulfate in liposomes, or ribonuclease dimer in liposomes, both latter prepared according to Example 1 of the present invention. Treatment was initiated at the same time as virus challenge with 100 $TCID_{50}$ of $HIV\text{-}1_{IIIB}$. hu-PBL-SCID mice were assessed for evidence of viral infection (virus isolation, PCR detection of proviral sequences) at 2, 4, and 6 weeks following viral infection. The results of the study show the percentage of animals in each treatment group from which virus was isolated:

phosphate buffer solution (blank test)	80%
ribonuclease dimer 0.001 mg/kg	92%
ribonuclease dimer 0.1 mg/kg	30%
ribonuclease dimer 10.0 mg/kg	63%
dextran sulfate 10 mg/kg	33%
dextran sulfate 10 mg/kg in liposomes	14%
ribonuclease dimer in liposomes	25%

The study results indicate protection of the majority of treated hu-PBL-SCID mice from HIV infection following q. 12 hr. injection of 0.1 mg/kg ribonuclease dimer, 10 mg/kg dextran sulfate, liposomes containing ribonuclease dimer, and liposomes containing dextran sulfate. Protection was marginal with ribonuclease dimer at 10 mg/kg, which might be due to the fact that, at higher dosage levels, ribonuclease dimer suppresses immunity. No protective effect of ribonuclease dimer at 0.001 mg/kg was seen. However, with ribonuclease dimer in liposomes according to the present invention, the success rate improved from 63% to 25% in spite of the high dosage. Further improvement is expected from optimum dosage levels in liposomes.

The same conclusions hold when mice with poorly functioning human PBL grafts at the end of the experiment are excluded from analysis, although it appears from analysis of human immunoglobulin levels and β-globin PCR results that treatment with either ribonuclease dimer liposomes or dextran sulfate liposomes interfered with the survival of human cells. Exclusion of hu-PBL-SCID mice on the basis of human function allows some distinction between direct anti-viral effects and immunomodulatory activity.

Example 10:

In a fusion test described by Lüscher and Glück (1990) (Anti-viral Research 14, 39 - 50), vesicles prepared according to Example 1 were compared with reconstituted influenza vesicles prepared according to the method of Kawasaki et al. in fusion activity with model membranes:
Fig.3 shows the kinetics of fluorescence de-quenching of R18-labelled influenza A virus with DOPC-cholesterol liposomes. The increase in fluorescence is expressed in % FDQ, calculated according to Lüscher & Glück (see above).

The initial fusion rates were obtained from the tangents to the fusion curves at time 0, when the fusion was initiated (dotted line in Fig.3). Curve 2 corresponds to the fusion activity of vesicles prepared according to the method of Kawasaki et al.

Claims

Claims for the following Contracting States : AT, BE, CH, LI, DE, DK, FR, GB, IT, LU, NL, SE

1. A phospholipid bi-layer vesicle comprising cell-specific markers on the membrane and containing at least one desired drug or pharmaceutically active substance, characterized in that the cell-specific markers have at least 90 % biological activity when measured according to Luescher & Glueck, Antiviral Research 14, 39-50.

2. A vesicle according to claim 1, characterized in that the cholesterol content in the membrane is less than 10%, preferably less than 2% by weight and/or the detergent content in the membrane is less than 10, preferably less than 1 ppb.

3. A vesicle according to claim 1 or 2, characterized in that its diameter is smaller than 100 nm and preferably is about 80 nm.

4. A vesicle according to any one of the preceding claims, characterized in that the cell-specific markers comprise at least one - preferably a monoclonal - antibody.

5. A vesicle according to any one of the preceding claims, characterized in that the cell-specific markers are selected from the group consisting of hemagglutinin trimer or monomer, one or both cleaved subunits thereof, glycopeptides HA1 and HA2, fusion peptide isolated from natural sources, synthetic "fusion peptide".

6. A vesicle according to claim 5, characterized in that the hemagglutinin stems from at least one member of the group consisting of influenza virus, preferably from the A-H_1N_1 subtype, rhabdovirus, parainfluenza virus and togavirus.

7. A vesicle according to any one of claims 1 to 3, characterized in that the cell-specific markers comprise at least one fusion peptide in the form of a sequence of aminoacids, at least one end of the sequence being formed by at least one cystein group, preferably by three cystein groups.

8. A vesicle according to any one of the preceding claims, wherein the phospholipid stems from at least one member of the group consisting of influenza virus, preferably from the A-H_1N_1 subtype, rhabdovirus, parainfluenza virus and togavirus, in combination with a 2 to 100-, preferably 5 to 15-fold quantity of phosphatidylcholine.

9. A vesicle according to any one of the preceding claims, characterized in that
   - the phospholipid in the membrane comprises 70 to 95 % by weight of phosphatidylcholine and 5 to 30, preferably 10 to 20 % by weight of phosphatidyl ethanolamine;
   - 5 to 10, preferably 6 to 8 % by weight of a crosslinker, preferably of a sulfosuccinimidyl derivate, and at least one cell-specific fusion peptide are linked to the membrane.

10. A process for the preparation of phospholipid bi-layer vesicles containing fusion peptides, and - optionally -other cell-specific markers, on the membrane and further containing at least one desired drug or pharmaceutically active substance, comprising the steps of
    a) dissolving phospholipids, preferably together with the fusion peptides, in the presence of a detergent,
    b) adding the desired drug or substance,
    c) removing the detergent, thereby causing the formation of vesicles,
    d) treating vesicles to achieve a desired size,
    e) adding the fusion peptides unless they have been provided under a), and
    f) optionally adding other cell-specific markers, preferably in combination with a crosslinker,
    characterized in that a non-ionic detergent, preferably octaethylene glycol monododecyl ether, is used that does not react with hemagglutinin, the detergent is removed by repeated treatment with polystyrene beaded microcarriers, and the desired size of the vesicles is achieved by ultrasonification.

11. Process according to claim 10, characterized in that the detergent is used in a concentration of between 10 and 250, preferably between 80 and 120 µmol/ml; and/or the detergent is removed by

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application of 1 to 2, preferably of about 1.5 g polystyrene beaded microcarriers per 100 mg detergent, the microcarriers preferably having a mesh size - wet - of 20 to 50;
and/or the desired vesicle size is 50 to 100 nm in diameter.

12. The use of vesicles according to any one of claims 1 to 9, or prepared according to claim 10 or 11, for the preparation of a pharmaceutical against AIDS.

13. Use according to claim 12 in which the vesicles contain at least one of the following substances: dextran sulfate, ribonuclease dimer, lysozyme dimer.

14. The use of vesicles according to any one of claims 1 to 9, or prepared according to claim 10 or 11, for the preparation of a pharmaceutical against carcinomas.

15. Use according to claim 14 in which the vesicles contain at least one of the following substances: imidazol-carboxamide, hydroxy-urea, adriplastin, endoxan, fluoro-uracil, colchicine.

Claims for the following Contracting States : ES, GR

1. A process for the preparation of phospholipid bi-layer vesicles containing fusion peptides, and - optionally - other cell-specific markers, on the membrane and further containing at least one desired drug or pharmaceutically active substance, comprising the steps of
   a) dissolving phospholipids, preferably together with the fusion peptides, in the presence of a detergent,
   b) adding the desired drug or substance,
   c) removing the detergent, thereby causing the formation of vesicles,
   d) treating vesicles to achieve desired size,
   e) adding the fusion peptides unless they have been provided under a), and
   f) optionally adding other cell-specific markers, preferably in combination with a crosslinker,
   characterized in that a non-ionic detergent, preferably octaethylene glycol monododecyl ether, is used that does not react with hemagglutinin, the detergent is removed by repeated treatment with polystyrene beaded microcarriers, and the desired size of the vesicles is achieved by ultrasonification.

2. Process according to claim 1, characterized in that the microcarriers have a mesh size - wet - of 20 to 50 and the desired vesicle size is 50 to 100 nm in diameter.

3. Process according to claim 1 or 2, characterized in that the detergent solution is used in a concentration of between 10 and 250, preferably between 80 and 120 µmol/ml and the detergent is removed by application of 1 to 2, preferably of about 1.5 g polystyrene beaded microcarriers per 100 mg detergent.

4. Process according to any one of the preceding claims, characterized in that the fusion peptides are selected from the group consisting of hemagglutinin trimer or monomer, one or both cleaved subunits thereof, glycopeptides HA1 and HA2, fusion peptide isolated from natural sources, and synthetic fusion peptide.

5. Process according to any one of the preceding claims, characterized in that the vesicles comprise at least one - preferably a monoclonal - antibody as a cell-specific marker, and preferably a crosslinker to which the antibody is bound in such a manner that it is still fully biologically active.

6. Process according to any one of the preceding claims, with hemagglutinin as fusion peptide, characterized in that the hemagglutinin is derived from at least one member of the group consisting of influenza virus, preferably from the A-H_1N_1 subtype, rhabdovirus, parainfluenza virus and togavirus.

7. Process according to any one of claims 1 to 5, characterized in that the fusion peptides comprise at least one fusion peptide in the form of a sequence of aminoacids, at least one end of the sequence being formed by at least one cystein group, preferably by three cystein groups.

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8. Process according to any one of the preceding claims, wherein part of the phospholipids is derived from at least one member of the group consisting of influenza virus, preferably from the A-H_1N_1 subtype, rhabdovirus, parainfluenza virus and togavirus, and applied in combination with a 2 to 100-, preferably 5 to 15-fold quantity of phosphatidylcholine.

9. Process according to any one of the preceding claims, characterized in that
   - the phospholipids comprise 70 to 95 % by weight of phosphatidylcholine and 5 to 30, preferably 10 to 20 % by weight of phosphatidyl ethanolamine;
   - 5 to 10, preferably 6 to 8 % by weight of a crosslinker, preferably of a sulfosuccinimidyl derivate,
   - at least one fusion peptide, and
   - at least one cell-specific antibody bound to the crosslinker.

10. Process according to any one of the preceding claims, wherein the vesicles contain at least one of the following substances: dextran sulfate, ribonuclease dimer, lysozyme dimer, imidazol-carboxamide, hydroxy-urea, adriplastin, endoxan, fluoro-uracil, colchicine.

Patentansprüche
Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, LI, DE, DK, FR, GB, IT, LU, NL, SE

1. Zweischichtiges Phospholipidvesikel mit zellspezifischen Indikatoren an der Membrane, welches wenigstens ein gewünschtes Arzneimittel bzw. eine pharmazeutisch aktive Substanz enthält, dadurch gekennzeichnet, die zellspezifischen Indikatoren - gemessen nach Luescher & Glueck, Anti-viral Research 14, 39-50 - zumindest zu 90% eine biologische Aktivität aufweisen.

2. Vesikel nach Anspruch 1, dadurch gekennzeichnet, daß der Cholesterolgehalt in der Membrane geringer als 10%, vorzugsweise geringer als 2 Gewichts-%, ist und/oder der Detergensgehalt in der Membrane geringer als 10, vorzugsweise geringer als 1 ppb, ist.

3. Vesikel nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß sein Durchmesser kleiner als 100 nm ist und vorzugsweise etwa 80 nm beträgt.

4. Vesikel nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß die zellspezifischen Indikatoren wenigstens einen - vorzugsweise monoklonalen - Antikörper aufweisen.

5. Vesikel nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß die zellspezifischen Indikatoren aus der aus Hämagglutinin-Trimer oder -Monomer, einem oder beiden seiner gespaltenen Untereinheiten, Glykopeptiden HA1 und HA2, einem aus natürlichen Quellen isoliertem Fusionspeptid und einem synthetischen "Fusionspeptid" bestehenden Gruppe ausgewählt sind.

6. Vesikel nach Anspruch 5, dadurch gekennzeichnet, daß das Hämagglutinin von wenigstens einem Vertreter der aus dem Grippevirus, vorzugsweise von der Unterart A-H_1N_1, dem Rhabdovirus, dem Paragrippevirus und dem Togavirus bestehenden Gruppe stammt.

7. Vesikel nach einem der Ansprüche 1 bis 3, dadurch gekennzeichnet, daß die zellspezifischen Indikatoren wenigstens ein Fusionspeptid in der Form einer Sequenz von Aminosäuren aufweist, wobei wenigstens ein Ende der Sequenz von mindestens einer Cysteingruppe gebildet wird, vorzugsweise von drei Cysteingruppen.

8. Vesikel nach einem der vorhergehenden Ansprüche, bei dem das Phospholipid von wenigstens einem Vertreter der aus dem Grippevirus, vorzugsweise von der Unterart A-H_1N_1, dem Rhabdovirus, dem Paragrippevirus und dem Togavirus bestehenden Gruppe stammt, in Kombination mit einer 2- bis 100-, vorzugsweise 5- bis 15-fachen, Menge an Phosphatidylcholin.

9. Vesikel nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß
   - das Phospholipid in der Membrane 70 bis 95 Gewichts-% Phosphatidylcholin und 5 bis 30, vorzugsweise 10 bis 20 Gewichts-%, Phosphatidyl-Äthanolamin aufweist;
   - 5 bis 10, vorzugsweise 6 bis 8 Gewichts-%, eines Vernetzers, vorzugsweise eines Sulfosuccinimidyl-Derivates, und mindestens ein zellspezifisches Fusionspeptid an die Membrane gebunden

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sind.

10. Verfahren für die Herstellung von Fusionspeptide - und gegebenenfalls andere zellspezifische Indikatoren - an der Membran enthaltenden zweischichtigen Phospholipidvesikeln, die ferner wenigstens ein gewünschtes Arzneimittel bzw. eine pharmazeutisch aktive Substanz enthalten, welches folgende Schritte aufweist
    a) Auflösen von Phospholipiden, vorzugsweise zusammen mit den Fusionspeptiden, in Gegenwart eines Detergens,
    b) Zugeben des gewünschten Arzneimittels bzw. Substanz,
    c) Entfernen des Detergens, wodurch die Bildung von Vesikeln verursacht wird,
    d) Behandeln der Vesikeln zur Erzielung einer gewünschten Größe,
    e) Zugeben der Fusionspeptide, außer sie wurden schon unter a) vorgesehen, und
    f) gegebenenfalls Zugeben anderer zellspezifischer Indikatoren, vorzugsweise in Kombination mit einem Vernetzer,
    dadurch gekennzeichnet, daß ein nicht-ionisches Detergens, vorzugsweise Octaäthylenglykol-Monododecyläther, benutzt wird, das mit Hämagglutinin nicht reagiert, das Detergens durch wiederholte Behandlung mit mit Polystyrol geperlten Mikroträgern entfernt wird, und die gewünschte Größe der Vesikeln durch Behandeln mit Ultraschall erzielt wird.

11. Verfahren nach Anspruch 10, dadurch gekennzeichnet, daß das Detergens in einer Konzentration zwischen 10 und 250, vorzugsweise zwischen 80 und 120 µmol/ml, verwendet wird; und/oder das Detergens durch Anwendung von 1 bis 2, vorzugsweise von etwa 1,5 g, von mit Polystyrol geperlten Mikroträgern pro 100 mg Detergens entfernt wird, wobei die Mikroträger vorzugsweise eine Mesh-Größe - im feuchten Zustande - von 20 bis 50 haben; und/oder die gewünschte Vesikelgröße im Durchmesser 50 bis 100 nm beträgt.

12. Verwendung der Vesikeln nach einem der Ansprüche 1 bis 9 bzw. welche nach den Ansprüchen 10 oder 11 hergestellt sind, für die Präparierung eines pharmazeutischen Stoffes gegen AIDS.

13. Verwendung nach Anspruch 12, bei der die Vesikeln wenigstens eine der folgenden Substanzen enthalten: Dextransulfat, Ribonuclease-Dimer, Lysozym-Dimer.

14. Verwendung der Vesikeln nach einem der Ansprüche 1 bis 9 bzw. welche nach den Ansprüchen 10 oder 11 hergestellt sind, für die Präparierung eines pharmazeutischen Stoffes gegen Karzinome.

15. Verwendung nach Anspruch 14, bei der die Vesikeln wenigstens eine der folgenden Substanzen enthalten: Imidazol-Carboxamid, Hydroxy-Harnstoff, Adriplastin, Endoxan, Fluor-Uracil, Colchizin.

Patentansprüche für folgende Vertragsstaaten : ES, GR

1. Verfahren für die Herstellung von Fusionspeptide - und gegebenenfalls andere zellspezifische Indikatoren - an der Membran enthaltenden zweischichtigen Phospholipidvesikeln, die ferner wenigstens ein gewünschtes Arzneimittel bzw. eine pharmazeutisch aktive Substanz enthalten, welches folgende Schritte aufweist
    a) Auflösen von Phospholipiden, vorzugsweise zusammen mit den Fusionspeptiden, in Gegenwart eines Detergens,
    b) Zugeben des gewünschten Arzneimittels bzw. Substanz,
    c) Entfernen des Detergens, wodurch die Bildung von Vesikeln verursacht wird,
    d) Behandeln der Vesikeln zur Erzielung einer gewünschten Größe,
    e) Zugeben der Fusionspeptide, außer sie wurden schon unter a) vorgesehen, und
    f) gegebenenfalls Zugeben anderer zellspezifischer Indikatoren, vorzugsweise in Kombination mit einem Vernetzer,
    dadurch gekennzeichnet, daß ein nicht-ionisches Detergens, vorzugsweise Octaäthylenglykol-Monododecyläther, benutzt wird, das mit Hämagglutinin nicht reagiert, das Detergens durch wiederholte Behandlung mit mit Polystyrol geperlten Mikroträgern entfernt wird, und die gewünschte Größe der Vesikeln durch Behandeln mit Ultraschall erzielt wird.

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2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß die die Mikroträger eine Mesh-Größe - im feuchten Zustande - von 20 bis 50 haben und die gewünschte Vesikelgröße im Durchmesser 50 bis 100 nm beträgt.

3. Verfahren nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß die Detergenslösung in einer Konzentration zwischen 10 und 250, vorzugsweise zwischen 80 und 120 µmol/ml, verwendet wird und das Detergens durch Anwendung von 1 bis 2, vorzugsweise von etwa 1,5 g, von mit Polystyrol geperlten Mikroträgern pro 100 mg Detergens entfernt wird.

4. Verfahren nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß die Fusionspeptide aus der aus Hämagglutinin-Trimer oder -Monomer, einem oder beiden seiner gespaltenen Untereinheiten, Glykopeptiden HA1 und HA2, einem aus natürlichen Quellen isolierten Fusionspeptid und einem synthetischen Fusionspeptid bestehenden Gruppe ausgewählt sind.

5. Verfahren nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß die Vesikeln wenigstens einen - vorzugsweise monoklonalen - Antikörper als zellspezifischen Indikator aufweisen, und vorzugsweise einen Vernetzer, an den der Antikörper derart gebunden ist, daß er biologisch noch voll aktiv ist.

6. Verfahren nach einem der vorhergehenden Ansprüche, mit Hämagglutinin als Fusionspeptid, dadurch gekennzeichnet, daß das Hämagglutinin von wenigstens einem Vertreter der aus dem Grippevirus, vorzugsweise von der Unterart A-H_1N_1, dem Rhabdovirus, dem Paragrippevirus und dem Togavirus bestehenden Gruppe abgeleitet ist.

7. Verfahren nach einem der Ansprüche 1 bis 5, dadurch gekennzeichnet, daß die Fusionspeptide wenigstens ein Fusionspeptid in der Form einer Sequenz von Aminosäuren aufweist, wobei wenigstens ein Ende der Sequenz von mindestens einer Cysteingruppe gebildet wird, vorzugsweise von drei Cysteingruppen.

8. Verfahren nach einem der vorhergehenden Ansprüche, bei dem ein Teil der Phospholipide von wenigstens einem Vertreter der aus dem Grippevirus, vorzugsweise von der Unterart A-H_1N_1,dem Rhabdovirus, dem Paragrippevirus und dem Togavirus bestehenden Gruppe abgeleitet ist und in Kombination mit einer 2- bis 100-, vorzugsweise 5- bis 15-fachen, Menge an Phosphatidylcholin angewandt wird.

9. Verfahren nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß
   - die Phospholipide 70 bis 95 Gewichts-% Phosphatidylcholin und 5 bis 30, vorzugsweise 10 bis 20 Gewichts-%, Phosphatidyl-Äthanolamin aufweisen;
   - 5 bis 10, vorzugsweise 6 bis 8 Gewichts-%, eines Vernetzers, vorzugsweise eines Sulfosuccinimidyl-Derivates,
   - mindestens ein Fusionspeptid, und
   - wenigstens einen zellspezifischen, an den Vernetzer gebundenen Antikörper.

10. Verfahren nach einem der vorhergehenden Ansprüche, bei dem die Vesikeln wenigstens eine der folgenden Substanzen enthalten: Dextransulfat, Ribonuclease-Dimer, Lysozym-Dimer, Imidazol-Carboxamid, Hydroxy-Harnstoff, Adriplastin, Endoxan, Fluor-Uracil, Colchizin.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, LI, DE, DK, FR, GB, IT, LU, NL, SE

1. Vésicule de couche double de phospholipides comprenant des marqueurs spécifiques des cellules sur la membrane et contenant au moins un médicament ou une substance pharmaceutiquement active désirée, caractérisée en ce que les marqueurs spécifiques des cellules ont une activité biologique, mesurée selon Luescher & Glueck, Antiviral Research 14, 39-50, d'au moins 90 %.

2. Vésicule selon la revendication 1, caractérisée en ce que la teneur en cholestérol de la membrane est inférieure à 10 %, de préférence inférieure à 2 % en poids, et/ou la teneur en détergent de la membrane est inférieure à 10, de préférence inférieure à une partie par milliard (ppb).

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3. Vésicule selon les revendications 1 ou 2, caractérisée en ce que son diamètre est inférieur à 100 nm et est de préférence d'environ 80 nm.

4. Vésicule selon l'une quelconque des revendications précédentes, caractérisée en ce que les marqueurs spécifiques des cellules comprennent au moins un anticorps, de préférence monoclonal.

5. Vésicule selon l'une quelconque des revendications précédentes, caractérisée en ce que les marqueurs spécifiques des cellules sont choisis parmi le groupe constitué de l'hémagglutinine trimère ou monomère, d'une de ses sous-unités coupées ou des deux, des glycopeptides HA1 et HA2, d'un peptide de fusion isolé de sources naturelles, d'un "peptide de fusion" synthétique.

6. Vésicule selon la revendication 5, caractérisée en ce que l'hémagglutinine provient d'au moins un membre du groupe constitué du virus de l'influenza, de préférence du sous-type A-H_1N_1, du rhabdovirus, du virus du parainfluenza et du togavirus.

7. Vésicule selon l'une quelconque des revendications 1 à 3, caractérisée en ce que les marqueurs spécifiques des cellules comprennent au moins un peptide de fusion sous la forme d'une séquence d'aminoacides, au moins une extrémité de la séquence étant formée par au moins un groupe cystéine, de préférence par trois groupes cystéine.

8. Vésicule selon l'une quelconque des revendications précédentes, dans laquelle le phospholipide provient d'au moins un membre du groupe constitué du virus de l'influenza, de préférence du sous-type A-H_1N_1, du rhabdovirus, du virus du parainfluenza et du togavirus, en association avec une quantité 2 à 100 fois, de préférence 5 à 15 fois supérieure de phosphatidylcholine.

9. Vésicule selon l'une quelconque des revendications précédentes, caractérisée en ce que
   - le phospholipide de la membrane comprend 70 à 95 % en poids de phosphatidylcholine et 5 à 30, de préférence 10 à 20 % en poids de phosphatidyl éthanolamine ;
   - de 5 à 10, de préférence de 6 à 8 % en poids d'un agent de réticulation, de préférence un dérivé sulfosuccinimidylé, et au moins un peptide de fusion spécifique des cellules, sont liés à la membrane.

10. Procédé pour la préparation de vésicules à double couche de phospholipides contenant des peptides de fusion et, si on le désire, d'autres marqueurs spécifiques des cellules, sur la membrane, et contenant en outre au moins un médicament ou une substance pharmaceutiquement active désirés, comprenant les stades consistant à :
    a) dissoudre les phospholipides, de préférence avec les peptides de fusion, en présence d'un détergent,
    b) ajouter le médicament ou la substance désirée,
    c) éliminer le détergent, provoquant ainsi la formation de vésicules,
    d) traiter les vésicules pour obtenir la taille désirée,
    e) ajouter les peptides de fusion à moins qu'ils n'aient été prévus en a) et
    f) si on le désire, ajouter d'autres marqueurs spécifiques des cellules, de préférence en association avec un agent de réticulation,
    caractérisé en ce qu'on utilise un détergent non ionique, de préférence l'éther monododécylique de l'octaéthylène-glycol, qui ne réagit pas avec l'hémagglutinine, en ce que le détergent est éliminé par des traitements répétés par des microsupports de perles de polystyrène et en ce que la taille désirée pour les vésicules est obtenue par traitement par les ultrasons.

11. Procédé selon la revendication 10, caractérisé en ce que le détergent utilisé a une concentration comprise entre 10 et 250, de préférence entre 80 et 120 µmoles/ml ; et/ou en ce que le détergent est éliminé par application de 1 à 2, de préférence d'environ 1,5 g de microsupports de perles de polystyrène pour 100 mg de détergent, les microsupports ayant de préférence une granularité, au mouillé, de 20 à 50 ; et/ou en ce que la taille désirée pour les vésicules est de 50 à 100 nm de diamètre.

12. Utilisation de vésicules selon l'une quelconque des revendications 1 à 9, ou préparées selon les revendications 10 ou 11, pour la préparation d'un produit pharmaceutique contre le SIDA.

13. Utilisation selon la revendication 12, dans laquelle les vésicules contiennent au moins une des substances suivantes : sulfate de dextrane, dimère de la ribonucléase, dimère du lyzozyme.

14. Utilisation des vésicules selon l'une quelconque des revendications 1 à 9, ou préparées selon les revendications 10 ou 11, pour la préparation d'un produit pharmaceutique contre les cancers.

15. Utilisation selon la revendication 14, dans laquelle les vésicules contiennent au moins une des substances suivantes : imidazol-carboxamide, hydroxy-urée, adriplastine, endoxane, fluoro-uracile, colchicine.

Revendications pour les Etats contractants suivants : ES, GR

1. Procédé de préparation de vésicules à double couche de phospholipides contenant des peptides de fusion et, si on le désire, d'autres marqueurs spécifiques des cellules, sur la membrane, et contenant en outre au moins un médicament ou une substance pharmaceutiquement active désirés, comprenant les stades consistant à :
   a) dissoudre les phospholipides, de préférence avec les peptides de fusion, en présence d'un détergent,
   b) ajouter le médicament ou la substance désirée,
   c) éliminer le détergent, provoquant ainsi la formation de vésicules,
   d) traiter les vésicules pour obtenir la taille désirée,
   e) ajouter les peptides de fusion à moins qu'ils n'aient été prévus en a) et
   f) si on le désire, ajouter d'autres marqueurs spécifiques des cellules, de préférence en association avec un agent de réticulation,
   caractérisé en ce qu'on utilise un détergent non ionique, de préférence l'éther monododécylique de l'octaéthylène-glycol, qui ne réagit pas avec l'hémagglutinine, on élimine le détergent par des traitements répétés avec des microsupports de perles de polystyrène et on obtient la taille désirée pour les vésicules par traitement par les ultrasons.

2. Procédé selon la revendication 1, caractérisé en ce que les microsupports ont une granularité - à l'état mouillé - de 20 à 50 et la taille désirée pour les vésicules est de 50 à 100 nm de diamètre.

3. Procédé selon les revendications 1 ou 2, caractérisé en ce que la solution de détergent est utilisée à une concentration comprise entre 10 et 250, de préférence entre 80 et 120 µmoles/ml et le détergent est éliminé par application de 1 à 2, de préférence d'environ 1,5 g de microsupports de perles de polystyrène pour 100 mg de détergent.

4. Procédé selon l'une quelconque des revendications précédentes, caractérisé en ce que les peptides de fusion sont choisis parmi le trimère ou le monomère de l'hémagglutinine, une de ses sous-unités coupées ou les deux, les glycopeptides HA1 et HA2, un peptide de fusion isolé de sources naturelles et un peptide de fusion synthétique.

5. Procédé selon l'une quelconque des revendications précédentes, caractérisé en ce que les vésicules comprennent au moins un anticorps - de préférence monoclonal - en tant que marqueur spécifique des cellules, et de préférence un agent de réticulation auquel l'anticorps est lié de telle manière qu'il ait toujours une activité biologique complète.

6. Procédé selon l'une quelconque des revendications précédentes, avec l'hémagglutinine comme peptide de fusion, caractérisé en ce que l'hémagglutinine est obtenue à partir d'au moins un membre du groupe constitué du virus de l'influenza, de préférence du sous-type A-H_1N_1, du rhabdovirus, du virus du parainfluenza et du togavirus.

7. Procédé selon l'une quelconque des revendications 1 à 5, caractérisé en ce que les peptides de fusion comprennent au moins un peptide de fusion sous la forme d'une séquence d'aminoacides, au moins une extrémité de la séquence étant formée par au moins un groupe cystéine, de préférence par trois groupes cystéine.

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8. Procédé selon l'une quelconque des revendications précédentes, dans lequel une partie des phospholipides est obtenue à partir d'au moins un membre du groupe constitué du virus de l'influenza, de préférence du sous-type A-H_1N_1, du rhabdovirus, du virus du parainfluenza et du togavirus, et appliquée en association avec une quantité de 1 à 100 fois, de préférence 5 à 15 fois supérieure de phosphatidylcholine.

9. Procédé selon l'une quelconque des revendications précédentes, caractérisé en ce que
   - les phospholipides comprennent 70 à 95 % en poids de phosphatidylcholine et 5 à 30, de préférence 10 à 20 % en poids de phosphatidyl éthanolamine ;
   - de 5 à 10, de préférence de 6 à 8 % en poids d'un agent de réticulation, de préférence d'un dérivé sulfosuccinimidylé ;
   - au moins un peptide de fusion, et
   - au moins un anticorps spécifique des cellules lié à l'agent de réticulation.

10. Procédé selon l'une quelconque des revendications précédentes, dans lequel les vésicules contiennent au moins une des substances suivantes : sulfate de dextrane, ribonucléase dimère, lysozyme dimère, imidazol-carboxamide, hydroxy-urée, adriplastine, endoxane, fluoro-uracile, colchicine.