

In addition, solubility of progesterone in DSPE-PEG micelles was approximately five to ten times larger than bile salt micelles (from 21 $\mu\text{g}/\text{ml}$ to $198 \pm 7 \mu\text{g}/\text{ml}$) for the same total lipid concentration, thereby suggesting that DSPE-PEG micelles have a greater potential as an efficient vehicle for insoluble drugs. However, this dispersion contained both SSM (at approximately 17 nm) and SSC (at approximately 150 nm).

EXAMPLE 13

According to the present example, enhanced solubility of normally water-insoluble compounds was further investigated using a DSPE-PEG micelle composition. In addition, a method for preparing micelles comprising a targeting agent in addition to an encapsulated water-insoluble compound was designed. Drug solubility was determined as follows.

Active drug loading was carried out by adding an excess of drug in powder form to a polyethylene microfuge tube containing PC/bile salt or DSPE-PEG prepared using the film method as previously described in Example 1. Excess drug was removed by centrifugation and the supernatant was analyzed by HPLC. In the case of progesterone, HPLC conditions included a YMC-CN (A-503, 250 \times 4.6 mm inner diameter) column, a mobile phase comprising acetonitrile and water (40:60), and a flow rate of 1.5 ml/minute. HPLC eluent was measured with adsorption at 254 nm. For PC/bile salt mixed micelles, the progesterone to lipid ratio was determined to be 0.0156. For DSPE-PEG micelles, the progesterone to lipid ratio was found to be 0.17.

Results indicated that progesterone (essentially insoluble in water as discussed above) in 10 mg/ml DSPE-PEG was soluble up to 198.5 $\mu\text{g}/\text{ml}$. This result was consistent with results using betulinic acid, sparingly soluble in water (see Merck Index, 12th Edition, p. 1213), which was soluble up to 200 $\mu\text{g}/\text{ml}$ in 10 mg/ml DSPE-PEG. In similar experiments with betulinic acid (also insoluble as defined in USP), solubility was calculated at 250 $\mu\text{g}/\text{ml}$ in either SSM or SSC.

In design of a targeted drug delivery system comprising micelles, a desired compound is incorporated in micelle compositions as described above. The resulting micelle compositions are then incubated with an amphiphilic compound to allow incorporation of the compound at, and into, the micelle surface as described in Example 1. The membrane associated compound in this arrangement acts as a targeting agent for the entire micelle composition to be delivered to, for example, a receptor for the membrane associated compound. In an alternative approach, the amphiphilic compound is linked, preferably through covalent modification, to one or the lipid components of the micelle. Through either of these mechanisms, the micelles can carry and deliver the incorporated drug to a target cell or tissue type expressing the cognate receptor.

For example, breast cancer cells express higher levels of VIP receptor than normal breast cells. Micelles comprising membrane associate VIP will therefore preferentially bind to a breast cancer cell rather than a normal cell. Since Taxol® has been shown to kill breast cancer cells, incorporating Taxol® in a VIP/micelle provides a targeted drug delivery for selective killing of the carcinoma cell type.

EXAMPLE 14

According to this aspect of the invention, the effect of infusion of enthelin-1 (ET-1) alone or in a SSM formulation

on mean arterial pressure (MAP), cardiac output (CO), total peripheral resistance (TPR), and regional blood circulation in anesthetized rats was examined using a radioactive microsphere technique. SSM with or without ET-1 were prepared according to the method described in Example 11 using DSPE-PEG in saline. Treatments for individual groups consisted of: (i) control, SSM at 2.7 mg/ml (n=6); (ii) ET-1 infusion at 50 ng/kg/min (n=5), and (iii) ET-1 at 50 ng/kg/min in SSM (n=8). Drugs were infused at 0.1 ml/min for 30 minutes.

Results showed that SSM did not affect MAP, CO, TPR or gastrointestinal tract (GIT) blood flow, however, an increase in blood flow to the kidneys (approximately 25%) and brain (approximately 19%) was observed compared to baseline. Blood flow decreased and vascular resistance increased in the kidney, GIT, and brain. ET-1 in SSM produced a significantly marked cardiovascular effect as compared to ET-1 alone. The increase in TPR was 102% in the ET-1 group and 227% in the group treated with ET-1 in SSM. Renal vascular resistance was 76% in the ET-1 group and 281% in the group treated with ET-1 in SSM. However, in brain, vascular resistance was 62% in the ET-1 group and 20% in the group treated with ET-1 in SSM. These results indicated that changes in MAP, TPR and regional vascular resistance are potentiated by ET-1 in SSM.

EXAMPLE 15

According to this aspect of the invention, the ability of micelle products of the invention to enhance cellular viability following cryopreservation was examined. In this experiment, cells were incubated with either DMSO, DSPE-PEG micelle products, or DSPE-PEG micelles products including VIP for 30 minutes prior to storage for 48 hours in liquid nitrogen. Follow removal from the liquid nitrogen, cells were thawed and viability was measured using Trypan blue using standard techniques.

Results indicated that cell viability following treatment with micelles, with or without associated VIP, was equal to or greater than cell viability following treatment with DMSO. Because DMSO is well known and routinely used in the art for cell cryopreservation, these results indicate that micelles can afford equal or better protection, thereby providing an alternative protective agent for cell storage.

Numerous modifications and variations in the invention as set forth in the above illustrative examples are expected to occur to those skilled in the art. Consequently only such limitations as appear in the appended claims should be placed on the invention.

What is claimed is:

1. A method of preparing a biologically active micelle product comprising one or more biologically active amphiphilic compounds in association with a micelle; said method comprising the steps of:

- a) mixing one or more lipids wherein at least one lipid component is covalently bonded to a water-soluble polymer;
- b) forming sterically stabilized micelles from lipids;
- c) incubating micelles from step (b) with one or more biologically active amphiphilic compound(s) under conditions in which said compound(s) becomes associated with said micelles in a more biologically active conformation.