

the ICR mice. The plasma was analyzed for ^{14}C -lipid after 1, 2 and 5 hours.

FIG. 20 shows the clearance of DNA encapsulated in particles composed of DOPE:DODAC:PEG-Cer(C_{20}) ((83.5:6.5:10 mole %) (83.5:6.5:10 mole %) The DNA and lipid are cleared much less rapidly from the circulation than when PEG-Cer(C_{14}) is used (see FIG. 21). Nearly 50% of the lipid and DNA are present after 1 hour. A significant amount of DNA and lipid were still present after 5 hr. The amount of DNA and lipid injected was 1.8 μg and 853 μg , respectively. Control particles exhibited a clearance similar to that of the plasmid-lipid particles.

FIG. 21 shows the clearance of DNA encapsulated in particles composed of DOPE:DODAC:PEG-Cer(C_{14}) ((83.5:6.5:10 mole %) Both DNA and lipid are cleared rapidly from the circulation with only about 20% of the lipid and 10% of the DNA present in the plasma after 1 hr. The amount of DNA and lipid injected was 2.7 μg and 912 μg , respectively. Control particles exhibited a clearance similar to that of the plasmid-lipid particles.

In Vivo Transfection in Lung, Liver and Spleen

Three groups of four IRC mice were injected via tail vein with pCMV4-CAT encapsulated in lipid particles composed of DOPE:DODAC:PEG-Cer(C_{14}) (83.5:6.5:10 mole %, "A") or DOPE:DODAC:PEG-Cer(C_{20}) (83.5:6.5:10 mole %, "B"), prepared as described above. The mice were sacrificed after 2, 4 and 8 days and the lung, liver and spleen were assayed for CAT activity according to a modification of Deigh, *Anal. Biochem.* 156:251-256 (1986). The amount of plasmid injected was 2.6 μg for the particles containing PEG-Cer(C_{14}) and 1.5 μg for the particles containing PEG-Cer(C_{20}).

FIG. 22 shows the results of in vivo transfection achieved in the lung. As can be seen from this figure, treatment with formulation "A" provided excellent transfection efficiency (based on CAT activity) up to 4 days. Formulation "B", while resulting in overall lower levels of CAT activity, provided relatively constant levels of enzyme activity over 8 days.

FIG. 23 shows the results of transfection achieved in the liver. For both formulations, transfection (and CAT activity) reached a maximum at 4 days.

FIG. 24 shows the results of transfection achieved in the spleen wherein the maximum transfection was found for both formulations to occur after 2 days.

VII. Conclusion

As discussed above, in accordance with one of its aspects, the present invention provides methods for preparing serum-stable plasmid-lipid particles which are useful for the transfection of cells, both in vitro and in vivo.

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

What is claimed is:

1. A nucleic acid-lipid particle, said particle comprising a cationic lipid; a non-cationic lipid; a PEG-lipid conjugate; and a nucleic acid.
2. The nucleic acid-lipid particle of claim 1, further comprising a sterol.
3. The nucleic acid-lipid particle of claim 1, wherein said cationic lipid is selected from the group consisting of DODAC, DDAB, DOTAP, DOTMA, DOSPA, DOGS, DC-Chol, and combinations thereof.
4. The nucleic acid-lipid particle of claim 1, wherein said non-cationic lipid is selected from the group consisting of DOPE, POPC, EPC, and combinations thereof.
5. The nucleic acid-lipid particle of claim 1, wherein said cationic lipid comprises from about 2% to about 55% by weight of the total lipid present in said particle.
6. The nucleic acid-lipid particle of claim 1, wherein said cationic lipid comprises from about 5% to about 45% by weight of the total lipid present in said particle.
7. The nucleic acid-lipid particle of claim 1, wherein said cationic lipid comprises from about 5% to about 15% by weight of the total lipid present in said particle.
8. The nucleic acid-lipid particle of claim 1, wherein said cationic lipid comprises from about 40% to about 50% by weight of the total lipid present in said particle.
9. The nucleic acid-lipid particle of claim 1, wherein said non-cationic lipid comprises from about 37% to about 89% by weight of the total lipid present in said particle.
10. The nucleic acid-lipid particle of claim 1, wherein said non-cationic lipid comprises from about 37% to about 75% by weight of the total lipid present in said particle.
11. The nucleic acid-lipid particle of claim 1, wherein said PEG-lipid comprises from about 1% to about 15% by weight of the total lipid present in said particle.
12. The nucleic acid-lipid particle of claim 1, wherein said PEG-lipid comprises about 10% by weight of the total lipid present in said particle.
13. The particle of claim 1, wherein said nucleic acid is DNA.
14. The particle of claim 13, wherein said DNA is a plasmid.
15. The nucleic acid-lipid particle of claim 1, wherein the nucleic acid in said nucleic acid-lipid particle is not substantially degraded after incubation of said serum in serum at 37° C. for 30 minutes.
16. A pharmaceutical composition comprising a nucleic acid-lipid particle comprising a cationic lipid; a non-cationic lipid; a PEG-lipid conjugate; and a nucleic acid; and, a pharmaceutically acceptable carrier.
17. The pharmaceutical composition of claim 16, wherein the nucleic acid-lipid particle further comprises a sterol.
18. The pharmaceutical composition of claim 16, wherein said nucleic acid is DNA.
19. A method of introducing a nucleic acid into a cell, said method comprising contacting said cell with a nucleic acid-lipid particle comprising a cationic lipid, a non-cationic lipid, a PEG-lipid conjugate, and a nucleic acid.
20. The method of claim 19, wherein the nucleic acid-lipid particle further comprises a sterol.

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