International Journal of Pharmaceutics, 69 (1991) 77–79

ADONIS 0378517391001152

IJP 02314

Note

Liposomes as carriers of ^{99m}Tc glucoheptonate for liver imaging

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(Received 18 July 1990)
(Modified version received 15 September 1990)
(Accepted 30 September 1990)

Key words: Liposome; ^{99m}Tc-GHA; Detergent dialysis; Biodistribution; Blood clearance; Scintigraphy

Summary

Liposomes were prepared by the detergent dialysis method and ^{99m}Tc-GHA, a radiopharmaceutical of choice for kidney imaging, was entrapped in SUV of different surface charges. The organ specificity of the agent with and without encapsulation in liposomes was studied in experimental animals. Results obtained show that ^{99m}Tc-GHA entrapped SUV can be targetted to liver. The effect of surface charge of liposomes on its targetting is discussed.

Liposomes are phospholipid vesicles which were discovered about three decades ago (Bangham et al., 1965), and have since been used as a carrier for delivery of various agents. More recently, liposomes have been used as diagnostic tools in the investigation of a variety of diseases. The diagnostic value of liposomes depends primarily on their ability to entrap a detector marker molecule, either within their cavity/cavities, or to attach to their membrane (Miller, 1975). For in vivo detection it is preferable for the marker to be a gamma emitter (radiopharmaceutical) which can be detected from the outside by suitable devices for investigations (De Kieviet, 1980). ^{99m}Tc-GHA is a radiopharmaceutical of choice for kidney imaging (Caride et al., 1976). In the present investigation the biokinetics of ^{99m}Tc-GHA have been altered by entrapping it in SUV. ^{99m}Tc-GHA entrapped SUV of different charges have been successfully targetted to the liver to various extents.

Liposomes were prepared by the method of detergent dialysis. Neutral, negative and positive charged liposomes were prepared with phosphatidylcholine, cholesterol and dicetyl phosphate (for negative charge) or stearylamine (for positive charge) in the molar ratio of 7 : 2 : 1 (Maierhofer, 1988). ^{99m}Tc-GHA was used in a kit form containing 5 mg GHA and 250 μg stannous chloride. The radiopharmaceutical preparation was used to dissolve the previously prepared and desiccated film of lipids. The resulting micelle solution was loaded on a Liposomat (Dianorm, Germany) and dialysed for 2–3 h against PBS. This resulted in the formation of ^{99m}TC-GHA entrapped SUV. The unentrapped agent was removed by passing the preparation through a Sephacryl HR-300 superfine column. The entrapment efficiency of neutral,

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positively and negatively charged SUV was found to be 28, 35 and 33%, respectively. The size of the SUV was determined by electron microscopy to be 20–50 nm.

Free and SUV entrapped ^{99m}Tc-GHA was injected intravenously in BALB/c mice and the biodistribution studied in various organs at time intervals of 30 min, 3 h and 18 h (complete data not shown). The organs that were studied included liver, spleen, lungs, heart, kidneys and stomach. A gamma counter (Iso-Data; Texas Instruments, U.S.A.) was used for quantitative analysis of the radiopharmaceutical in blood and other organs. Radioactivity of the whole organ was measured and expressed in terms of percentage per whole organ. The data collected indicates in confirmation with earlier reports that ^{99m}Tc-GHA is targetted to the kidneys (Caride et al., 1976); 23.1% of the injected dose was seen to concentrate in kidneys at 3 h, while only 11.6% was detected in liver at the same time interval (Fig. 1a–d). ^{99m}Tc-GHA entrapped SUV were targetted maximally to the liver. The percentage of radioactivity detected in liver after 3 h was 20.9, 12.8 and 11.9% for liposomes bearing a negative, neutral and positive charge, respectively. Only 2–3% of the radioactivity was detected in the kidneys after the same time interval. The other organs studied for biodistribu-

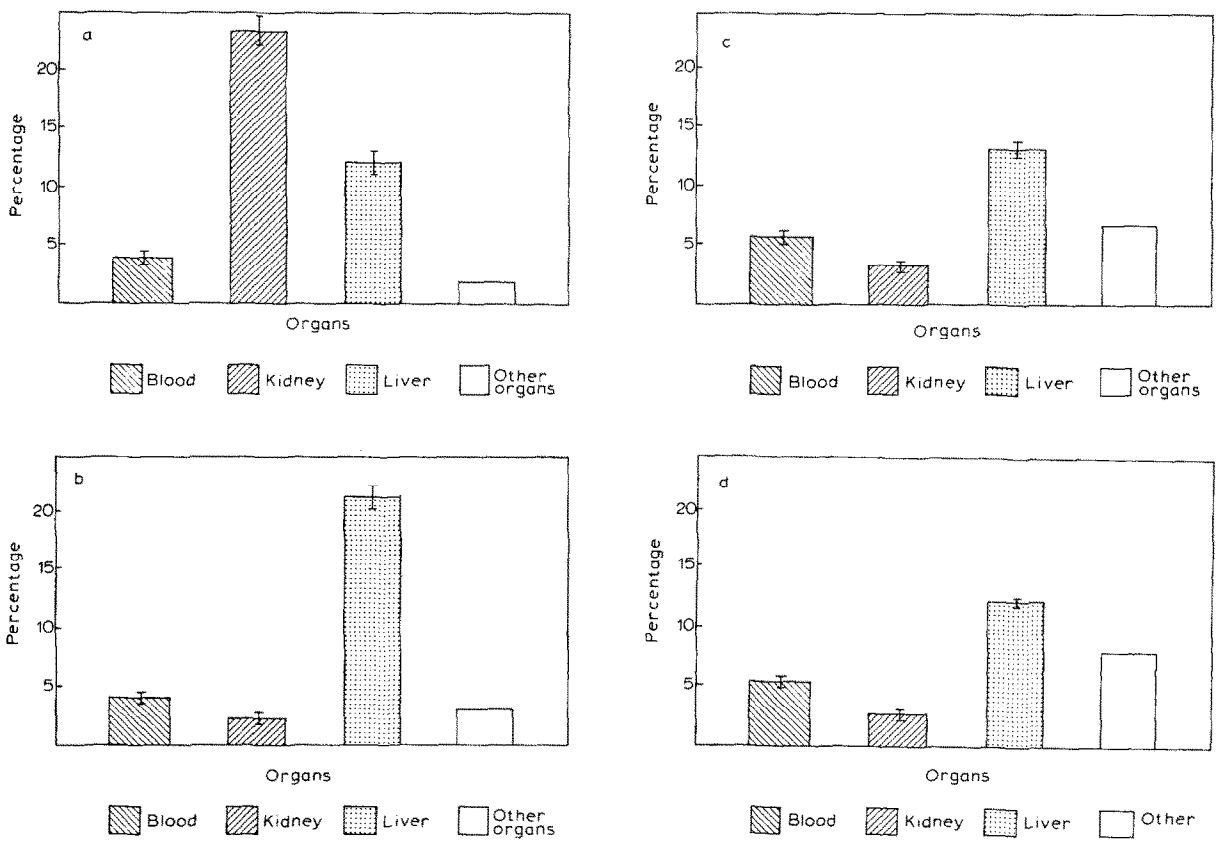

Fig. 1(a). Biodistribution of ^{99m}Tc-GHA in mice at 3 h (percentage of dose injected per whole organ). Each value is the mean ± SE of four mice. (b). Biodistribution of ^{99m}Tc-GHA entrapped SUV of negative charge in mice at 3 h (percentage of dose injected per whole organ). Each value is the mean ± SE of four mice. (c). Biodistribution of ^{99m}Tc-GHA entrapped SUV of neutral charge in mice at 3 h (percentage of dose injected per whole organ). Each value is the mean ± SE of four mice. (d). Biodistribution of ^{99}mTc-GHA entrapped SUV of positive charge in mice at 3 h (percentage of dose injected per whole organ). Each value is the mean ± SE of four mice.

tion showed a negligible amount of radioactivity. One of the reasons for targetting of liposomes to the liver may be due to the presence of sinusoidal fenestrations of about 100 nm size into which the liposomes become deposited (Spanjer and Rooke, 1980). Among SUV of various charges, ^{99m}Tc-GHA entrapped SUV of negative charge are targetted to the liver to a maximum extent. A hypothesis for this finding is that the hepatic cell (parenchymal and Kupffer) surface, like most of the other living surfaces, is negatively charged, and due to presence of an electrostatic interaction, the wall of the negatively charged SUV is disrupted and its contents are released, which are subsequently taken up by the liver cells.

The blood clearance studies of free and entrapped ^{99m}Tc-GHA conducted in rabbits show that the half-life of the agent, when entrapped in SUV, was greater by a factor of three than that of the agent in its free state. Enhancement of the residence time of the agent in blood makes the imaging of the liver more convenient. This was corroborated by performing scintigraphic studies which revealed that the entrapped agent was concentrated in the liver.

It is concluded that ^{99m}Tc-GHA entrapped SUV of negative surface charge are targetted to the liver. Thus, in addition to the free agent being used clinically for imaging of kidneys, the entrapped agent may also be used for hepatic imaging.

Acknowledgements

The authors would like to thank the Institute of Nuclear Medicine and Allied Sciences, New Delhi and Council of Scientific and Industrial Research, Biomedical Unit, New Delhi for providing necessary research facilities. M.J. thanks the UGC for financial assistance.

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