Annals of the Rheumatic Diseases, 1987; **46**, 314–318

Synovial accumulation of technetium labelled liposomes in rheumatoid arthritis

B D WILLIAMS,[1] M M O'SULLIVAN,[2] G S SAGGU,[3] K E WILLIAMS,[4] L A WILLIAMS,[5] AND J R MORGAN[4]

From the Departments of [1]Medicine, [2]Rheumatology, [3]Medical Physics, [4]Medical Microbiology, and [5]Radiology, University Hospital of Wales, Heath Park, Cardiff

SUMMARY Technetium labelled, negatively charged, unilamellar liposomes were given by intravenous injection to patients with rheumatoid arthritis and the joints scanned 20–22 hours later. Positive scintigraphy was obtained in all six patients with active disease, and, with the exception of the small interphalangeal joints, all clinically involved joints could be visualised.

Key words: scintigraphy, reticuloendothelial system.

Liposomes are small microscopic spheres composed of one or more concentric phospholipid bilayers.[1] These particles are taken up by the cells of the reticuloendothelial system and after intravenous injection are found mainly in the Kupffer cells of the liver and the macrophages of the bone marrow and spleen. Their distribution in vivo can be determined by labelling the liposomes with gamma emitting radionuclides such as technetium.[2]

The inflamed synovial tissue of patients with rheumatoid arthritis is rich in phagocytic cells. This preliminary study was undertaken to determine whether labelled liposomes could be used to identify inflamed joints in patients with rheumatoid arthritis.

Patients and methods

Two control subjects and eight patients with rheumatoid arthritis were studied. The patients fulfilled the criteria of the American Rheumatism Association for definite/classical rheumatoid arthritis.[3] Six patients with rheumatoid arthritis had active disease with synovitis and were receiving treatment with either Myocrisin or penicillamine. The two patients with inactive disease were chosen because they had had an excellent clinical response to treatment. There was no evidence of synovitis on clinical examination and the laboratory measures of disease activity were entirely normal. Ethical committee approval was obtained for this study.

PREPARATION OF LIPOSOMES

Negatively charged liposomes were prepared from phosphatidylcholine (egg lecithin), cholesterol, and phosphatidic acid at a molar ratio of 7:2:1. Liposomes were formed by evaporating the chloroform/methanol solvent containing 250 mg of lipid. A 4 ml solution of sterile, phosphate buffered saline was then propelled onto the glass vial containing the dried phospholipid and held at 50°C. The resulting liposome suspension was ultrasonicated in seven 30 second periods with a 9 mm probe attached to a Soniprobe type 1130A. All aqueous solutions used were sterile, pyrogen free, and suitable for injection. The procedure was carried out in a sterile laminar Flow cabinet (Microflow 102743). The liposome suspension was then labelled with technetium-99m using stannous chloride in oxygen free water.[4 5] The free isotope was separated from labelled liposomes by Sephadex G75 gel filtration. The liposome suspension was then passed through a 220 nm Millipore filter and taken up in 8 ml of saline for intravenous injection. The liposomes were sized by electron microscopy (negatively stained), and their diameter varied between 20 and 60 nm.

An aliquot was routinely tested for sterility by inoculation into a pair of blood culture bottles, which were then incubated for three weeks at 37°C and examined for aerobic and anaerobic microorganisms.

Accepted for publication 15 October 1986.
Correspondence to Dr B D Williams, Rheumatology Research Centre, University Hospital of Wales, Heath Park, Cardiff CF4 4XW.

JOINT IMAGING

Each patient received 380 MBq of technetium labelled liposomes administered as a slow intravenous injection. The effective dose equivalent for 380 MBq of technetium was 4 mSv. Joint scintigraphy was performed 20–22 hours after the infusion using an IgE 400 A gammacamera with a general purpose parallel whole beam collimator.

DISTRIBUTION OF LIPOSOMES WITHIN INFLAMED JOINTS

In one patient we followed up the accumulation of radioactivity after the intravenous injection of liposomes by aspirating synovial fluid at 10, 30, 60, and 90 minutes postinjection. At 20 hours an attempt was made to determine whether the radioactivity was associated with the synovial tissue or the synovial fluid. Surface counting over the knee joint was carried out before and after the aspiration of synovial fluid, the procedure being controlled by performing surface counts over the other non-aspirated knee.

Results

No side effects were observed in any of the patients or control subjects who received the liposome preparation.

Even though the scans obtained at four hours showed accumulation of radioactivity within the liver and spleen there was very little accumulation within synovial tissue. A lateral view of the knee joint is shown in Fig. 1, and it can be seen that most of the radioactivity was located within the blood vessels and the bone marrow at the lower end of the femur. At 20 hours bone marrow uptake was still visible, there was increased activity in synovial tissue, but the blood vessels were no longer identifiable (Fig. 1b).

Radioactivity was detectable in synovial fluid 10 minutes after the intravenous injection of liposomes (synovial fluid radioactivity was 0·7% of blood radioactivity), and during the subsequent 90 minutes the activity rose to 13% of that present in the peripheral blood. The isotope was non-dialysable

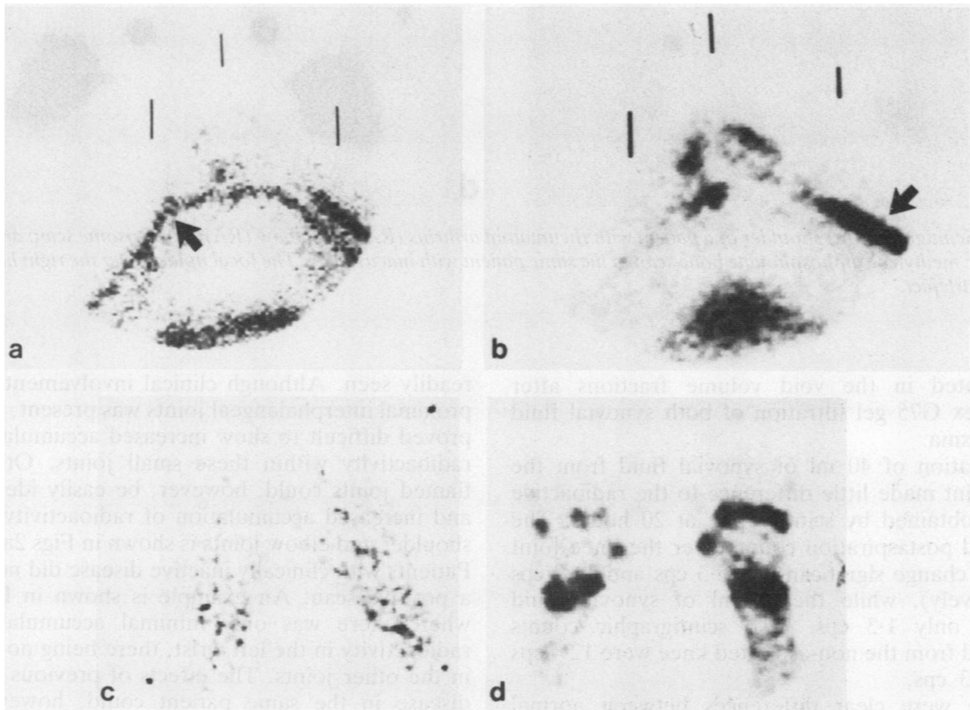

Fig. 1 *Scintigraphs—lateral view of the knee of a patient with rheumatoid arthritis. (a) Four hour scan. The arrow delineates liposomes within blood vessels. (b) Twenty hour scan showing accumulation of liposome within femoral bone marrow (arrow) and synovium. (c) Scintigraphs of the hands and wrists of a normal control. (d) Scintigraphs of the hands and wrists of a patient with rheumatoid arthritis.*

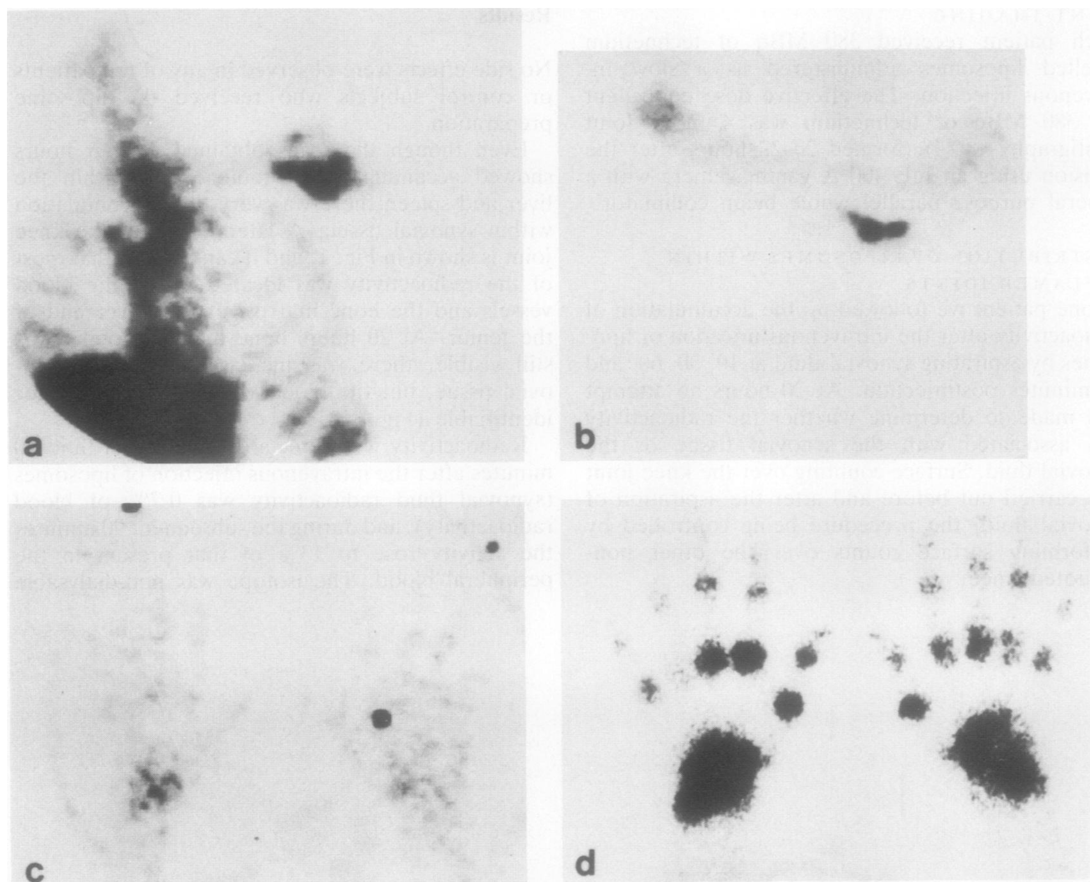

Fig. 2 *Scintigraphs: (a) shoulder of a patient with rheumatoid arthritis (RA); (b) elbow (RA); (c) liposome scan; and (d) ^{99m}Tc methylene diphosphonate bone scan in the same patient with inactive RA. The focal uptake over the right hand in (c) is an artefact.*

and eluted in the void volume fractions after Sephadex G75 gel filtration of both synovial fluid and plasma.

Aspiration of 40 ml of synovial fluid from the knee joint made little difference to the radioactive counts obtained by scintigraphy at 20 hours. The pre- and postaspiration counts over the knee joint did not change significantly (15·3 cps and 14·9 cps respectively), while the 40 ml of synovial fluid yielded only 1·3 cps. Two scintigraphic counts obtained from the non-aspirated knee were 12·4 cps and 12·3 cps.

There were clear differences between normal individuals (Fig. 1c) and patients with rheumatoid arthritis (Fig. 1d). Focal accumulations of radioactivity in the metacarpophalangeal joints and in the wrists of patients with rheumatoid synovitis were readily seen. Although clinical involvement of the proximal interphalangeal joints was present, it often proved difficult to show increased accumulation of radioactivity within these small joints. Other inflamed joints could, however, be easily identified, and increased accumulation of radioactivity in the shoulder and elbow joints is shown in Figs 2a and b. Patients with clinically inactive disease did not yield a positive scan. An example is shown in Fig. 2c, where there was only minimal accumulation of radioactivity in the left wrist, there being no uptake in the other joints. The effects of previous erosive disease in the same patient could, however, be demonstrated either by x ray or by a technetium methylene diphosphonate bone scan (Fig. 2d), which showed the extent of the bone destruction produced by the rheumatoid disease process.

Discussion

This study shows that after the intravenous injection of radiolabelled liposomes positive scintigraphs are obtained in patients with active rheumatoid arthritis and that the uptake of isotope corresponds to areas of synovial proliferation. When disease remission is induced and active synovitis disappears no accumulation of the isotope is observed. In common with other studies in man we have shown that liposomes are safe and easily prepared.[6 7]

Enhanced uptake of liposomes in tumour tissue has been shown to occur in experimental animals,[4] though tumour localisation has not been satisfactorily achieved in man using similar techniques.[2 6] The successful demonstration of uptake in rheumatoid synovium is probably due to two factors. We used small liposomes which were removed slowly from the circulation, could cross the capillary barrier in inflamed tissue, and gain access to the cells in the synovial tissue. The second factor reflects the presence in synovial tissue of 'reticuloendothelial phagocytes', which serve as natural targets for the injected liposomes. We presume that the accumulation of radioactivity in the synovial tissue represents the endocytosis of liposomes by these cells, though we do not know which cell type is responsible for the uptake. Since indium labelled neutrophils have been shown to accumulate in the knee joints of patients with rheumatoid arthritis[8] we are unable to exclude the possibility that some of the liposome localisation may be due to the migration into the joints of polymorphs which have endocytosed these particles in the circulation.

Although our results are consistent with the idea that positive scintigraphs represent endocytosis of radiolabelled liposomes by phagocytic cells, some caution is required in interpreting our findings. The behaviour of liposomes after intravenous injection is complex and it is known that their properties can be substantially modified after their interaction with a number of plasma proteins.[9] Although cholesterol, which was present in the liposomes that we used, reduces the damaging effects of interaction with high density lipoprotein, phospholipid exchange may still occur. This exchange could lead to a loss of the stannous pertechnate complex from the liposome surface and its association with other serum proteins. Such a loss would not be detectable by fractionation on Sephadex G75 columns. Very little is known about the subsequent fate of the liposomal phospholipid acquired by serum lipoproteins, but there is some evidence which suggests that it may be deposited in the parenchymal cells of the liver.[10] If this did occur then a positive scan could be produced even though the isotope was no longer attached to the surface of the liposome. Additional studies are therefore required to establish how much of the technetium remains attached to the liposome surface in vivo.

Our preliminary observations do, however, raise two interesting points. The first concerns the potential use of liposomes to monitor fluctuations in the activity of the phagocytic cells in the synovial tissue, fluctuations which may either follow the natural history of the disease or be induced by treatment. The second arises out of the studies on the natural targeting of liposomes in parasitology.[11] *Leishmania donovani* is taken up, and resides in, the same phagocytic cells that remove liposomes from the bloodstream. Liposome encapsulated antimonial drugs can therefore be delivered directly to the host cells of the reticuloendothelial system and dramatic increases in the therapeutic efficiency of these drugs have been achieved in this way. Similarly, in the rheumatic diseases it may be possible to improve the efficacy and safety of drugs which are currently in use and are believed to exert their therapeutic effect by altering the function of mononuclear phagocytes.[12 13] In the rat egg phosphatidylcholine vesicles containing sodium aurothiomalate have been shown to be suitable carriers of sodium aurothiomalate for parenteral administration, and they provide a more effective way of increasing the uptake of gold at sites of inflammation.[14] In view of the critical role of macrophages in both the induction and expression of immune mediated inflammation an examination of the properties of liposome entrapped drugs administered parenterally in man should be made.

References

1 Gregoriadis G. The carrier potential of liposomes in biology and medicine. *N Engl J Med* 1976; **295:** 704–10, 765–77.

2 Richardson V J, Ryman B E, Jewkes R F, *et al.* Tissue distribution and tumour localization of ^{99m}technetium labelled liposomes in cancer patients. *Br J Cancer* 1979; **40:** 35–43.

3 Ropes M W, Bennett G A, Cobb S, Jacox R, Jessar R A. Revision of the diagnostic criteria for rheumatoid arthritis. *Bull Rheum Dis* 1958; **9:** 175–6.

4 Richardson V J, Jeysingh K, Jewkes R F, Ryman B E, Tattersall M H N. Properties of ^{99m}technetium-labelled liposomes in normal and tumour bearing rats. *Biochem Soc Trans* 1977; **5:** 290–3.

5 Morgan J R, Williams K E, Davies R L C, Leach K, Thomson M, Williams L A. Localization of experimental staphylococcal abscesses by ^{99m}technetium-labelled liposomes. *J Med Microbiol* 1981; **14:** 213–7.

6 Lopez-Berestein G, Kasi L, Rosebleum M G, *et al.* Clinical pharmacology of ^{99m}Tc labelled liposomes in patients with cancer. *Cancer Res* 1984; **44:** 375–8.

7 Morgan J R, Williams L A, Howard C B. Technetium-labelled liposome imaging for deep-seated infection. *Br J Radiol* 1985; **58:** 35–9.

8 Segal A W, Thakur M L, Arnot R N, Lavender J P. Indium

111-labelled leucocytes for localisation of abscesses. *Lancet* 1976; **ii:** 1056–8.

9 Scherphof G, Damen J, Hoekstra D. The interactions of liposomes with plasma proteins and components of the immune system. In: Knight C G, ed. *Liposomes from physical structure to therapeutic applications.* North Holland: Elsevier, 1981: ch 10.

10 Scherphof G, Damen J, Hoekstra D, van Renswoude A J, Roerdink F H. In: Daems W, Burger E H, Afzelius B A, eds. *Cell biological aspects of disease: the plasma membrane lysosomes.* The Hague: Nijhoff, 1981: 281–97.

11 Alving C R, Steck E A, Chapman Jr W L, *et al.* Therapy of leishmaniasis: superior efficacies of liposome encapsulated drugs. *Proc Natl Acad Sci USA* 1978; **75:** 2959–63.

12 Rosenberg S A, Lipsky P E. Inhibition of pokeweed mitogen-induced immunoglobulin production in humans by gold compounds. *J Rheumatol [Suppl]* 1979; **6:** 107–11.

13 Vernon-Roberts B, Doré J L, Jessop J D, Henderson W J. Selective concentration and localisation of gold in macrophages of synovial and other tissue during and after chrysotherapy in rheumatoid patients. *Ann Rheum Dis* 1976; **35:** 477–86.

14 Cleland L G, Shandling M, Percy J S, Poznansky M J. Liposomes: a new approach to gold therapy? *J Rheumatol [Suppl]* 1979; **6:** 154–63.