Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 296 (2005) 34–43
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Scintigraphic imaging of radiolabelled drug delivery systems in rabbits with arthritis

Selcan Türker [a], Suna Erdoğan [a], Asuman Yekta Özer [a,*], Eser Lay Ergün [b], Murat Tuncel [b], Hasan Bilgili [c], Salih Deveci [d]

[a] *Department of Radiopharmacy, Faculty of Pharmacy, Hacettepe University, 06100 Sıhhıye/Ankara, Turkey*
[b] *Department of Nuclear Medicine, Faculty of Medicine, Hacettepe University, 06100 Sıhhıye/Ankara, Turkey*
[c] *Department of Orthopaedics and Traumatology, Faculty of Veterinary Medicine, Ankara University, 06110 Dışkapı/Ankara, Turkey*
[d] *Department of Pathology, Gülhane Military Medical Academy, School of Medicine, 06018 Etlik/Ankara, Turkey*

Received 11 November 2004; received in revised form 31 January 2005; accepted 9 February 2005
Available online 12 April 2005

Abstract

This paper describes a novel approach for designing drug delivery systems for intra-articular (i.a.) treatment of rheumatoid arthritis. Retention of these systems was evaluated by radiolabeling with Tc-99m and gamma scintigraphy in arthritic rabbits. Liposome, niosome, lipogelosome and niogelosome formulations of Diclofenac Sodium (DFNa) have been prepared and drug release properties and in vitro characterisation studies have been carried out. According to characterisation results L1 (DMPC: CHOL: DCP (7:1:2)), L1J1 (DMPC: CHOL: DCP (7:1:2) in C-940 1:1 (w/w)), N (SUR I: CHOL: DCP (7:1:2)) and NJ1 (SUR I: CHOL: DCP (7:1:2) in C-940 1:1 (w/w)) formulations were chosen for the further studies. Retention time of these formulations was evaluated by gamma scintigraphic imaging studies. Rabbits with antigen-induced arthritis were injected intra-articularly with Tc-99m labelled drug delivery systems. Serial scintigraphic images were obtained to investigate the retentions of labelled drug delivery systems in the arthritic joints and choose a suitable formulation for the treatment protocol of arthritis.

At the end of the scintigraphic imaging studies it was observed that radiolabelled lipogelosome formulation containing DFNa (L1J1) retained much longer in the experimentally arthritic knee joints of the rabbits. This formulation was used for the treatment protocol of arthritis. Mono articular arthritis was induced in the knee joints of rabbits and it was monitored at regular time intervals by measuring changes in knee joint diameter. Also macroscopic and histopathologic evaluations were performed for further evaluation of arthritis. Great retention of DFNa in the arthritic joint might reduce potential adverse systemic effects of the drug because of local administration into the diseased area. It appeared to be a promising drug delivery system for intra-articular drug delivery.

Keywords: Intra-articular drug delivery; Scintigraphic imaging; Drug delivery systems; Rheumatoid arthritis; Diclofenac sodium

* Corresponding author. Tel.: +90 312 305 21 96; fax: +90 312 311 47 77.
E-mail address: ayozer@yahoo.com (A.Y. Özer).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.02.017

1. Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory condition of unknown aetiology that affects about 1% of the general population (Feldmann et al., 1996) and is the most common cause of chronic inflammatory synovitis (Watson-Clark et al., 1998). Although spontaneous remission can occur, it often progresses to a chronic state associated with significant functional disability (Geletka and Clair, 2003). The number of drugs used in the treatment of RA has increased over the past 10–20 years. An ideal therapy in RA should ameliorate disease, prevent the development of extra-articular complications such as vasculitis, serositis, and lung fibrosis and prevent premature death (Rabinovich, 2000). However, there is still an urgent need for more effective drugs with reduced side effects (Corvo et al., 2002). Non steroidal analgesic anti-inflammatory drugs (NSAIDs) have been used extensively for treatment of RA owing to their quick onset of analgesic effects and mild anti-inflammatory properties despite the potential associations with various side effects such as ulceration of gastrointestinal tract and kidney toxicity with systemic administration (Jung-Ah Lee and Kavanaugh, 2003; Srinath et al., 2000). Intra-articular (i.a.) drug delivery is chosen for the rationale treatment of arthritic joints with drugs due to attaining high concentrations of drug in the joint and causing minimal systemic toxicity (Dingle et al., 1978).

A major limitation in the administration of soluble drugs by the i.a. route is their rapid clearance into the systemic circulation (Wigginton et al., 1980). Various particulate systems, which are retained effectively within the joint, have been investigated. Webb et al. (1969) showed that i.a. injected colloidal particles are taken up by both normal and inflamed synovium; however there was still some degree of extra-articular spread with these materials. Ratcliffe et al. (1986) and other workers (Barrera et al., 2000) have shown that factors such as particle size, physical form and degree of inflammation may influence the clearance rate. It is assumed that incorporation of drugs in drug delivery systems such as liposomes and niosomes may increase accumulation in the sites of inflammation, possibly by extravascularization through the gaps formed between the endothelial cells of the vasculature and reduce the side effects by reducing the availability of the drug in systemic circulation (Simionescu, 1980). Also treatment of arthritis by i.a. administration of anti-inflammatory drugs encapsulated in drug delivery systems prolongs the residence time of the drugs in the joint (Trif et al., 2001). It was therefore anticipated that liposome/niosome entrapment would enhance the efficacy of drugs in the inflammatory sides (Mönkkönnen et al., 1995; Pennanen et al., 1995).

Current treatment of RA frequently includes the use of NSAIDs, such as Diclofenac Sodium (DFNa) (Pittier, 2000 and Motta et al., 2003). DFNa has a very short plasma half-life and most common side effects. Due to short biological half-life and adverse effects including gastrointestinal side effects such as gastritis, peptic ulcer and bleeding (Gupta and Kumar, 2000) DFNa is considered as an ideal candidate for controlled drug release formulations. It has been shown to be effective for relieving pain in rheumatic and non-rheumatic diseases (Torres-Lòpez et al., 1997) with a good safety profile (Utsinger et al., 1985).

In the presented study our aims were: (1) To develop and choose an optimum drug delivery system with reduced systemic effects for the treatment of antigen-induced arthritis (AIA) with assessing the retention kinetics of i.a. administered radiolabelled drug delivery systems. (2) To observe the value of scintigraphic imaging in the detection of the retention amounts of different drug delivery systems. Based on our purpose we prepared drug delivery systems containing DFNa and we performed characterization, in vitro release and scintigraphic imaging studies of these systems.

2. Materials and methods

2.1. Materials

Dimyristoyl phosphotidyl choline (DMPC) and distearyl phosphotidyl choline (DSPC) are representative of liquid and gel state phospholipids, respectively, and were obtained as gift from Nattermann GmBH (Germany). Surfactant I (polyglyceryl-3 cethyl ether (SUR I)) was supplied by L'Oreal. Dicethyl phosphate (DCP), HEPES, ovalbumin, cholesterol (CHOL), Freud's Complete Adjuvant (FCA) and carboxhymethyl cellulose (CMC-Na) were purchased from Sigma (USA). Chloroform was obtained from Merck (Germany). All chemicals used in experiments were of analytical grade.

2.2. Preparation of liposome/niosome dispersions

Liposome and niosome dispersions were prepared by film technique (Bangham et al., 1965). Briefly, liposomes and niosomes were prepared by dissolving the 40 μmol mL^{-1} of phospholipids and/or the surfactant in 30 mL chloroform in a round-bottom flask. The chloroform was removed using a rotary evaporator (Buchi 461, Switzerland) under reduced pressure to form a thin film over the wall of the flask. The dried film was then hydrated over a water bath with 10 mM HEPES (pH 7.4) containing 10 mg mL^{-1} DFNa. Free DFNa was removed by centrifugation three times at 17,500 rpm for 45 min in each. The pellets that were obtained after the centrifugations were treated with detergent (Triton X-100 in 10 mM HEPES (pH 7.4) buffer) and then final clear solutions were analysed for drug content spectrometrically at λ = 303 nm.

Encapsulation efficiency was calculated as a fraction of drug in the liposome/niosome pellet expressed as a percentage of total drug content.

2.3. Preparation of lipogelosome and niogelosome formulations

Lipogelosome and niogelosome formulation were prepared by incorporation of liposomes and niosomes in structured vehicles. Carbopol 940 (C-940) at the concentration of 1% in distilled water (Farshi et al., 1996) and CMC-Na at the concentration of 2.5% (Paavola et al., 1995) was used as gel forming agents because of their good bioadhesive properties.

For preparation lipogelosomes/niogelosomes, gel formulations of C-940 and CMC-Na and liposomal/niosomal DFNa were mixed 1:1 ratio on weight basis. pH measurements were done by using pH meter (Inolab, Germany). All the pH of formulations was adjusted to pH 7.4.

Composition, ratio and code of the liposome, niosome, lipogelosome and niogelosome formulations are given in Table 1.

2.4. Characterization of liposome/niosome dispersions

2.4.1. Particle size measurement

Mean particle size and size distributions of the dispersions were measured by dynamic light scattering method (Malvern Mastersizer 2000, UK).

2.4.2. Determination of liposomal phospholipid

Determination of lipid content gives an idea about the efficiency of method of preparation of liposomes. Liposomal phospholipid content was determined by the colorimetric method of Rouser et al., 1970.

2.4.3. In vitro release of DFNa from liposome and niosome formulations

In vitro release of DFNa from liposome and niosome formulations was measured spectrophotometrically at λ = 275 nm by incubation of 0.1 mL of liposome and niosome formulations in 10 mL 10 mM HEPES (pH 7.4) buffer at 37 °C in mild shaking water bath. Samples were withdrawn at fixed time intervals and absorbance was noted of 275 nm with 1 cm path length quartz cuvettes by using UV spectrophotometer (Schimadzu UV 160A, Japan). No interference of the empty formulations was detected at this wavelength.

Table 1
Composition, ratio and code of the liposome, niosome, lipogelosome and niogelosome formulations containing DFNa

Formulation	Composition	Ratio	Code
Liposome	DMPC: CHOL: DCP	7:1:2	L1
Liposome	DMPC: CHOL: DCP	7:1:2	L2
Niosome	DSPC: CHOL: DCP	7:1:2	N
Lipogelosome	(DMPC: CHOL: DCP) + C-940	1:1 (w/w)	L1J1
Lipogelosome	(DMPC: CHOL: DCP) + CMC-Na	1:1 (w/w)	L1J2
Lipogelosome	(DSPC: CHOL: DCP) + C-940	1:1 (w/w)	L2J1
Lipogelosome	(DSPC: CHOL: DCP) + CMC-Na	1:1 (w/w)	L2J2
Niogelosome	(SUR I: CHOL: DCP) + C-940	1:1 (w/w)	NJ1
Niogelosome	(SUR I: CHOL: DCP) + CMC-Na	1:1 (w/w)	NJ2

2.5. Characterization of lipogelosome and niogelosome formulations

2.5.1. Viscosity measurements

Viscosity measurements of lipogelosomes and niogelosomes were performed by using rheometer (Brookfield, USA) at 25 and 37 °C. The shear rate was increased from 0 to 100 rpm. The viscosity was determined from the flow curve obtained at different values of shear rate.

2.5.2. In vitro release of DFNa from lipogelosomes and niogelosomes

In vitro release properties of liposome and niosome dispersions were evaluated by using Franz-type diffusion cell. About 0.5 of sample was introduced into a donor compartment separated by a cellophane membrane (Thomas Sci. Comp., USA) from the receptor compartment, 20 mL of HEPES buffer (pH 7.4). The whole assembly was placed in a water bath, maintained at 37 °C and continuously well stirred. Care was exercised to remove any air bubble from the under side of the membrane and the receiving solution.

At specified time intervals 1 mL samples were removed from the receiver compartment, i.e. partial sampling and refilled with an equal volume fresh buffer. All samples were analysed for DFNa content spectrophotometrically using the wavelength of 275 nm.

2.6. Labelling and quality control of liposome and niosome formulations

L1, L1J1, N and NJ1 formulations were labelled with ^{99m}Tc by stannous reduction method (Erdoğan et al., 1998). Tc-99m pertechnetate ($^{99m}TcO_4^-$) was obtained from a generator (CIS Biointernational, France). Briefly 0.2 mL $SnCl_2$ (1 mg mL^{-1}) and 74–111 MBq $^{99m}TcO_4^-$ were added to liposome/niosome formulations. The mixture then allowed to stand 20 min at room temperature and radiochemical purity (RCP) was determined by impregnated thin-layer chromatography (ITLC) using ready silica-gel plates of ITLC-SG (Gelman Sci., Germany) with acid citrate dextrose (ACD) (Erdoğan et al., 2000; Ergun et al., 2002). The labelled liposomes/niosomes stayed at the origin and free $^{99m}TcO_4^-$ and hydrolysed-reduced ^{99m}Tc moved with the solvent front (Saha, 1992; Rubin, 1989).

2.7. Animal experiments

2.7.1. Induction of arthritis

New Zealand rabbits weighing 2.5–3 kg were used for the in vivo experiments. A physiological solution (0.9% NaCl) of ovalbumin at a concentration of 20 mg mL^{-1} was emulsified with an equal volume of Freud's Complete Adjuvant (Foong and Green, 1993; Čeponis et al., 2001). In order to obtain homogenous dispersion, the emulsion was passed through a homogeniser for three times. To obtain inflammation 1 mL of the emulsion sample was injected into right knee joints of each rabbit. Collateral knees used as the control joints were injected 1 mL of 0.9% NaCl solution. Monoarticular arthritis developed in 10 days after the i.a. injection.

2.7.2. Assessment of the arthritis

Inflammation of the knee joints was assessed for the degree of swelling (Richards et al., 1999). For histopathological evaluation, the joint capsule of the right knee was removed fixed in 10% formalin and processed through routine paraffin embedding and sectioning. Calcified tissue was decalcified in formic acid. After staining by haematoxylin and eosin, the sections were examined under light microscopy (Lòpez-Garcia et al., 1993). Pathological results were graded on a scale (0–3) as follows: (1) normal sinovium with a few plasma cells and lymphocytes; (2) increased hyperplasia and cellular infiltrate with vasculitis; (3) cellular infiltration of whole sinovium, pannus formation, cartilage and bone erosion (Foong and Green, 1988).

2.8. Scintigraphic imaging studies

^{99m}Tc labelled formulations were used to demonstrate which formulation would stay longest in the arthritic joint cavity before therapy protocol. After induction of arthritis, each rabbit was administered with 37 MBq of ^{99m}Tc labelled formulations (L1, L1J1, N, NJ1) intra-articularly into the inflamed right knee joints. The rabbits were placed under a single-headed gamma camera (ADAC, Cirrus, Gamma camera). Sequential gamma images from the anterior projection of the knees were carried out at 5, 15, 30 min, 1, 2, 3, 4 and 24 h. ROIs were drawn over the arthritic knee joints on the sequential images. Counts calculated from ROIs were corrected by decay factor and plotted on a

graph for retention kinetics for each radiolabelled formulation. Additionally whole body scintigraphic images from the anterior projection were performed for each rabbit at 20 min to determine the systemic leakage of the Tc-99m labelled formulations.

3. Results and discussion

Liposomes have been proposed as efficient carriers for controlled drug delivery. They are able to entrap hydrophilic drugs in the large aqueous interior and lipophilic drugs inserted in the lipid bilayer. Derived from naturally occurring, biodegradable, and nontoxic lipids, they are good candidates for local targeting of therapeutic agents to the site of interest, while reducing systemic toxicity. In the case of arthritic diseases, it has been reported that the i.a. administration of anti-inflammatory drugs encapsulated in liposomes shows prolonged residence in the joint and reduction of inflammation. Moreover, the greater retention of the drug at the injection site should reduce potential adverse effects (Trif et al., 2001). Film method was used due to its simplicity, reproducibility and high efficiency of aqueous phase encapsulation. Extrusions were used for homogenisation of size distribution of MLV liposomes and niosomes obtained by the Film method. Approximately 10% of the total drug in the aqueous phase was entrapped with the liposome and niosome formulations. It was observed that formulation L1 (DMPC: DCP: CHOL (7:1:2)) has the highest encapsulation efficiency, phospholipid content and entrapped volume. Table 2 illustrates the characterization studies of DFNa containing liposome and niosome formulations. All the formulations have a monophasic and similar particle size distribution (200–250 nm).

A vehicle used for preparation of lipogelosome and niogelosome formulations should provide adequate pH value and rheological characteristics (Škalko et al., 1988). Due to good physical, chemical and biological properties of hydrophilic polymer gels (Knuth et al., 1993) we have chosen polymers of acrylic acid (Carbopol resins) and cellulose derivatives. The viscosity of C-940 and CMC-Na lipogelosomes and niogelosomes was determined at various shear rates (Figs. 1 and 2). As the shear rate increased, the viscosity of the lipogelosome and niogelosome formulations decreased. All the formulations were displayed a non-Newtonian

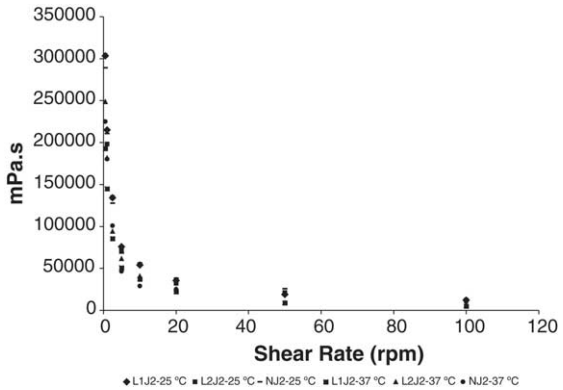

Fig. 1. Rheological behaviour of C-940 lipogelosomes and niogelosomes.

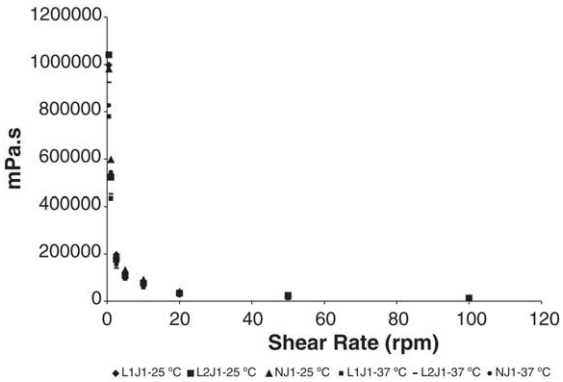

Fig. 2. Rheological behaviour of CMC-Na lipogelosomes and niogelosomes.

Table 2
Characterization of DFNa loaded liposome and niosome formulations

Formulation	Size (nm)	Encapsulation efficiency (%)	Phospholipid content (%)	Entrapped volume (μL mL^{-1})
L1	235 ± 0.011	10.8 ± 0.002	97.3 ± 2.230	4.38 ± 0.06
L2	242 ± 0.027	9.7 ± 0.002	92.2 ± 3.025	4.19 ± 0.01
N	272 ± 0.017	9.6 ± 0.002	–	3.86 ± 0.02

Each value represents the mean value ± S.D. of six experiments.

Table 3
Cumulative amounts of DFNa released from different drug delivery systems at the end of 24 h

Formulation		DFNa released (%)
Liposome formulation	L1	86.7
	L2	99.5
Niosome formulation	N	78.9
Lipogelosome formulation	L1J1	48.2
	L1J2	42.8
	L2J1	58.2
	L2J2	42.8
Niogelosome formulation	NJ1	50.4
	NJ2	41.8

Each value represents the mean value ± S.D. of six experiments.

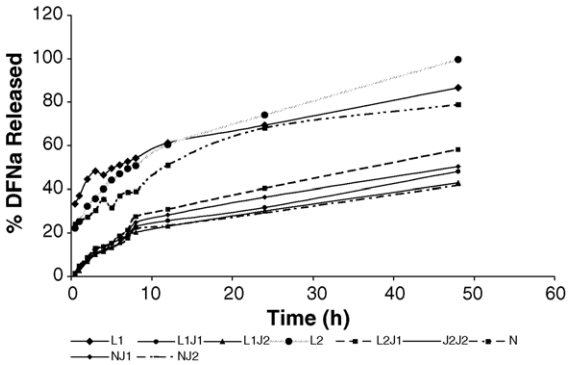

Fig. 3. Release profile of DFNa loaded liposome, niosome, lipogelosome and niogelosome formulations.

behaviour at both 25 and 37 °C. Difference between the viscosities of lipogelosome and niogelosome formulations was found statistically significant ($P < 0.05$)

The drug release profiles of DFNa from the formulations were examined in 10 mM HEPES (pH 7.4) buffer. The cumulative amount of DFNa released in 48 h from the formulations was given in Table 3. As expected, liposome and niosome formulations showed higher release of DFNa than lipogelosome and niogelosome formulations. The lipogelosome and niogelosome formulations significantly ($P < 0.05$) prolonged the DFNa released compared with the liposome and niosome formulations (Fig. 3). The release of DFNa from the lipogelosome and niogelosome formulations is a combination of the release of free, surface-bound and encapsulated drug through the micellar network channel structures of the gel. Also the release rate of a drug dissolved in the liquid phase of lipogelosome and niogelosome formulations may be affected by the type and concentration of gelling agent and by processing conditions (Realdon et al., 1996).

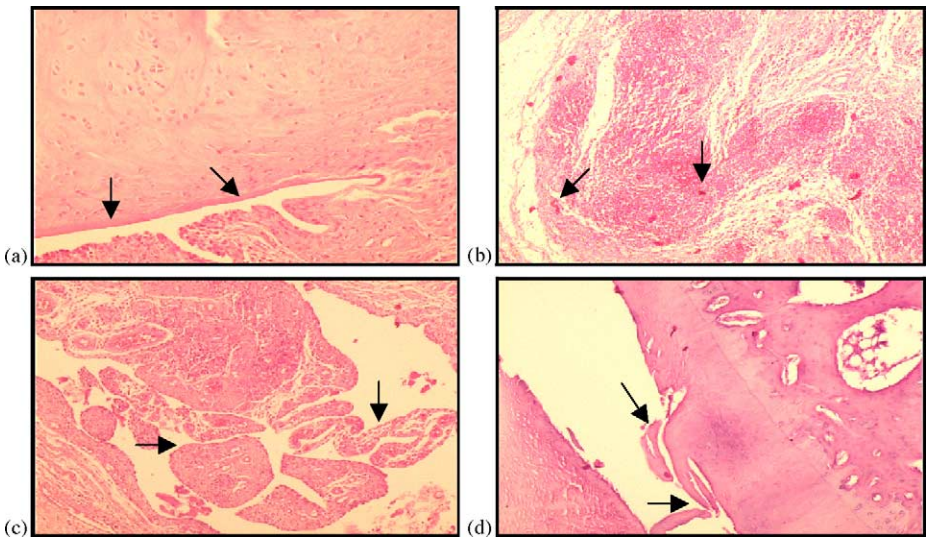

Fig. 4. Histopathological analysis in paraffin wax section of knee joints isolated from untreated rabbits. Normal articular cartilage before induction of arthritis (a); lymphocytes 1 week after induction of arthritis (b); synovial hyperplasia 2 weeks after induction of arthritis (c); cartilage destruction at last stage of arthritis (d).

At the end of the characterisation studies L1, L1J1, N and NJ1 formulations were chosen for scintigraphic studies according to release, viscosity properties and in vitro characterisation results.

AIA in rabbits provides one of the best models of RA available. The rabbit knee joint is anatomically similar to the human knee joint, the area of the body with the highest incidence of RA (Meijers, 2003). The joint histopathology of AIA closely resembles RA and its responsiveness to anti-rheumatic and anti-inflammatory drugs is similar to that of the clinical disease (Glynn, 1968; Zvaifler, 1973; Foong and Green, 1993). The injection of ovalbumin and Freud's Complete Adjuvant emulsion resulted in an increase of the diameter of the knee joint of the rabbits, from 13 ± 0.16 to 19 ± 0.08 mm, 2 weeks after the i.a. injection. Both knee measurements and histopathological (Fig. 4) and macroscopic assay indicate that AIA was successfully induced in rabbits.

Scintigraphic imaging studies were performed to detect the retention times of the formulations and to choose the optimum formulations for the further in vivo studies. For this purpose, selected formulations for scintigraphic studies were labelled with $^{99m}TcO_4^-$ by stannous reduction method and RCP of these systems were checked with thin-layer chromatography. According to the quality control results it is concluded that the labelling efficiency was found higher than 90% each all formulation. The efficiency has to be more than 90% for a good labelling. Our results are in agreement with literature (Erdoğan, 2001; Billinghurst et al., 2004).

According to the scintigraphic imaging results, it was observed that L1 and L1J1 formulation stayed in the arthritic joint longer in comparison with the other formulations (Fig. 5). L1 and L1J1 were further evaluated in arthritic joints because of the similar results. It was found that Tc-99m labelled L1J1 formulation was the most significantly retained radiopharmaceutical

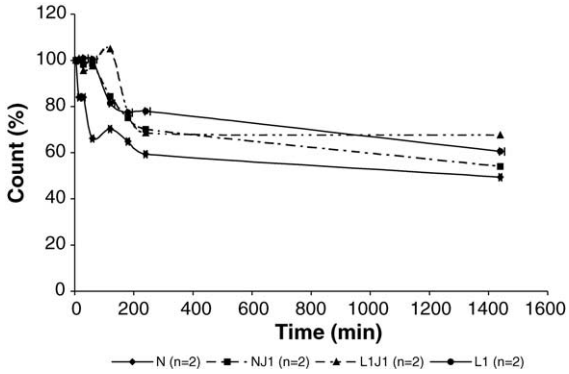

Fig. 5. Time–active curves which shows retentions of Tc-99m labelled DFNa formulations in the arthritic knee joints.

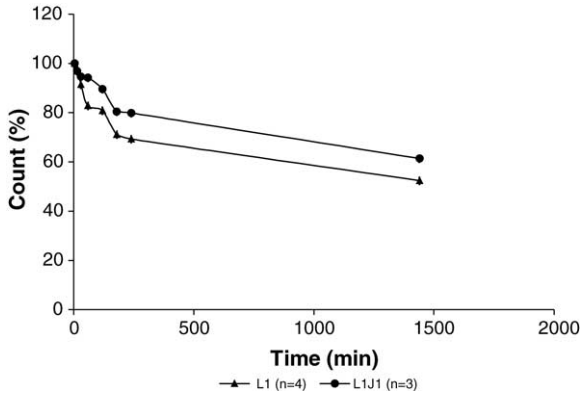

Fig. 6. Time–active curves which shows retentions of Tc-99m labelled L1 and L1J1 formulations in the arthritic knee joints.

Table 4
Percentage retention amounts of L1, N, L1J1 and NJ1 formulations in the arthritic knee joints

Formulation	Count (%)							
	5 min	15 min	30 min	1 h	2 h	3 h	4 h	24 h
L1	100	100	101	100	81	77	78	60
N	100	84	84	66	70	64	59	49
L1J1	100	100	95	97	104	77	68	67
NJ1	100	98	98	84	75	70	70	54

Formulation	Count (%)							
	5 min	15 min	30 min	1 h	2 h	3 h	4 h	24 h
L1	100	97	91	83	81	71	69	52
L1J1	100	97	95	94	90	80	80	61

at the end of 24 h with 67% of the injected activity (Fig. 6, Table 4). Enhancing the retention time of the drugs is very important in i.a. therapy. It was shown that liposome-entrapped methotrexate injected i.a. approximately 10-fold more effective than the free drug in arthritis (Foong and Green, 1988). Dingle et al. (1978) first proposed the use of liposome-entrapped drugs for i.a. injection, and subsequently showed that the retention of liposome-entrapped corticosteroids in the injection was greatly enhanced.

Fig. 7 displays scintigraphic images throughout at 5, 15, 30 min and 1, 2, 3, 4 and 24 h. These images showed that at the end of 24 h, Tc-99m radiolabelled L1J1 formulation was still significantly present in the arthritic knee joint. Extra-articular uptakes in whole body scintigrams such as kidneys indicated that a

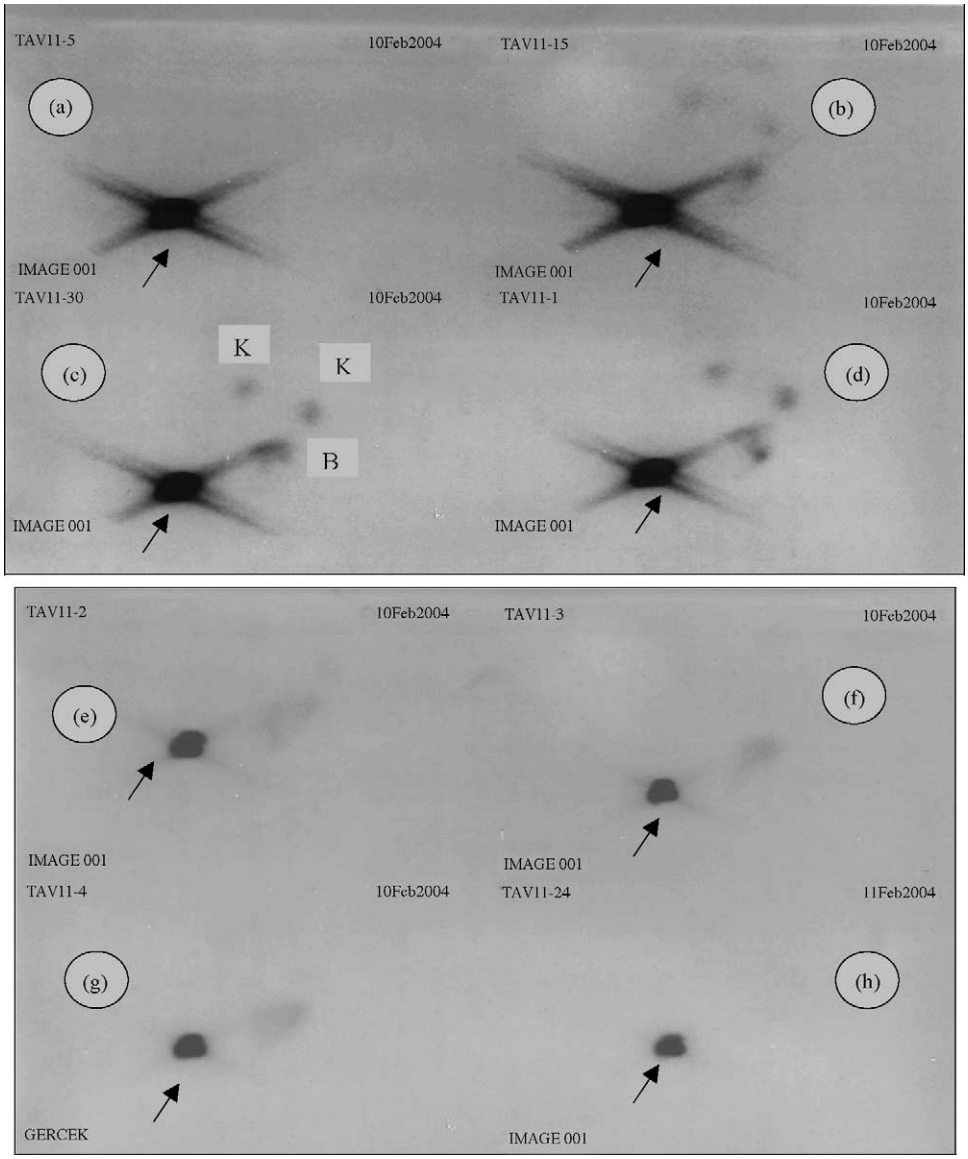

Fig. 7. Scintigrams of i.a. Tc-99m L1J1 injected arthritic rabbit in different time intervals: 5 min (a); 15 min (b); 30 min (c); 1 h (d); 2 h (e); 3 h (f); 4 h (g); and 24 h (h). K: kidneys; B: bladder; arrow: arthritic knee joint.

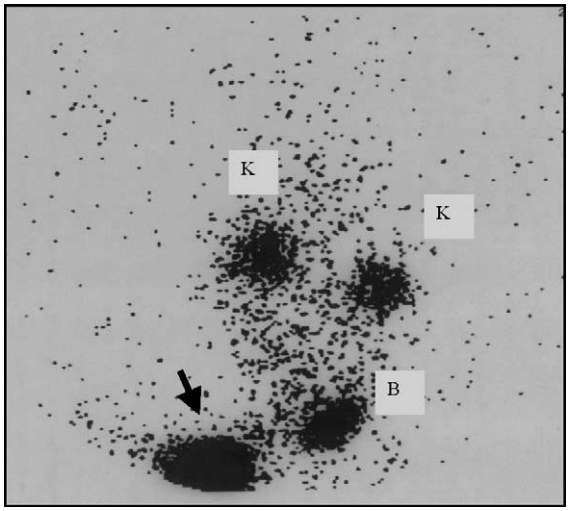

Fig. 8. Whole body scintigram of i.a. L1J1 injected arthritic rabbit. K: kidneys; B: bladder; arrow: arthritic knee joint.

negligible amount of radiolabelled formulation passed into the systemic circulation (Fig. 8). Free $^{99m}TcO_4^-$ is physiologically taken up by the thyroid gland and stomach in the body. In the whole body images, no $^{99m}TcO_4^-$ uptake was observed in these organs. These results indicated that the radioactivity accumulations in kidneys and bladder were due to the excretion of the leaked Tc-99m labelled formulation not the free $^{99m}TcO_4^-$. This was confirmed with the quantitative results obtained from ROIs which showed that Tc-99m labelled L1J1 formulation is the formulation that accumulated significantly higher than the other formulations in the arthritic knee joints (Table 4, Fig. 5 and Fig. 6). The quality control results, labelling efficiency higher than 90% are in agreement with these data.

4. Conclusions

This study has shown that DFNa loaded lipogelosome formulation retained longer within arthritic knee joints and this type of system can be used to retain molecules within the knee joint for a prolonged period of time. This system will have the advantages of regional therapy with intra-artricular injection together with an increased efficiency and decreased systemic side effects. Scintigraphy is a good imaging tool to study such a system. On the basis of in vitro and in vivo results treatment and biodistribution studies with L1J1 formulation with rabbits are being performed in our departments.

Acknowledgements

Authors are grateful to Pharm. Özge İnal (B.U) and Ferhad Farshi (BU) for the valuable help in performing rheological and the particle size measurements. We also would like to thank Nattermann GmbH for supplying phospholipids and L'Oreal and Deva for supplying surfactants and active substances, respectively.

References

Bangham, A.D., Standish, M.M., Watkins, J.C., 1965. Diffusion of univalent ions across lamellae of swollen phospholipids. J. Mol. Biol. 13, 238.

Barrera, P., Blom, A., Lent, P.L.E.M., Bloois, L., Beijnen, J.H., Rooijen, N., Malefijt, M.C.W., Putte, L.B.A., Storm, G., Berg, W.B., 2000. Synovial macrophage depletion with clodronate-containing liposomes in rheumatoid arthritis. Arthritis Rheum. 43, 1951–1959.

Billinghurst, M.W., Eckert, K., Mang'era, K., 2004. Alternative quality control for technetium-99m IDA complexes. Appl. Radiat. Isotopes 61, 1151–1155.

Čeponis, A., Waris, E., Mönkkönen, J., Laasonen, L., Hyttinen, M., Solovieva, S.A., Hanemaaijer, R., Bitsch, A., Konttinen, Y.T., 2001. Effects of low-dose, noncytotoxic, intra-articular liposomal clodronate on development of erosions and proteoglycan loss in established antigen-induced arthritis in rabbits. Arthritis Rheum. 44, 1908–1916.

Corvo, M.L., Jorge, C.S., Hof, R.V., Cruz, M.E.M., Crommelin, D.J.A., Storm, G., 2002. Superoxide dismutase entrapped in long-circulating liposomes: formulation design and therapeutic activity in rat adjuvant arthritis. Biochim. Biophys. Acta 56, 227–236.

Dingle, J.T., Gordon, J.L., Hazleman, B.L., Knight, C.G., Page-Thomas, D.P., Philips, N.C., Shaw, I.H., Fildes, F.J.T., Oliver, J.E., Jones, G., Turner, E.H., Lowe, J.S., 1978. Novel treatment for joint inflammation. Nature 271, 372–373.

Erdoğan, S., Özer, A.Y., Ercan, M.T., Aydın, K., Hıncal, A.A., 1998. Biodistribution and computed tomography studies on iopromide liposomes. S.T.P. Pharma. Sci. 8, 133–137.

Erdoğan, S., Özer, A.Y., Ercan, M.T., Hıncal, A.A., 2000. Scintigraphic imaging of infections with 99m-Tc labelled glutathione liposomes. J. Microencapsul. 17, 459–465.

Erdoğan, S., 2001. In vitro and in vivo studies on drug delivery systems developed for diagnosis and scintigraphic imaging of deep vein thrombosis. Ph.D. Thesis, Hacettepe University, Turkey.

Ergun, E.L., Bozkurt, M.F., Ercan, M.T., Ruacan, Ş., Şener, B., 2002. Detection of inflammatory lymph nodes in rabbits by ^{99m}Tc-HIG lymphoscintigraphy. Nucl. Med. Commun. 23, 1177–1182.

Farshi, F., Özer, A.Y., Tavassoli, S., Sungur, A., Hıncal, A.A., 1996. A clinical trial: in vivo studies on dexamethasne sodium phosphate liposomes in the treatment of human aphthous stomatitis. J. Liposome Res. 6, 699–712.

Feldmann, M., Brennan, M.F., Maini, R.N., 1996. Rheumatoid arthritis. Cell 85, 307–310.

Foong, W.C., Green, K.L., 1993. Treatment of antigen-induced arthritis in rabbits with liposome-entrapped methotrexate injected intra-articularly. J. Pharm. Pharmacol. 45, 204–209.

Foong, W.C., Green, K.L., 1988. Retention and distribution of liposome-entrapped [^{3}H] methotrexate injected into normal or arthritic rabbit joints. J. Pharm. Pharmacol. 40, 464–468.

Geletka, R., Clair, E.W., 2003. Treatment of early rheumatoid arthritis. Best Pract. Res. Cl. RH. 17, 791–809.

Glynn, L.E., 1968. The chronicity of inflammation and its significance in rheumatoid arthritis. Ann. Rheum. Dis. 27, 105–121.

Gupta, K.C., Kumar, M.N.V.R., 2000. Drug release behavior of beads and microgranules of chitosan. Biomaterials 21, 1115–1119.

Jung-Ah Lee, S., Kavanaugh, A., 2003. Pharmacological treatment of established rheumatoid arthritis. Best Pract. Res. Cl. RH. 17, 811–829.

Knuth, K., Amiji, M., Robinson, J.R., 1993. Hydrogel delivery system for vaginal and oral applications: formulations and biological consideration. Adv. Drug. Deliv. Rev. 11, 137–167.

Lòpez-Garcia, F., Văzquez-Autòn, J.M., Gıl, F., Latoore, R., Moreno, F., Villalan, J., Gòmez-Fernàndez, J.C., 1993. Intra-articular therapy of experimental arthritis with a derivative of triamcinolone acetonide incorporated in liposomes. J. Pharm. Pharmacol. 45, 576–578.

Meijers, T., 2003. (www.arthritisnetwork.ca/products_and_services/inflammatory.asp).

Mönkkönnen, J., Liukkonen, J., Taskinen, M., Heath, T.D., 1995. Studies on liposome formulations for intra-articular delivery of clodronate. J. Controlled Rel. 35, 145–154.

Motta, A.F., Junior, N.G.B., Fonseca, J.C.P., Tonussi, C.R., 2003. The antinociceptive effect of iontophoretic direct application of diclofenac to arthritic knee-joints of rats. Life Sci. 73, 1995–2004.

Paavola, A., Kilpeläinen, I., Yliruusi, J., Rosenberg, P., 1995. Controlled release injectable liposomal gel of ibuprofen for epidural analgesia. Int. J. Pharm. 199, 85–93.

Pennanen, N., Lapinjoki, S., Urtti, A., Mönkkönen, J., 1995. Effect of liposomal and free bisphosphonates on the IL-1β, IL-6 and TNF-α secretion from RAW 264 cells in vitro. Pharmaceut. Res. 12, 916–922.

Pittier, A., 2000. Current advances in rheumatoid arthritis therapy. T. S. M. J. 1, 72–76.

Rabinovich, G.A., 2000. Apoptosis as a target for gene therapy in rheumatoid arthritis. Mem. Inst. Oswaldo Cruz, Rio de Janerio 95, 225–233.

Ratcliffe, J.H., Hunneyball, J.M., Wilson, C.G., Smith, Davis, S.S., 1986. Albumin microspheres for intra-articular drug delivery: investigation of their retention in normal and arthritic knee joints of rabbits. J. Pharm. Pharmacol. 39, 290–295.

Realdon, N., Zotto, M.D., Ragazzi, E., Fini, G.D., 1996. Drug release from lipogels according to gelling conditions and mechanical treatment. Drug. Dev. Ind. Pharm. 22, 125–134.

Richards, P.J., Amos, N., Willams, A.S., Willams, B.D., 1999. Pro-inflammatory effects of the aminobisphosphonate ibandronate in vitro and in vivo. Rheumatology 38, 984–991.

Rouser, G., Fleisner, S., Yamamoto, Y., 1970. Lipids 5, 494–499.

Rubin, A., 1989. Contributions of cognitive science and educational technology to training in radiology. Invest. Radiol. 24, 729–732.

Saha, G.B., 1992. Characteristics of specific radiopharmaceuticals. In: Saha, G.B. (Ed.), Fundamentals of Nuclear Pharmacy. Springer Verlag, New York, pp. 109–142.

Simionescu, M., 1980. Blood cells and vessel wall: Functional Interactions Ciba Found Symp. 71.

Škalko, N., Čajkovac, M., Jalšenjak, I., 1988. Liposomes with metronidazole for topical use: the choice of preparation method and vehicle. J. Liposome Res. 8, 283–293.

Srinath, P., Chary, M.G., Vyas, S.P., Diwan, P.V., 2000. Long-circulating liposomes of indomethacin in arthritic rats-a biodisposition study. Pharm. Acta Helv. 74, 399–404.

Torres-Lòpez, J.E., Lòpez-Muñoz, F.J., Castañeda-Hernández, G.C., Flores-Murrieta, F., Granados-Soto, V., 1997. Pharmacokinetic-Pharmacodynamic modelling of the antinociceptive effect of diclofenac in the rat. J. P. E. T. 282, 685–690.

Trif, M., Guillen, C., Vaughan, D.M., Telfer, J.M., Brewer, J.M., Roseanu, A.V., Jeremy, H.B., 2001. Liposomes as possible carriers for lactoferrin in the local treatment of inflammatory diseases. E. B. M. 226, 559–564.

Utsinger, P.D., Zvaitler, N.J., Ehrlich, G.E., 1985. Rheumatoid arthritis etiology-diagnosis-management. J.B. Lippincott, Philadelphia.

Watson-Clark, R.A., Banquerigo, M.L., Shelly, K., Hawthorne, M.F., Brahn, E., 1998. Model studies directed toward the application of boron neutron capture therapy to rheumatoid arthritis. Boron delivery by liposomes in rat collagen-induced arthritis. Proc. Natl. Acad. Sci. 95, 2531–2534.

Webb, F.W.S., Lowe, J., Bluestone, E., 1969. Uptake of colloidal radioactive Yttrium by synovial membrane. Ann. Rheum. Dis. 28, 300–302.

Wigginton, S.M., Chu, B.C., Weisman, H., Howell, S.B., 1980. Methotrexate pharmacokinetics after intra-articular injection in patients with rheumatoid arthritis. Arthritis Rheum. 23, 119–122.

Zvaifler, N.J., 1973. Immunopathology of joint inflammation in rheumatoid arthritis. Adv. Immunol. 16, 265–336.