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Liposomes containing blue dye for preoperative lymph node staining: Distribution and stability in dogs after endolymphatic injection

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Summary

Homogeneous unilamellar liposomes consisting of lecithin and cholesterol in a molar ratio of 3:1 were produced using a detergent dialysis technique. The water-soluble blue dye Isosulfan blue was subsequently incorporated into these liposomes. For in vitro stability tests, 111-Indium was incorporated into liposomes of identical lipid composition. Stability in the lymph and excised lymph nodes was examined using gamma-ray angular correlation spectroscopy. The final liposome solution, consisting of 32 mg/ml lipid and 2.7 mg/ml dye, was used in dog experiments. The lymphatic uptake of liposomes after subcutaneous (s.c.) injection was not satisfactory. Only a slight staining of lymph nodes with dye concentrations between 3.5 and 15 μg/g occurred.Intensive staining of lymph nodes was, however, achieved by endolymphatic injection of 4 ml of liposome suspension. Dye content was 99, 122, and 281 μg/g tissue in paraaortal, pelvic, or popliteal lymph nodes, respectively. The reduction of injection time from 1 to 0.5 h resulted in elevated dye content in paraaortal lymph nodes (110 μg/g). The intravenous infusion and pad massage led to accessory spillover of dye, with a slight reduction of its content in lymph nodes. The liposomes used here are suitable for preoperative lymph node staining. They increase the radicality and the selectivity of lymphonodectomy. The described method is also suitable for local lymph node treatment after replacing blue dye with drugs, preferably cytostatics.

Introduction

The inaccessibility of retroperitoneal lymph nodes for therapy is one of many still unsolved clinical problems. In patients undergoing surgery, the identification of lymph nodes in the surrounding fatty tissue is important to achieve the maximum radicality connected with maximum selectivity of lymphonodectomy. In patients treated with conservative methods, the possibility of local deposition of highly concentrated cancer therapeutic agents may be important for control of lymphogenous tumor spread. For both of these indications a potent drug carrier for stains and cytostatics must be found. These substances are commonly water soluble, escaping into the surrounding tissue just after endolymphatic injection.

Initial experiments with oily suspensions (Hirnle, 1985), emulsions (Hirnle and Heide, 1985)

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and solutions (Harzmann et al., 1989) showed that all these forms of drug delivery have disadvantages which reduce their clinical applicability. Our preliminary experiments with liposomes (Hirnle et al., 1985, 1986) indicated that these vesicles may be suitable drug carriers for endolymphatic use. The liposomally encapsulated dark blue dye Isosulfan blue was used in the studies presented here. This dye was intended to be used as a lymph node stain, as well as a model substance for other water-soluble drugs, particularly cytostatics. For animal experiments dye was encapsulated into large unilamellar vesicles. The obvious advantage of the liposomes used here is the biocompatibility of the materials; egg yolk lecithin and cholesterol were the only components. Moreover, a minimum of lamellarity of the liposomes enhanced the dye/lipid ratio in the liposomes, thus minimizing metabolic problems with injected lipids.

This kind of liposomes is known to be preferably captured by macrophages of liver or spleen depending on their cholesterol content (Moghimi and Patel, 1988), but the data about their behaviour in lymph nodes are incomplete. It can be, however, expected, that these liposomes are nontoxic when applied in a single dose (Allen, 1988).

After injection of vesicles having these properties into lymphatic vessels we expected sustained deposition of liposomes in the retroperitoneal lymph nodes with slow release of entrapped substances.

The amount of liposomes necessary for sufficient lymph node staining is much higher than that produced with routine laboratory scale methods. We therefore modified the method of detergent dialysis (Schwendener, 1986) and developed a scale-up method with a high trapping efficiency of Isosulfan blue, with which concentrated preparations of liposomes with defined and uniform size and negligible residual amounts of detergent can be produced. The most important quality criterion of these liposomes was their stability in lymph as well as in lymph nodes.

Liposome stability, especially in tissues and body fluids, was estimated by perturbed angular spectroscopy. In this method the tumbling rate of a radioactive ion such as $^{111}In^{3+}$ (Mauk and Gamble, 1979) is measured. When entrapped into liposomes, surrounding small solutes allow fast tumbling of the probe. Release from liposomes during membrane disturbances leads to absorption of indium by large molecules (e.g. proteins) or cellular structures and results in a dramatic decrease of probe tumbling.

Liposomes analogous to that, which were found to be stable in perturbed angular spectroscopy for 57 h were used in a dog experiment to determine their distribution kinetics after endolymphatic application.

Materials and Methods

Materials

All chemicals were of the highest purity. Lecithin from egg yolk, cholesterol and Isosulfan blue were purchased from Sigma Chemical Co., St. Louis, MO; sodium cholate and polyethylene glycol (M_r 40 000) were from Serva, Heidelberg, Germany.

Methods

Preparation of liposomes containing Isosulfan blue

Preparation was performed in sterile phosphate-buffered saline (10 mM phosphate, 150 mM NaCl, pH adjusted to 7.35). Liposomes of defined size were prepared by modifying the method of fast and controlled dialysis of mixed detergent/lipid micelles using a capillary dialyser. Lecithin and cholesterol (3 : 1 mol/mol) were mixed with sodium cholate in a molar lipid/detergent ratio of 0.55. Lipids and detergents were dissolved in methanol, and solvent was completely removed by reduced pressure. The dry mixtures were redissolved in 600 ml buffer to yield a final lipid concentration of 25 mM. The slightly opalescent solution was sterilized through 0.2 μm filters (Millipore, Molsheim, France). Mixed micelle solution was then dialysed four times against a continuous flow of buffer, using a sterile hollow fiber cartridge originally designed for blood dialysis (Travenol, 23.08, 5M1789). The membrane of this cartridge has a cutoff of approx. 5000 Da. Flow rates were

12 ml/min in the inner (mixed micelle) compartment and 24 ml/min in the external (buffer) compartment.

The intensively opalescent liposome solution contained less than 0.6 mM cholate and was concentrated by dialysis against a solution of 10% polyethylene glycol in buffer (w/v) instead of pure buffer, using the same hollow fibre cartridge. This further reduced cholate content and the volume of the liposome solution was reduced from 700 to 110 ml within 90 min without any alterations of the liposomes.

Entrapment of dye into liposomes was performed by detergent-induced liposome loading, the original method, which is based on a formation of transient membrane holes in liposomes (Schubert et al., 1986). To 100 ml of formerly prepared, concentrated solution of liposomes, 11.2 g of Isosulfan powder was added under continuous mixing. Subsequently, 12 ml of cholate solution (0.2 M) in buffer were added and the suspension was mixed immediately to avoid local concentration peaks, thereby avoiding foam formation. After 10 min, the dark blue liposome suspension was intensively dialysed against 8 l of buffer, again using this same hollow fiber cartridge. After 2 h, the increased volume of the liposome solution was reduced from 300 to 54 ml using a 5% (w/v) solution of polyethylene glycol with the same procedure described above.

Before using of blue liposomes for animal experiments following quality controls were performed: analysis of dye trapping efficiency on Sepharose CL-4B (Pharmacia, Uppsala, Sweden); electron-microscopical studies of lamellarity and size; stability measurement; sterility control.

Preparation of ^{111}In liposomes

Stability of liposomes in lymph and lymph nodes was studied by perturbed angular spectroscopy after loading of the liposomes with ^{111}In as described elsewhere (Hwang, 1984). The preparation of these liposomes was performed under similar conditions as described for blue liposomes.

Lecithin and cholesterol (3:1 mol/mol) were mixed with sodium cholate in a molar lipid/detergent ratio of 0.55. After dissolving in methanol and drying under reduced pressure, the lipid-detergent film was redissolved in 1 ml buffer containing 1 mM nitrilotetraacetic acid (NTA) to yield a lipid concentration of 25 mM. Mixed micelle solution was dialyzed three times for 8 h against 50 ml of NTA-containing buffer, using a small rotating dialysis device with a highly permeable dialyzing membrane with a cutoff of 10 000 Da (Mini-Lipoprep, Dianorm, Munich). During this dialysis, very homogeneous liposomes with a mean diameter of 74 nm and entrapping NTA were formed. The liposomes were subsequently separated from the non-entrapped NTA on Sepharose CL-4B. The final lipid concentration was 10 mM.

^{111}InCl to be entrapped into the preformed liposomes was purified before use; 1 ml liposome solution with a lipid concentration of 10 mM was incubated for 1 h with a mixture containing 16.7 μl of purified ^{111}InCl (in 3 mM HCl), 33 μl of 1 M acetylacetone (in 5 mM Tris and 0.9% NaCl, pH 7.6) and 60 μl buffer (20 mM Tris-HCl in 0.9% NaCl). Non-entrapped material was subsequently separated from ^{111}In-containing liposomes on an ion-exchange resin (AG1-X8, Biorad) in phosphate form. Trapping efficiency of ^{111}In was 90%. Trapping into the aqueous inner liposomal compartment was distinguished from membrane adsorption in a parallel experiment by an unloading procedure. Tropolone as ionophore (100 μM) and NTA (10 mM) were added to the purified ^{111}In-containing liposome preparation. After incubation for 20 min, almost the total amount of ^{111}In could be separated from liposomes by ion-exchange chromatography on AG1-X8, indicating negligible membrane adsorption of ^{111}In.

Perturbed angular spectroscopy

The above-described liposomes, which were similar to those produced for animal experiments, were investigated for their stability properties in lymph and lymph node. Lymph was obtained from the ductus thoracicus; lymph node was excised from the popliteal region of an anesthetized dog. Immediately thereafter, 250 μl liposomes were mixed with 400 μl dog lymph (separated from cells by centrifugation for 10 min, 4000 $\times$ g). Another 250 μl of liposomes were injected with a sterile 27-gauge sterile needle into different parts

of the lymph node. Both samples were incubated at 37°C and measured repeatedly by perturbed angular spectroscopy.

Determination of dye and lipid concentrations

Isosulfan blue The dye content in liposome suspension, body fluids and tissues was measured by HPLC (Kontron) at 630 nm. The fluids were diluted with methanol to destroy liposomes. The solid tissues were homogenized (Micro-Dismembrator, Braun, Germany), diluted with 4 volumes of buffer and centrifugated (100 000 × *g*, 90 min, 4°C) to separate a pellet, which contained less than 4% of the total dye. The supernatant was mixed with the same volume of aqueous guanidium chloride solution (6 M), transferred onto a C18 Bond Elut column (Analytichem Int.) and eluted with 2 × 200 μl acetonitrile (Baker, Deventer). The column was equilibrated with methanol and water in ratios 1 : 1 (v/v) prior to sample injection.

The injected volume of 20 μl was separated on a 125 mm × 4.6 mm I.D. HPLC column Spherisorb ODS-2 (5 μm). Solvent was distilled water, acetonitrile and 85% phosphoric acid in ratios of 82:18:0.1 (v/v) with a flow rate of 2 ml/min.

Lecithin Because of the constant lecithin/cholesterol ratio (3:1 mol/mol) in liposomes only the lecithin content was measured by HPLC at 203 nm using a standard curve. The liposome suspension was diluted with methanol to destroy liposomes.

A 10 μm Radial-Pak silicon column, 100 mm × 8 mm I.D., was used with a silicon-guard Pak pre-column in an RCM-100 module (Waters, Milford, MA) Solvent was acetonitrile, methanol and 85% phosphoric acid in ratios of 130 : 9 : 1.5 (v/v). The column was equilibrated for 4 h with 2 ml/min prior to 20 μl sample injection. The flow rate was 2 ml/min.

Methods of animal experimentation

Blue liposomes were used on eight anesthesized dogs. Experiments performed on these dogs were divided in two groups, depending on whether liposomes were injected intracutaneously or endolymphatically.

The site of subcutaneous injections (first group) was the dorsal hind limbs; 27-gauge needles were inserted just under the skin of both limbs and 2 ml of liposome suspension were automatically injected subcutaneously into each foot within 1 h. Immediately after the completion of injection, tissue from the injection site and lymph nodes were removed. The representative parts of lungs, kidneys, liver and spleen, as well as serum and lymph were obtained. In controls, we used free instead of liposomally entrapped dye.

To compare the values of different injection rates, a subcutaneous bolus injection of 0.5 ml liposomes was performed. In this case the ductus thoracicus was cannulated in the left neck region and lymph fractions were collected up to 2 h after injection.

Prior to the endolymphatic injections (second group) the ductus thoracicus was cannulated and lymph was collected. The identification of lymph vessels was possible using 0.5 ml blue liposomes injected subcutaneously. Subsequently, the lymph vessel on the dorsal site of the foot was cannulated according to standard technique (Kinmonth, 1982). To compare the influence of the injection rate on the dye distribution, 4 ml of blue liposomes were injected over 60 or 30 min. In the last experiment, the accessory spillover of liposomes already deposited in lymph nodes was attempted using massage and fast fluid infusion. The dye concentrations in lymph, blood, lymph nodes and internal organs were determined in all experiments.

Results

Liposomes

The technique of liposome production used here was found to be very effective when highly concentrated, sterile unilamellar liposomes are needed in large amounts. The final liposome solution was sterile and contained 32 mg lipid/ml and 2.7 mg Isosulfan/ml. After separation on Sepharose CL-4B it was found that the dye was distributed approximately equally inside and outside of the liposomes. The residual cholate concentration in liposomes used here was less than 200 μg/ml, as determined in a parallel experiment using radioactive cholate.

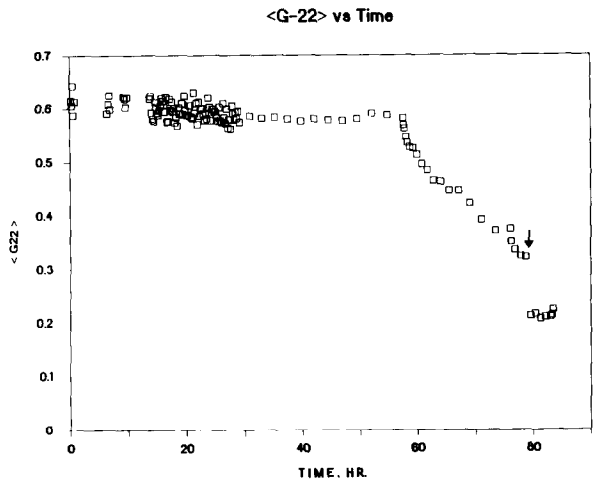

Fig. 1. Time course showing the stability of liposomes during incubation with cell-free dog lymph at 37°C measured with perturbed angular correlation spectroscopy. The $\langle G22 \rangle$ values represent the amount of liposomally entrapped, freely tumbling ^{111}In. Beginning of membrane leakage after 57 h. Addition of 10 µl Triton X-100 (arrow) serves as control for total liposome disruption.

Electron-microscopical analysis of specimen, using a negative staining technique with uranyl acetate, showed that liposomes were nearly exclusively unilamellar. Freeze-fracture replicas showed a homogeneous size of approx. 74 nm.

The results of perturbed angular spectroscopy indicated that the liposomes used in this experiment have a high stability in lymph and lymph node at body temperature. In particular, liposomes incubated with lymph remained stable up to 57 h (Fig. 1).

Liposomes in excised lymph node were stable at least for 8 h and were totally destroyed after 36 h.

Animal experiments

Subcutaneous injections The result of slow subcutaneous injection of liposomes (2 ml per injection site within 1 h) was unsatisfactory. Immediately after completion of injection, a large amount of dye (470 µg/g tissue) was found on the injection site. Nearly the same amount (454 µg/g) was found in the control experiment with aqueous dye solution. However, considerable differences could be found in serum concentrations (liposomal dye, 0.062 µg/ml; free dye, 0.472 µg/ml). The 8-fold lower content of liposomal dye in serum can be explained by its higher concentration in lymph nodes. In the paraaortal lymph nodes, which are the intended target, the liposomal dye concentration (3.4 µg/g tissue) was 70-fold higher than the concentration of free dye (0.047 µg/g), but still too low for appropriate lymph node staining. It should be noted that the dye concentration in lymph nodes represented only 0.7% of the dye concentrations on the injection site. Concentrations in other tissues and blood were less than 0.001 µg/g or not detectable.

The bolus subcutaneous injection of blue liposomes was more successful. Although the injected amount was 4-fold lower (0.5 ml), the dye amount in paraaortal lymph nodes was nearly 4-fold higher (15 µg/g). This value was obtained from lymph nodes removed 6 h after injection. However, dye concentration was still too low to stain these lymphs satisfactorily.

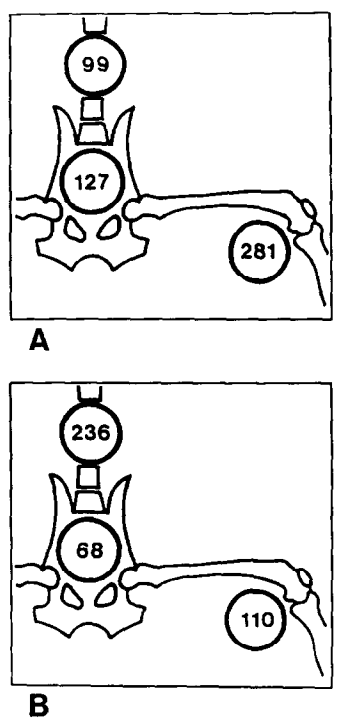

Fig. 2. Isosulfan concentrations in lymph nodes (µg/g) 1 h after endolymphatic injection of 4 ml blue liposomes: (A) within 60 min; (B) within 30 min.

The passage of subcutaneously injected liposomes through the lymph system was very quick. Already in the time between 10 and 15 min after injection, an 8.3 ml lymph fraction with 64.4 μg dye/ml was collected. The analysis of lymph from the ductus thoracicus showed that the blue dye was still incorporated in the liposomes to the same extent as before entering the lymph system.

The total dye amount measured in ductus-thoracicus-lymph varied in different experiments between 0.53 and 1.12% of the injected amount. The bulk of liposomes remained at the injection site, where 6 h after injection 159 μg/g could still be detected.

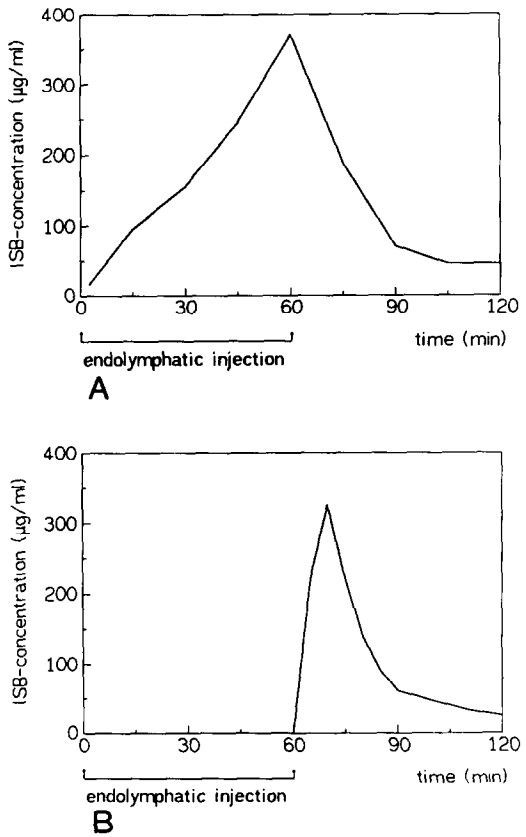

Fig. 3. Concentration of dye in the ductus thoracicus lymph during and after the injection of 4 ml liposomes containing 2.7 mg dye per ml into the lymphatic vessels of two dogs. (A) Increase of concentration during injection (B) No spillover of dye during injection. Independently of time differences in appearance of the dye in the ductus thoracicus, its peak concentrations were reached after 1 h and were approximately equal at 350 μg/ml.

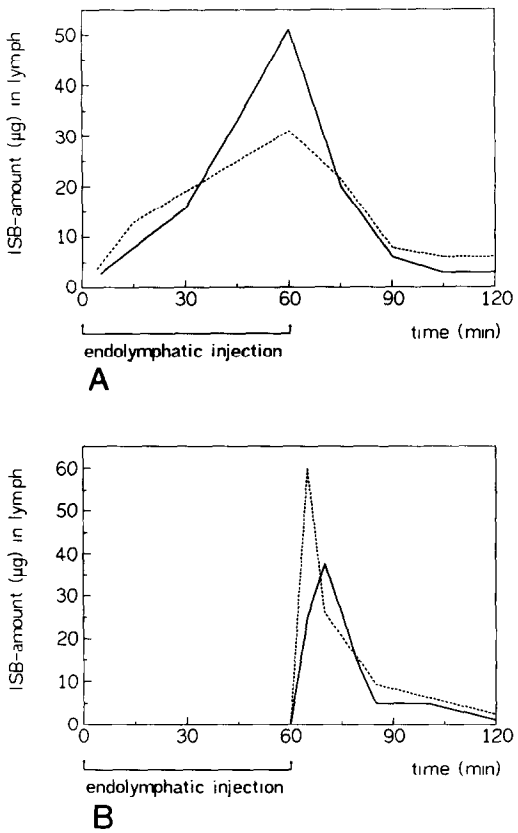

Fig. 4. Gel separation on Sepharose CL-4B of the ductus thoracicus lymph obtained during and after endolymphatic injection of blue liposomes. The ratio free (———)/entrapped (— — —) dye was approximately the same as in the preparation originally injected. The amounts shown here were determined in fractions collected every 5 min. (A) Analysis of the case from Fig. 3A; (B) analysis of the case from Fig. 3B.

Endolymphatic injections After experiments with subcutaneous injection it became clear that only direct injection of blue liposomes led to significant lymph node staining. All following injections were performed in this way.

After direct endolymphatic injections of blue liposomes, all retroperitoneal lymph nodes became blue, independently of experimental design. The following differences were, however, noted:

The paraaortal lymph nodes examined after completion of 1 h injection of 4 ml liposomes were less stained than the pelvic and popliteal lymph

nodes. The dye content was found to be 99 or 122 or 281 μg/g, respectively. In contrast, injection of the same amount of liposomes twice as rapidly resulted in respective concentrations of 236 or 68 or 110 μg/g (Fig. 2).

At this time, liposomes were distributed uniformly in the lymph node, and could not be identified as having affinity with any particular areas of lymph nodes. As an unspecific reaction, sinus hysteocytosis was found in all examined lymph nodes.

A time course of concentration of the dye which escaped from the lymphatic system into serum is shown in Fig. 3A. Dye concentration increased rapidly during the injection time, and decreased in a similar way during the following hour.

This time course was, however, dependent on the individual anatomic and physiologic alterations. Extreme alteration was demonstrated in the other dog in which the storage capacity of the lymph system was large enough to retain the entire injected amount up to the completion of injection (Fig. 3B). Thereafter, a rapid elevation of dye concentration occurred. The duration of this high recovery of dye was 5 min, in which 875 μg dye was collected.

During the following hour the dye amount recovered from lymph decreased rapidly. At the end of this period, however, a total of 26.4% of the amount injected was collected together with ductus-thoracicus lymph. The chromatographic analysis of this blue lymph showed that the in-

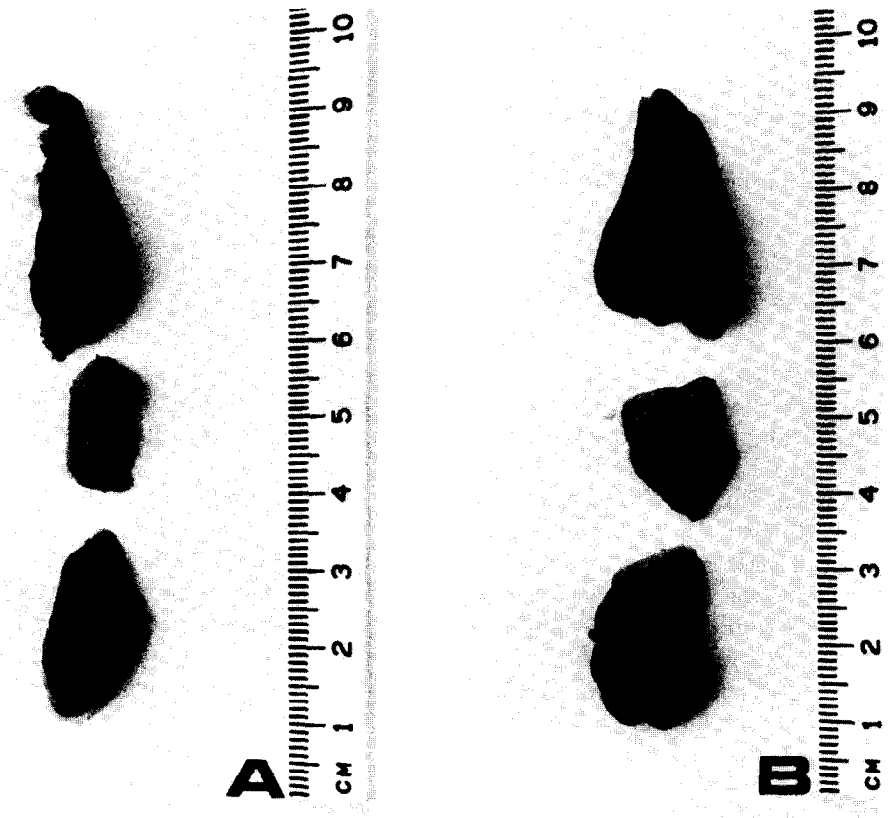

Fig. 5. Dog lymph nodes excised 3 h after extensive removal of liposomes, using massage and saline infusion. Lymph nodes from bottom to top: popliteal, pelvic, paraaortal. The dye contents are 191, 102 and 103 μg/g, respectively. (A) Lymph nodes as found in surrounding tissue; (B) these same lymph nodes incised to demonstrate their macroscopically intact structure.

jected liposomes passed the lymph system undamaged (Fig. 4).

In the fractions of liposomes spilled out of lymph nodes as an accessory bulk, the amount of intact liposomes was unchanged. The accessory spillover of liposomes into the ductus thoracicus was achieved with massage and fluid infusion.

Repeated pressure on the pad from distal to proximal with the thumb during the 1 h injection of 4 ml liposomes resulted in elevated spillover of 44%. Compared with 26.4% without massage, the increase was 67%. The following 30 min intravenous infusion of 500 ml saline resulted in the spillover of an additional 20.4%, for a further increase of 46%.

With these two methods a total fraction of 64.6% of the amount injected was found in the ductus thoracicus again. Even in this case the lymph nodes remained dark-blue stained, with dye content between 102 and 191 $\mu g/g$ (Fig. 5).

A dye concentration of about 100 $\mu g/g$ was found to be sufficient for lymph node staining. The injection technique used here allows filling of most of the lymph nodes to this extent or more, independent of injection rate alterations, massage or infusions.

Discussion

Published experiments on the action of liposomes in the lymph system were limited for the most part to subcutaneous injections in animal experiments. After s.c. injections of ^{125}I-labelled liposomes into mice and rats (Mangat and Patel, 1985) it was found that 1% of the injected amount was detectable 6 h after injection in the primary lymph nodes, and only 0.25% in the secondary nodes. However, after the liposomes were coated with IgG, the uptake was increased to 4.5 and 1.8%, respectively. The lower uptake in the secondary nodes was observed in another study using rats (Tumer et al., 1983). In the latter experiment, the small liposomes (30–60 nm) penetrated better into the lumbar lymph nodes than the larger liposomes (400 nm).

The electrical charge of liposomes also affects their uptake by the lymph nodes. After s.c. injection of different charged liposomes into rats using the aqueous space marker, ^{125}I-labelled poly(vinyl pyrrolidone), it was found that maximal uptake can be achieved by negative liposomes, followed by positive and neutral species (Patel et al., 1984).

Although the lymphatic uptake after s.c. injection is low, the experimental tumors responded better after use of liposomal drugs (Kaledin et al. 1981; Khato et al., 1983), in particular when temperature-sensitive liposomes were combined with hyperthermia (Maekawa et al., 1987).

The indication that lymph nodes act as a filter for liposomes is supported by the observation that the s.c. injected ^{125}I-labelled liposomes are found in constant amounts independently of the concentration used; 3–6- and 12-fold dilution with saline resulted in the same uptake (Kaledin et al., 1982). In only one reported instance (Perez-Soler et al., 1985) were multilamellar vesicles injected directly endolymphatically into dogs and compared with subcutaneous injection. Endolymphatic injection resulted in immediate uptake by the popliteal, inguinal and pelvic lymph nodes.

After s.c. injection, all activity remained for up to 4 h at the injection site. After this time, some activity was found in the popliteal lymph nodes; this increased during the next 24 h. No uptake was found in the other nodes. The value of these results, which were obtained with the membrane marker ^{99m}Tc, is diminished by the observation that this marker probably dissociates up to 97% from liposomes and thus does not indicate the presence of intact liposomes in lymph nodes (Patel et al., 1984).

The analysis of the above results gives no clear indications regarding the optimal mode of lymphatic drug delivery. However, the decision to use unilamellar liposomes of defined size acquired from pure natural lipids and injected endolymphatically was supported by many of these results. In addition, the problem of detection of intact liposomes in lymph and lymph nodes was solved by using perturbed angular spectroscopy.

This technique was found to be valuable for the examination of the stability of liposomes in fresh biological material (Hwang and Mauk, 1977) and to be useful for screening different liposome preparations designed for animal experiments.

Comparison of 57 h stability of liposomes in cell-free lymph with only 8 h stability of liposomes in excised lymph node indicates that released enzymes or metabolic products originating from lysed cells must be responsible for the destruction of liposomes. Bacterial contamination, however, may also be considered during the long incubation times.

The good stability of liposomes in cell-free lymph indicates that the influence of lipoproteins and other substances present in body fluids on liposome destabilisation (Tall et al., 1986) is probably less extensive in lymph than reported.

Since preliminary studies (Hirnle et al., 1988) proved the sustained deposition of blue liposomes in rabbit lymph nodes over 28 days, the present study was designed rather to answer pharmacokinetic questions on the time during and shortly after endolymphatic injection. The lymphatic filter system does not retain all substances injected, independent of their chemical properties. One-fourth of injected liposomes leave the lymph system during and just after the injection. This amount is similar to those of oily drug suspensions (Hirnle, 1985) injected under the same experimental conditions.

Liposomes have, however, many important advantages. They are not deposited in lungs, and there is no hazard of embolization of capillary vessels. They are nontoxic for lymph node parenchyme and are fully biocompatible. They cause neither pain nor fever. They have low vicosity and are stable in suspension.

The residual cholate concentration in liposomes reaching no more than 200 μg/ml is higher than the physiological cholate concentration in serum (Matern and Gerok, 1979). However, after dilution of the liposome suspension in lymph similar low cholate concentrations will be promptly achieved.

These properties make liposomes optimal drug carriers for endolymphatical injections. Liposomes are much less appropriate for subcutaneous injection. The fraction of liposomes found in the lymphatic system was unsatisfactory, and the majority of the liposomes remained on the injection site. The liposomes were, however, shown to be lymphotropic. After the subcutaneous injection of Isosulfan liposomes, the blue staining of foot lymph vessels is evident and sufficient for their easy identification prior to lymphography. The staining of these vessels is similar in extent to that after the routine s.c. injection of 2.5% aqueous dye, which is 10 times more concentrated.

The area of potential clinical interest is, however, direct endolymphatic injection. This study indicates that the fate of liposomes in the human lymph system will vary from the commonly known behavior of oily contrast media used for over 30 years for lymphography (Kinmonth, 1982). The advantages to be expected are:

(1) The possibility of rapid endolymphatic injection. Because of low viscosity, even an injection rate of 0.14 ml/min will probably not lead to any damage of the lymphatic system. This will benefit patients due to the brevity of the procedure and higher drug concentrations which can be achieved in paraaortal lymph nodes.

(2) There is no hazard of lung damage. Liposomes are not stored with predilection in the lungs. The fraction of liposomes which escape from the lymph system via the ductus thoracicus pass unhindered through the lung capillaries. There are no respiratory complications to be expected, with the result that the general anesthesia required for subsequent surgery is not contraindicated.

The importance of sufficient preoperative lymph node staining is best visible in lymphangiograms made after radical lymphonodectomy, in which the remaining enlarged lymph nodes are present (Piver et al., 1971).

The results of lymph node staining can be extended to oncological lymph node treatment, using cytostatics incorporated into the liposomes. Because of their considerable toxicity, additional precautions originating from results of animal experiments should be respected:

The drug should be injected into the lymphatic vessel as close as possible to the target nodes.

The amount of drug injected endolymphatically should not exceed the maximum amount of this drug which may be injected as a daily routine intravenous dose. This dose can be only increased after obtaining statistical data on the maximal spillover on humans.

If an increased drug dose is indicated, the

ductus thoracicus may be cannulated and the drug excess can be removed in this way. With this 'isolated perfusion' most of the drug escaping from the lymph system can be evacuated.

In conclusion, endolymphatic application of liposomes for diagnostic and therapeutic purposes seems to be a safe, effective and non-toxic method of drug transport and its storage in the lymph nodes.

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