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Histological Findings in Rabbit Lymph Nodes After Endolymphatic Injection of Liposomes Containing Blue Dye

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Abstract—Liposomes produced from phosphatidylcholine and cholesterol and containing Patent Blue V have been injected endolymphatically in the rabbit. The lymph nodes stained dark blue and retained this colour until termination of the experiment after 28 days. No toxic effects were observed histologically. The liposomes were deposited within the macrophages in a fine granular pattern. Because endolymphatic injection of such liposomes produces excellent contrast between retroperitoneal lymph nodes and their surrounding fatty tissue, it can improve both the radicality and the selectivity of lymphonodectomy in oncological surgery.

Radical retroperitoneal lymphonodectomy usually confronts surgeons with the problem of distinguishing between lymph nodes and surrounding fatty tissues. Repeated attempts in the past to remedy this by staining lymph nodes preoperatively have met with little success due to inadequate contrast or unacceptable toxicity (Numers et al 1965; Davidson et al 1967; Gregl et al 1968; Schmidt-Mende et al 1968; Spelsberg et al 1968; Zerbino et al 1975; Ludvik 1965).

In one recent trial, a dark blue dye, Guajazulen, was dissolved in Lipiodol Ultra-Fluid (Harzmann et al 1989; 1990). When injected endolymphatically, it afforded excellent blue staining of lymph nodes. However, local necrosis, tissue compression caused by oil droplets, and lung involvement with potential respiratory distress prompted further search for a carrier of endolymphatic dyes.

The encapsulation of blue dye in liposomes was investigated as a possible solution to this problem.

Material and Methods

The liposomes were manufactured by detergent dialysis as previously described (Milsmann et al 1978), using sodium cholate as detergent. Chromatographically pure phosphatidylcholine (Sigma) and cholesterol (Sigma), mixed 3:1 (mol/mol), were added to sodium cholate in a molar lipid/detergent ratio of 0·55. All three components were dissolved in methanol and subsequently dried in a rotary evaporator. The lipid film on the inner surface of a round bottom flask was redissolved in buffer containing a saturated solution of Patent Blue V (Guerbet) and sterilized through 0·2 μm filters (Millipore). This mixed micelle solution was dialysed in a special device (Liposomat, Dianorm) over 48 h to remove most of the detergent and non-entrapped dye.

The resulting diluted liposome suspension was dialysed against a 20% polyethylene glycol solution (Serva) to achieve a dye concentration of 0·8 mg L^{-1}, which preliminary experiments showed to be adequate for appropriate staining of lymph nodes.

The prepared liposomes were examined in a laser light correlation spectrometer (Coulter Nanosizer) and found to be homogeneous, with a mean diameter of 74 nm. In electron microscopical examination, the liposomes were found to be unilamellar. Sterility was demonstrated by microbiological assay. The stability of these liposomes in-vitro at 20°C was 80% after 28 days.

Endolymphatic injection of blue liposomes was performed on six Giant German rabbits (7·0±0·7 kg). The rabbits received an average of 2·1 mL (0·3 mL kg^{-1}) of liposomes within 30 min. The animals were killed by the intravenous overdoses of pentobarbitone.

The lymph nodes were removed 6 h and 2, 7, 14, 21 and 28 days after injection.

Results

The identification of lymph nodes was simple up to the 28th day because they were stained dark blue, in contrast to the unstained surrounding fatty tissues. Especial care was needed during removal of the lymph nodes up to two days after injection because the nodes were enlarged and maximally filled with blue liposomes (Fig. 1A). The mechanical damage of the node capsule, which occurred 6 h after injection of the liposomes, led to dye extravasation and staining of surrounding fatty tissue. This complication was not observed in any subsequent lymphnodectomies.

Except for dark blue staining, the excised lymph nodes showed no macroscopic signs of damage which might have been caused by liposomes (Fig. 1B). Twenty eight days after injection, dye concentrations in lungs, spleen, liver and kidneys were above 5 μg g^{-1}. At this time, the dye concentration in the lymph nodes was 172 μg g^{-1} and was five times lower (22%) than that found 24 h after injection.

Six hours after injection, the extracellular space was stained uniformly blue, and no histological structures hosting liposomes could be identified. In the following days, the liposomes moved gradually out of the extracellular space into the macrophages, whose filling with blue granulations was observable in full spectrum after 14 days, and remained virtually unchanged up to termination of the experiment after 28 days. Although the lymph nodes remained macroscopically homogeneously blue after 28 days, a wide spectrum of histological findings was observed. In some pictures,

only single accumulations of blue cells were observed, surrounded by a wide, dye-free area (Fig. 1C, D). Most of the pictures, however, showed extensive staining of lymph node tissue. In these areas, numerous groups of macrophages were filled with blue granulations (Fig. 1E, F). The lymph node tissue was normal; no inflammation and no necroses occurred.

Discussion

Encapsulation of blue aqueous dye into the inner spaces of unilamellar liposomes was found to be a good method for sustained blue staining of lymph nodes, with no measureable side effects. The blue background, best visible 6 h after injection, was probably composed of intact blue liposomes. The granulations visible within the macrophages in later examinations are attributed to clumped liposomes stored there for more than a month.

The method of staining lymph nodes described here is of potential clinical relevance in that it permits clear identification of the retroperitoneal lymph nodes during surgery thereby making their radical, but selective removal possible.

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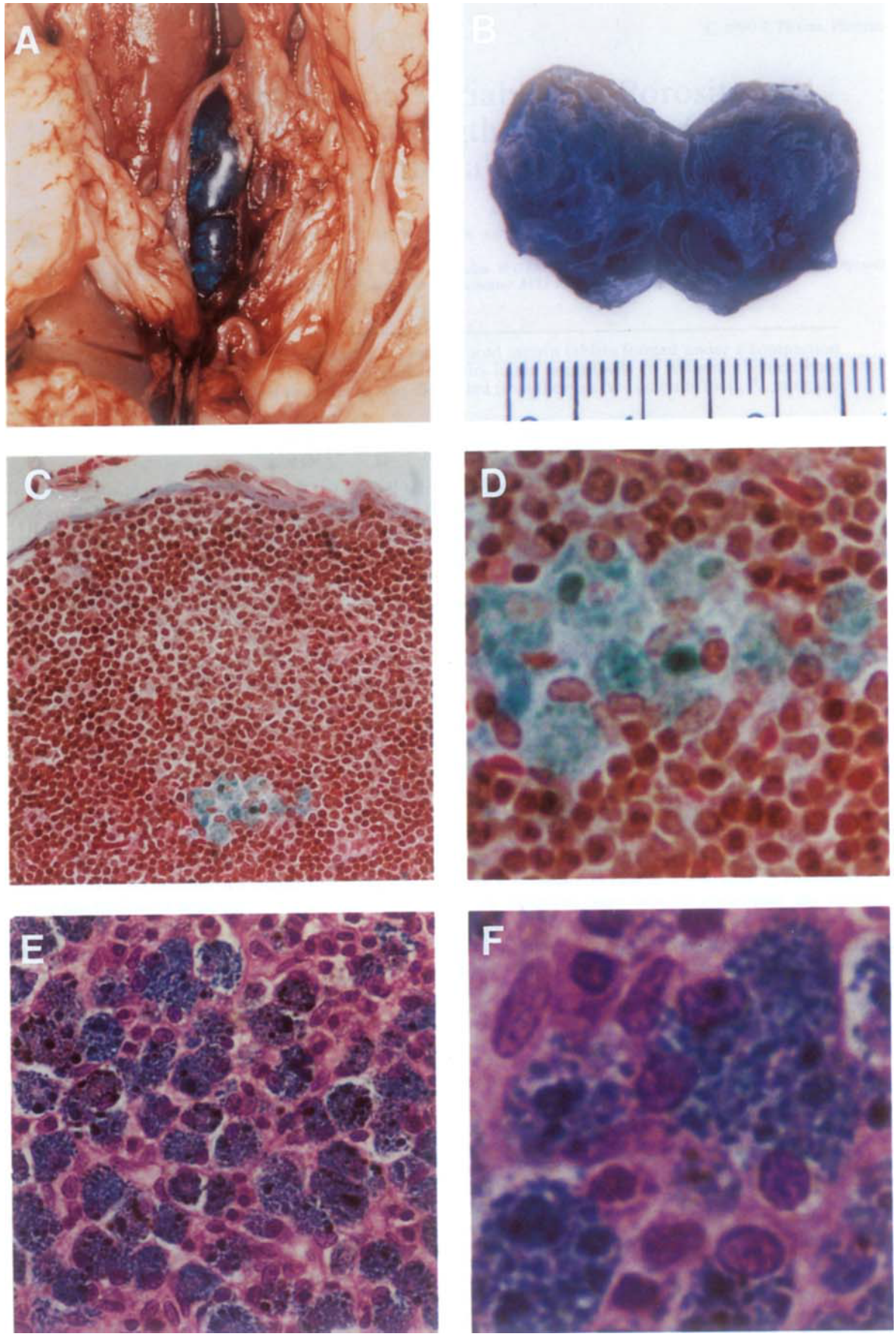

FIG. 1. A. Enlarged retroperitoneal rabbit lymph nodes saturated with blue liposomes on 2nd day after endolymphatic injection of blue liposomes. Excellent visibility in the surrounding fatty tissues.
B. Dissected popliteal lymph node 28 days after endolymphatic injection of blue liposomes. Dark blue homogeneous staining of tissue, no evidence of necroses or inflammation. The lymph node is not enlarged.
C. Fragment of a retroperitoneal lymph node with a single group of macrophages containing blue granulations. Giemsa-staining.
D. Enlarged picture of a single group of macrophages containing blue granulations interpreted as intact, clumped liposomes. Giemsa-Staining.
E. Large accumulation of macrophages from the central part of a retroperitoneal lymph node filled with blue liposomes. Haematoxillin-eosin staining.
F. Enlarged image of macrophages from the central part of a retroperitoneal lymph node. The liposomes are distributed uniformly in cytoplasm in a fine granular pattern.