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PATENT BLUE V ENCAPSULATION IN LIPOSOMES: POTENTIAL APPLICABILITY TO ENDOLYMPATIC THERAPY AND PREOPERATIVE CHROMOLYMPHOGRAPHY

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ABSTRACT

The water soluble dark blue stain Patent Blue V was incorporated into liposomes to increase its depot properties. Experiments with intraperitoneal and intramuscular application in rats and with intralymphatic injections in rabbits showed a substantial difference between the pharmacokinetics of aqueous and liposomal solutions. Independent of the route of administration, the dye-stain remained much longer in the tissues using liposomes. Most notably, retroperitoneal lymph nodes in rabbits remained dark blue up to 28 days after hindlimb endolymphatic instillation of liposomal patent blue. This vastly superior depot effect may be clinically adaptable for endolymphatic therapy with cytostatic drugs to enhance nodal drug retention and concentration or for preoperative chromolymphography to facilitate nodal visualization at laparotomy.

Endolymphatic application of cytostatic drugs may be a useful method for treating lymph nodal metastases. Water soluble drugs, however, are retained in lymph nodes only in connection with lipids, that is, suspended (1) or emulsified in oil (2). In either instance a large amount of oil and additives is required and lung oil embolism is therefore a potentially dangerous side effect of conventional endolymphatic injection.

To circumvent this drawback we tested use of liposomes as a new carrier system for endolymphatic drugs. We found it possible to load these small vesicles built of lipid bilayers with model substances and thereby study the modified pharmacokinetics of the drugs. In this report, we examined tissue retention of Patent Blue V after incorporation into liposomes and compared the findings with administration of the dye-stain in aqueous solution.

MATERIALS AND METHODS

Liposomes were generated with a detergent dialysis technique using a Liposomat made by the Dianorm Company *(Fig. 1)*. The Patent Blue concentration in liposomes was set at 1.6mg/ml for rat experiments and 0.8mg/ml for rabbit experiments. This material was detected by 630nm absorption. Using these liposomes preparations, 3 animal studies were performed:

1. Intraperitoneal injection in 1ml liposomal Patient Blue (4 rats).
2. Intramuscular injection of 1ml liposomal Patent Blue (4 rats).
3. Hindlimb endolymphatic injection of 2ml liposomal Patient Blue (6 rabbits).

Each experiment included appropriate controls without liposomes which received an aqueous solution of Patent Blue in the same concentration at each application site.

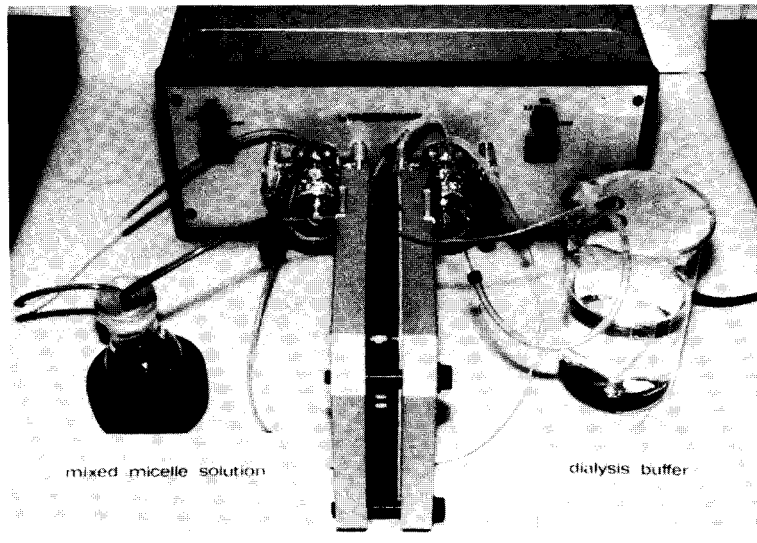

Fig. 1. Instrument for generating liposomes using a detergent dialysis technique.

RESULTS

The detergent dialysis technique makes it possible to produce homogeneous lipid vesicles with a diameter of approximately 170-200nm. The animal experiments showed that these liposomes have much better deposition properties than aqueous controls.

Experiment 1: 24 hours after intraperitoneal injection no toxicity was noted. *Fig. 2* shows the organ concentrations of Patent Blue at this time. The highest levels were found in the spleen and liver with only slight amounts detectable in the lungs. Note, moreover, that the Patent Blue deposition was considerably higher when the dye-stain was incorporated into liposomes.

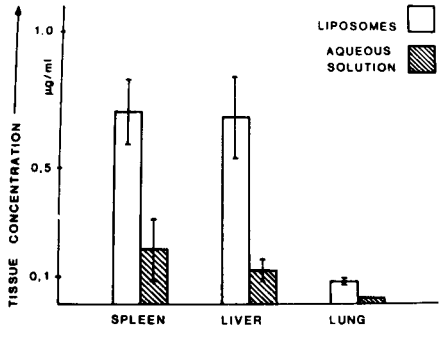

Fig. 2. Retention of Patent Blue in spleen, liver, and lung, 24 hours after intraperitoneal injection of the blue dye-stain either as an aqueous solution or after its incorporation into liposomes.

Experiment 2: The intramuscular injection site remained dark blue until the study ended on day 28. Patent Blue concentrations reached 1.52µg per gram of tissue. No persistent muscle staining was observed in the control group with Patent Blue aqueous solution.

Experiment 3: Rabbit lymph nodes were examined at intervals between the 2nd and 28th days after endolymphatic injection. The retroperitoneal nodes intended as targets remained dark blue up to the 28th day when the study was ended. Identification and selective removal of lymph nodes was therefore comparatively simple. The stained nodes were slightly larger than the control nodes *(Fig. 3)*. They otherwise showed no sign of mechanical or toxic damage. Nodal histology showed no major alteration.

After 28 days, Patent Blue was found in the retroperitoneal lymph nodes

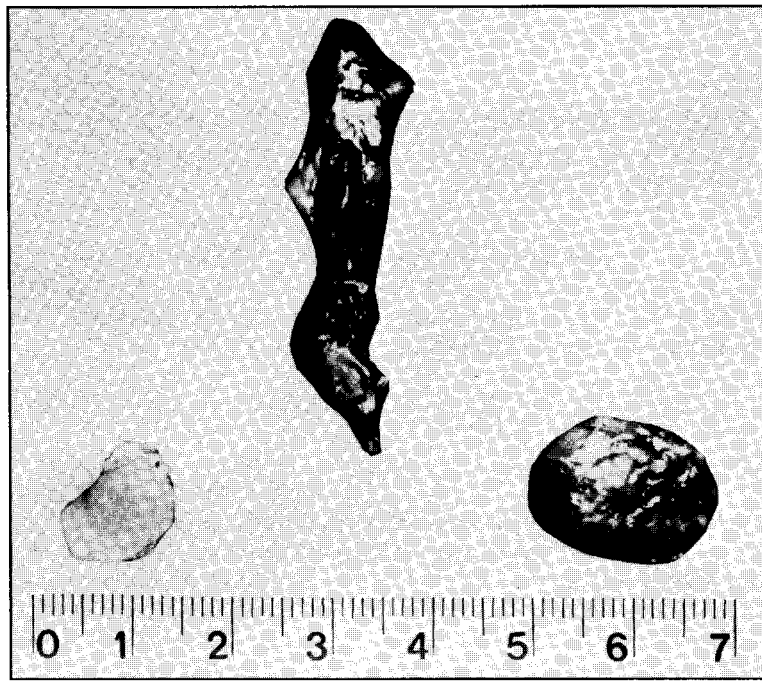

Fig. 3. Popliteal and retroperitoneal lymph nodes of rabbit 28 days after endolymphatic injection of patent blue incorporated into lysosomes. Upper: deeply blue stained retroperitoneal nodes. Right: deeply blue stained popliteal node on side of injection. Left: pale contralateral popliteal node.

in an average concentration of 172μg per gram tissue. This was 22% of the highest concentration found 24 hours after injection. The concentrations found in spleen, liver, lungs and kidneys were consistently above 5μg per gram tissue. No evidence of lung damage was observed.

COMMENT

Patent Blue incorporated in liposomes changed its pharmacokinetic qualities. Independent of route of administration, the dye-stain remained much longer in the tissues using liposomes than when administered as an aqueous solution. In particular, it remained for several weeks in retroperitoneal lymph nodes after hindlimb intralymphatic instillation. This latter finding is potentially clinically useful for two reasons. First, it may be possible to incorporate other water soluble drugs such as cytostatics into liposomes with similar prolongation of tissue deposition. Second, Patent Blue liposomes may be a superior and safe preparation for preoperative chromolymphography to facilitate visualization of retroperitoneal lymph nodes at operation.

ACKNOWLEDGEMENT

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REFERENCES

1. Hirnle, P: Endolymphatic application of bleomycin oil suspension in dog model. Lymphology 18 (1985), 56.
2. Hirnle, P, E Heide: Iodinated emulsions of cytostatic agents for combined diagnostic and therapeutic lymphography. In *Progress in Lymphology*, University of Adelaide Press (1985), 279.

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