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Cellulose microspheres as a sustained release system for parenteral administration

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Summary

Microspheres of modified cellulose (DEAE), 40–160 μm in diameter, were labelled with [^{131}I]rose bengal and administered subcutaneously to rabbits. The release of the rose bengal was followed using gamma scintigraphy over a period of 28 days. Significant sustained tissue and blood levels were obtained.

Introduction

The use of microspheres as sustained release targeting agents for anticancer drugs has received much attention during recent years (Widder et al., 1979). A variety of different biodegradable materials have been used including gelatin and albumin (Yoshioka et al., 1981; Sugibayashi, 1977). More recently agarose beads (Kojima et al., 1978) and starch microspheres (Lindell et al., 1978) have been described. Blanchard et al. (1965) have shown convincingly using rabbits, that radioactive ceramic or plastic microspheres (15 μm in diameter) lodged in tumours in higher concentrations than surrounding liver tissue when infused via the hepatic artery.

The intra-arterial infusion of non-degradable ethylcellulose microspheres containing mitomycin was found to be an effective means of producing chemoembolism (infarction and sustained drug action) in man (Kato et al., 1981).

Cellulose can be modified in a variety of ways to alter its solubility and binding characteristics. DEAE cellulose is a modified cellulose with basic groupings (Weaver, 1969). It is used widely in ion-exchange column chromatography and is available commercially in the form of microspheres (40–160 μm). Acidic drugs are bound

strongly to DEAE-cellulose and presently we are investigating its use as a non-degradable sustained release system for parenteral administration using the rabbit as an appropriate animal model. Our preliminary results are reported here.

Experimental

DEAE-cellulose as microspheres was purchased from Pharmacia Fine Chemicals (DEAE-Sephacel). This material has a particle size range of 40–160 μm and is provided in a preswollen condition in an aqueous ethanol dispersion. The binding capacity is approximately 0.7 meq./g. Acidic anticancer drugs such as methotrexate bind to DEAE-cellulose (Chamberlin et al., 1976). Furthermore, the microspheres can be labelled effectively with a gamma-emitting radiopharmaceutical such as [^{131}I]rose bengal that contains a carboxylic acid grouping. [^{131}I]Rose bengal was obtained from the Radiochemical Centre, Amersham, at a specific activity of 1 mCi (37 MBq) in 2 ml. DEAE-cellulose microspheres were washed and suspended in normal saline and sterilized by autoclaving. 60 mg, containing about 10^6 particles were labelled by the addition of approximately 50–150 μCi (1.8–5.5 MBq) of [^{131}I]rose bengal solution (concentration 0.29 mg/ml) and allowed to equilibrate for 18 h. The rose bengal was strongly bound to the DEAE-cellulose; the binding capacity in normal saline was 380 mg/g as determined by equilibrium adsorption studies. The labelled microspheres, in a dose volume of 1 ml, were administered subcutaneously as a bolus injection to New Zealand White rabbits (2–4 kg). Control experiments were conducted using 1 ml [^{131}I]rose bengal solution of the same concentration and activity.

The subcutaneous deposition of the microspheres and the sustained release of rose bengal was followed using external scintigraphic imaging (General Electric Maxi Camera II). Dynamic and static views were recorded and processed by computer (Gammascope-Link Systems). Blood samples were removed at suitable time intervals and were analyzed for radioactivity using a gamma-counter (Intertechnique CG4000). At the end of the experiments animals were killed and histological sections were taken of the injection site.

Results and discussion

Figs. 1 and 2 show the activity–time profiles obtained using the gamma camera following subcutaneous injection of [^{131}I]rose bengal-labelled DEAE-microspheres and free [^{131}I]rose bengal. Fig. 1 shows data for the injection site, while Fig. 2 shows data for the whole animal. The rose bengal solution is cleared quite rapidly from the administration site with a clearance half-time of about 7 h; the corresponding data for the DEAE-microspheres indicates that rose bengal is released more slowly after injection. This constitutes a sustained release process whereby the bound rose bengal is slowly leached from the microspheres. The half-time for clearance is of the order of 30 h. (The range of values for the microspheres was much greater than for the

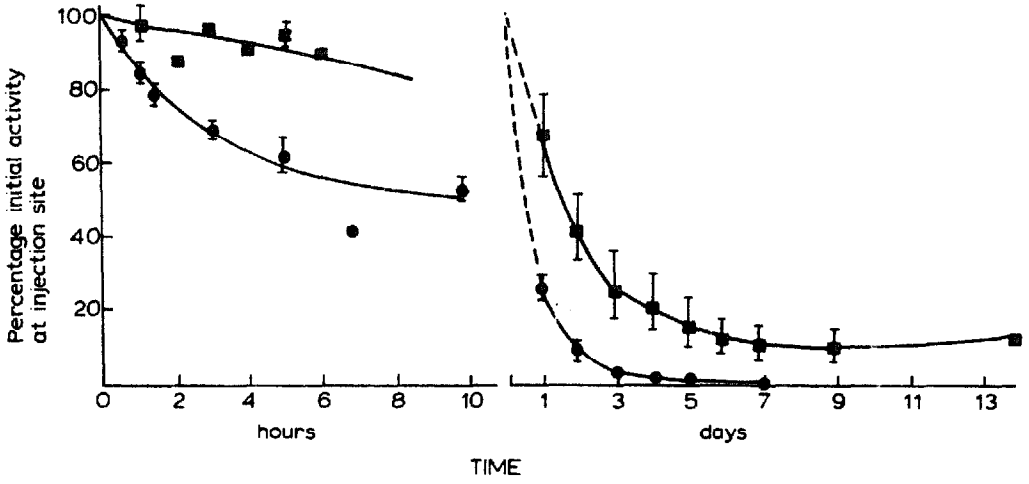

Fig. 1. Clearance of [^{131}]rose bengal from a subcutaneous injection site in rabbit (n = 3, mean + S.E.M.). ●, Rose bengal solution; ■, DEAE-microspheres labelled with rose bengal.

solution systems but it was noticed that administration of the total dose of microspheres to one injection site gave slower disappearance of activity from the site of injection than the same dose administered to three separate injection sites.) A much longer clearance phase is evident after 5 days and activity was still present at

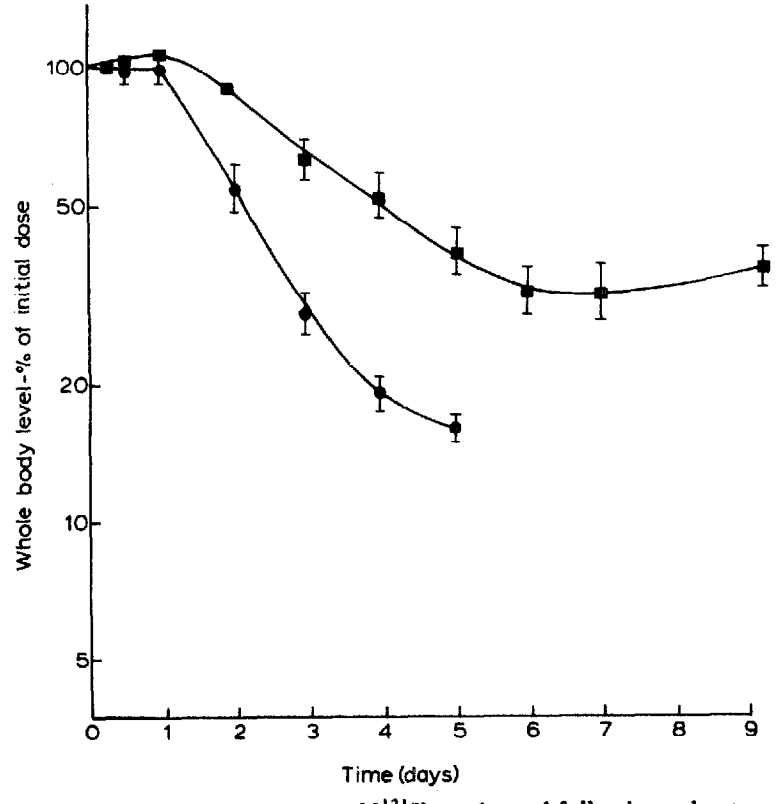

Fig. 2. Whole body clearance of [^{131}I]rose bengal following subcutaneous administration to rabbits (n = 3, mean + S.E.M.). ●, Rose bengal solution; ■, DEAE microspheres labelled with rose bengal

the site of injection after 27 days. The half-time for clearance of this phase is of the order of 10–12 days.

The whole body clearance half-times are 2 and 4 days for the rose bengal solution and DEAE-cellulose microsphere rose bengal systems, respectively (Fig. 2). It will be noticed that the scatter in the results for the microsphere system is much less than for that obtained from an examination of the injection site. Moreover, there was no evidence of a more rapid clearance pattern for multiple injection sites. 30% or more of the original dose of rose bengal resided in the animal 9 days after injection even though only 10% could be found at the injection site itself. Thus it can be concluded that the labelled microspheres diffuse from the injection site still retaining much of the rose bengal in a bound form.

The blood level–time profiles are shown in Fig. 3. As expected the data for the rose bengal solution show a rapid appearance of activity in the blood with a peak at about 30 min, while for the DEAE-cellulose system a much reduced peak occurs after 200 min and significant blood levels are maintained for a considerable period of time. The rate constants for elimination are very different; the half-lives determined from the final portions of the elimination curves are 1.2 and 5.0 days for the solution and microsphere systems, respectively, and are similar in magnitude to the whole body clearance values.

Histological examination of the injection site 6 weeks after administration of the microspheres indicated the presence of intact undegraded DEAE-cellulose. There was no evidence of any inflammatory tissue response.

These preliminary results suggest that DEAE-cellulose microspheres may have

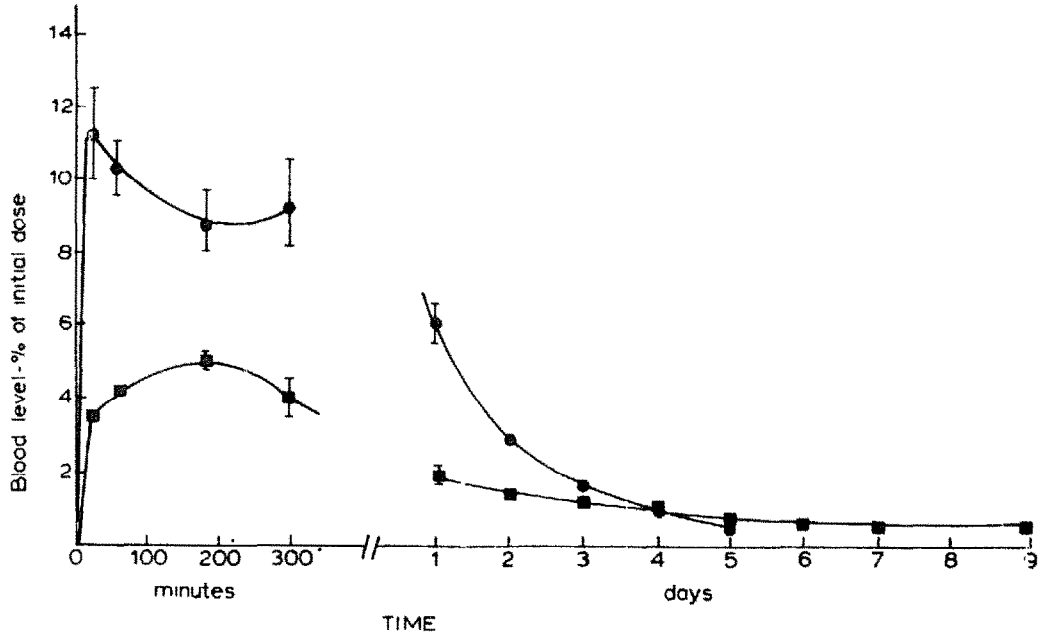

Fig. 3. Blood level activity profiles following the subcutaneous administration of [^{131}I]rose bengal to rabbits (n = 3, mean + S.E.M.). ●, Rose bengal solution; ■, DEAE microspheres labelled with rose bengal.

utility as sustained release system. The administration of microspheres containing bound methotrexate is now under study. Alternative routes of administration and other ion exchange systems (e.g. DEAE-agarose) are also to be investigated.

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