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Comparison of albumin and casein microspheres as a carrier for doxorubicin

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Doxorubicin (Adriamycin)-loaded casein and albumin microspheres, with diameters between 14 and 38 μm (50% weight average) were prepared by glutaraldehyde stabilization of the aqueous phase (containing protein and drug) of a water in oil emulsion. Physical properties, drug loading characteristics and release rates from microspheres in-vitro have been compared and correlated with effects on tumour growth when injected intratumourally in rats. Compared with albumin, the surface charge of the casein system was more negative and the microspheres exhibited a slower release of drug in-vitro. Both observations could be explained by the lower drug content of the casein system. There was evidence for the formation of a doxorubicin complex in the microspheres, the significance of which is not yet known. Casein microspheres containing 11 μg of doxorubicin had a similar inhibitory effect on tumour growth (growth delay = 20·7 days) to 85 μg of drug incorporated into albumin microspheres (growth delay = 18·6 days). The absence of a simple dose-response relationship shows that carrier matrix can influence potency of incorporated drug. The results are consistent with release rate of the drug from microspheres (obversely, rate of drug delivery to the tumour), being a determinant of potency in these systems.

In the chemotherapy of cancer, therapeutic effects are often limited by the toxicity of drug to healthy tissue and inadequate localization and duration of contact of the drug at the desired site of action. To enhance the therapeutic potency of anticancer drugs, carrier systems have been sought which might localize drug in an organ or be taken up selectively by malignant cells. Microspheres have received growing attention as drug carriers (Widder et al 1979; Tomlinson 1983; Tokes et al 1984). Many reports deal with the preparation and characterization in-vitro of protein, polysaccharide and other polymeric microspheres (Longo et al 1982; Artursson et al 1984; Tomlinson et al 1984; Juni et al 1985). A limited number of in-vivo investigations have described microsphere distribution, drug disposition and antitumour activity following administration of drug-loaded microspheres (for example Morimoto et al 1980; De Luca et al 1984; Willmott et al 1985b; Willmott & Cummings 1987).

We consider the carrier matrix to be an important factor in determining the in-vitro characteristics and in-vivo behaviour of microspheres. Previous studies from this laboratory focused on albumin as a carrier for the cytotoxic drug doxorubicin (Willmott et al 1985a, b). In an attempt to increase potency of that drug delivery system we have incorporated the drug into microspheres prepared using proteins other than albumin; this paper compares albumin with the amphiphilic protein casein (Dalgleish 1982; Fox & Mulvihill 1982) as a microsphere matrix. To our knowledge, only one publication has cited casein as a carrier system (Desoize et al 1986). We have compared the following features of albumin and casein microspheres: morphology by electron microscopy, incorporation of drug using fluorimetric methods, drug release rate in a continuous flow apparatus and electrophoretic mobility by a micro-electrophoresis technique in an attempt to identify factors of importance in the antitumour effect of this delivery system.

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MATERIALS AND METHODS

Preparation of casein microspheres

Casein microspheres containing doxorubicin were prepared by modifying the emulsification method of Willmott et al (1985a, b). Casein (200 mg) as the sodium salt (Sigma Chemical Co., Poole, UK) was wetted with absolute ethanol (400 μL), and dispersed by heating (95 °C for 1 min) in 600 μL of 1 mM phosphate buffer pH 7·5 containing 0·1% sodium dodecyl sulphate. It was then mixed with 0·5 mL of a doxorucibin (Adriamycin, Farmitalia Carlo Erba, Milan, Italy) solution 2% w/v with 50 mg of lactose as excipient. The disperse phase was added to the continuous phase which consisted of 60 mL cotton seed oil (Sigma Chemical Co.) and 40 mL light

petroleum (120–160 °C) (BDH Chemicals Ltd, Poole, UK) and 0·6% Span 80. A w/o emulsion was formed using a Silverson Mixer (Silverson Machines, Chesham, Bucks, UK) at a stirring speed of 3200 rev min^{-1} at room temperature (20 °C). 100 μL of a solution of glutaraldehyde, of a strength giving a final concentration between 1·5–2% w/v in the aqueous phase, was used to crosslink casein and stabilize the microspheres.

In one experiment the drug was incorporated as a complex with polyaspartic acid. Doxorubicin (10 mg) in 500 μL water was precipitated by the addition of polyaspartic acid (30 mg) in 400 μL 1 mM phosphate buffer containing 0·1 mM sodium dodecyl sulphate and the whole incubated at 37 °C for 30 min. The precipitated complex was dried in a vacuum desiccator and then mixed with casein (200 mg). The mixture was wetted with absolute ethanol (150 μL) and dispersed in 1 mL of 1 mM phosphate buffer containing 0·1% sodium dodecyl sulphate. This served as the aqueous phase in the emulsification procedure (see above).

After emulsification (1 h), the resulting microspheres were washed with light petroleum (×3), isopropanol (×2), phosphate buffered saline containing 0·5% Tween 80 (×2) and 60 mM phosphate buffer + 0·1% benzalkonium chloride pH 6·3 (×3). After centrifugation, the microspheres were filtered through wire mesh (aperture size 120 μm) and resuspended in 60 mM phosphate buffer plus 0·1% benzalkonium chloride.

Human serum albumin microspheres used for comparison with casein were prepared as described by Willmott et al (1985a, b). Glutaraldehyde concentration in the aqueous phase was the same as for the casein system (see above).

Microsphere morphology

Glutaraldehyde (5% w/v) was used to fix the microspheres on the same day that they were made. Their surface and internal structure were examined by scanning and transmission electron microscopy (Glauert 1967). They were also examined under the light microscope.

Particle size measurement

The size of the microspheres was measured on a Model D Coulter Counter (Coulter Electronics Ltd, Harpenden, Herts, England). The mean size quoted here is the 50% weight average (rather than number average) because this value, through its dependence on particle volume, more closely reflects drug content.

Drug content

The amount of doxorubicin incorporated into microspheres was assessed by analysing microspheres solubilized by trypsin digestion, using HPLC with fluorescence detection or measurements of total fluorescence for drug quantitation (Cummings et al 1984; Willmott et al 1985a, b).

Drug release rate in-vitro

A continuous flow system similar to that described by Chien (1982) was used to investigate drug release from microspheres in-vitro. Microspheres were immobilized on a column containing a fixed weight of glass wool (3·5 g) as support material and kept at 37 °C. They were subjected to a constant flow (5·5 mL h^{-1}) of 60 mM phosphate buffer (pH 6·3) with 0·1% benzalkonium chloride as preservative. Fractions were collected at hourly intervals, and the amount of drug eluted was determined by HPLC with fluorescence detection.

Surface charge

The electrophoretic mobility of the microspheres in water was measured using a microelectrophoresis apparatus (Rank Bros, Bottisham, Cambridge, UK) with a horizontal flat cell and platinum black electrodes. The velocity of at least 20 particles was measured in both directions.

Electrophoretic mobility (U), in $m^2s^{-1}\ V^{-1}$, was calculated from the equation $U = d \times Le/V \times t$ where Le is the effective length of the cell between two electrodes, V is the applied voltage, and t is the time for particles to cross the distance d. In our experiments Ka is large (Ka > 200), so the Smoluchowski equation, $U = \varepsilon\ G/g\pi\eta$, was used to calculate Zeta potential (G). For water at 25 °C, the permittivity $\varepsilon/4\pi = 6{\cdot}937 \times 10^{-10}\ C^2J^{-1}m^{-1}$ and viscosity $\eta = 8{\cdot}903 \times 10^{-4}\ Nsm^{-2}$. Therefore, for water at 25 °C the Smoluchowski equation becomes $12{\cdot}83 \times 10^5\ U = G$.

Antitumour effect of drug-loaded casein microspheres

Mammary carcinoma Sp 1017 is a non-immunogenic tumour transplantable in Wistar rats. After direct injection of doxorubicin-loaded casein microspheres into subcutaneously growing tumours, growth was measured (two diameters at right angles) twice weekly and a weight in grams obtained from the formula $(\text{lowest diameter in cm})^2 \times \text{highest diameter in cm}$ divided by 2. Controls were given the drug in solution.

RESULTS

Preparation and characteristics of microspheres

In preliminary experiments different types of surfactant (1·5% Tween 85; 1·5% Span 80 and 2·5% Span 80 + 0·4% Pluronic L81) were used to promote the formation of the w/o emulsion. With 1·5% Tween 85, a large amount of drug leaked out during the washing steps and with both the concentrations of Span 80 foaming was a problem and the microspheres tended to aggregate. When the concentration of Span 80 was decreased to 0·6%, foam formation was reduced and microsphere yield, starting from 200 mg casein, was generally between 80–100 mg. The resultant casein microspheres could be easily dispersed in aqueous buffers without surfactant.

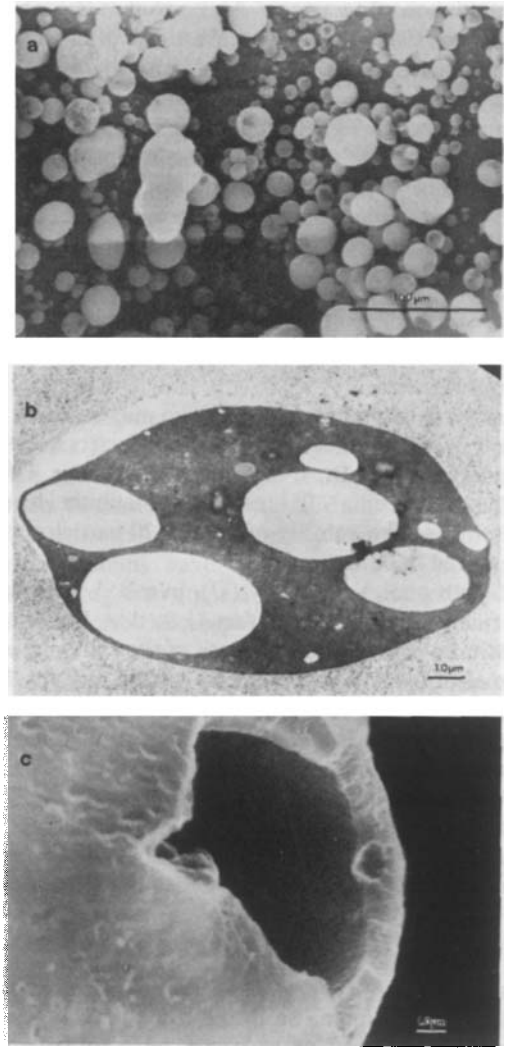

FIG. 1. a, Scanning electron photomicrograph of doxorubicin-loaded casein microspheres prepared with 1·6% glutaraldehyde. Note frequent departures from sphericity of particles. b, Transmission electron photomicrograph of drug-loaded casein microspheres prepared with 1·6% glutaraldehyde. Note the perforate appearance. c, Scanning electron photomicrograph of drug-loaded casein microspheres prepared with 1·6% glutaraldehyde. Note the fractured surface revealing internal discontinuity.

Fig. 1 shows doxorubicin-loaded casein microspheres under the scanning and transmission electron microscopes. Fig. 1a reveals that most of the particles are spherical, but some exhibit marked departures from sphericity, and there is a wide range of particle sizes. It is clear from Fig. 1b and c that there are large lacunae of regular shape in the matrix, thus giving cross-sections of microspheres a perforate appearance. Such discontinuities were never seen in albumin microspheres (Willmott et al 1984). Under the light microscope no pores were visible in drug-loaded microspheres; however, discrete patches of precipitated drug were readily discernible by their vivid red colour.

For use in in-vivo chemoembolization studies, microspheres must be greater than 7 μm (Kanke et al 1980) and ideally with a narrow size distribution—a condition clearly not fulfilled for the casein system (Fig. 1a), even with Span 80 in the continuous phase. As it has been reported (Ishizaka et al 1981) that surfactants of the span series can influence microsphere size and size distribution, we determined whether Span 80 had any effect on these parameters of the casein system. Fig. 2a shows that, in the absence of drug, Span 80 (0·6%) did, indeed, narrow the size range and decrease modal particle size. In contrast, when doxorubicin-loaded microspheres were prepared Span 80 had little effect on these parameters.

Drug content of and release from casein microspheres

Table 1 shows both the drug content expressed in μg mg^{-1} of microspheres and also the effect of incorporating polyaspartic acid into the carrier matrix. Drug content was assessed as the amount of doxorubicin in a sample of microspheres solubilized by trypsin digestion. Three points are apparent from Table 1. The content of doxorubicin in the casein microspheres, as assessed by HPLC, is significantly lower than in the equivalent albumin microspheres; incorporation of polyaspartic acid into the matrix increased the drug content of the casein microspheres; the drug content of microspheres, as assessed by HPLC, was lower than when measure-

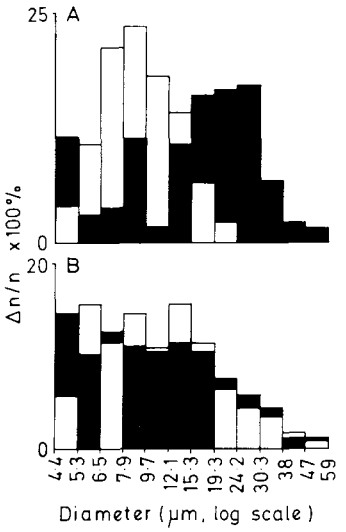

FIG. 2. Influence of surfactant on size distribution of casein microspheres. Following preparation, microsphere size distribution was obtained using a Model D Coulter counter. Δn is number of particles in each size increment and n is total number of particles measured. A, empty casein microspheres; B, drug-loaded casein microspheres; □ with Span 80; ■ without Span 80.

ments of total fluorescence in microsphere digests were used.

In an attempt to compare drug release rates from casein and albumin microspheres, a known weight of drug-loaded microspheres of each type was immobilized on the column, subjected to a continuous flow of aqueous buffer and the rate of elution of drug measured.

A problem with this type of comparative study is that by altering one feature (e.g. substituting casein for albumin as matrix protein) in an attempt to examine the effect of matrix protein on rate of drug release, the resultant microspheres differ in a number of features that influence drug release. For example, Table 1 shows that the drug contents of albumin and casein microspheres were significantly different. Nevertheless, in Fig. 3 we have examined comparable ranges of particle sizes (albumin 23–38 μm, casein 22–36 μm) and column loadings (albumin 20–60 mg, casein 20–88 mg) in an attempt to elucidate the factors determining drug release rate.

Depending on drug content (μg mg^{-1}), drug release rates for casein microspheres could be divided into two groups. The variation inherent in the system is shown by the shaded areas in Fig. 3. By comparing these ranges it was possible to conclude that for casein microspheres the initial drug release rate depended on the drug content of the system.

Table 1. Doxorubicin content of microspheres.

Material used as microsphere matrix[a] (n)	Particle size (μm)[b] range	Drug content (μg mg^{-1} microspheres)[c] [mean ± s.d.] Total fluorescence	HPLC
Casein (6)	22–36	7·1 ± 1·0	3·1 ± 1·0[d]
Casein + poly-aspartic acid (1)	27	13·8	9·1
Albumin (4)	23–38	13·8 ± 2·2	6·9 ± 1·1

[a] Microspheres prepared using 1·5–2% w/v of glutaraldehyde in aqueous phase of emulsion.

[b] Size (50% weight average) measured by Coulter counter.

[c] Following digestion and solubilization of a sample of microspheres with 0·4%–0·8% trypsin, aliquots of digest were extracted with chloroform–isopropanol and drug in organic layer estimated both by fluorescence measurements (μg fluorescence equivalents of drug) and by HPLC (μg of drug).

[d] As assessed by HPLC, drug content of casein microspheres was significantly lower than albumin microspheres ($P < 0·05$ by two-tailed Student's t-test).

Note that theoretical maximum doxorubicin content is 50 μg mg^{-1} (i.e. starting amounts of drug and protein were 10 mg and 200 mg, respectively).

Compared with this factor, column loading and particle size were not as important in determining drug release rate. Drug release from the albumin microspheres was faster than from casein for both the initial and later phases.

Because the drug content of the casein microspheres was lower than that of the albumin microspheres (Table 1), by examining comparable weight ranges of microspheres, as in Fig. 3, the range of amounts of doxorubicin on the column will be less for the casein than for the albumin system. This could explain the lower release rate from casein microspheres seen in Fig. 3. However, if comparable amounts of drug on the column are examined, e.g. by comparing a high weight of casein microspheres (88 mg, 255 μg drug) with a lower weight of albumin microspheres (34 mg, 279 μg drug) then a slower drug release from casein microspheres is manifest. The example of casein microspheres used here was from the slower drug release rate group. Thus, it appears that doxorubicin release from casein microspheres is slower than that from the albumin system.

We have previously reported (Willmott et al 1985a) that, with albumin microspheres, increasing particle size over the range 23–60 μm does not lead to slower doxorubicin release rates. This also appeared to be the case in this study (Fig. 3), the dependence on drug content being the major factor for the casein system.

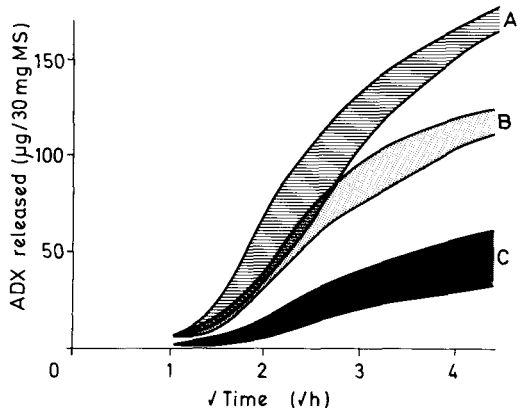

FIG. 3. In-vitro release of doxorubicin from microspheres. Microspheres prepared using 1·5–2% glutaraldehyde in aqueous phase were suspended in 60 mM phosphate buffer pH 6·3 with 0·1% benzalkonium chloride. Samples of 4 mL were applied to a glass wool column and drug release assessed when subjected to a continuous flow of buffer (5·5 mL h^{-1}) at 37 °C. Individual fractions, collected at hourly intervals, were analysed by HPLC with fluorescence detection. Results are expressed as µg drug eluted per 30 mg microspheres on column. Note that drug release from all systems had both an initial and terminal phase. Key: A, albumin (n = 4) 8·2–5·9 µg mg^{-1} (38–23 µm); B, casein (n = 2) 4·5–3·8 µg mg^{-1} (36–27 µm); C, casein (n = 4) 2·9–1·7 µg mg^{-1} (24–22 µm).

Table 2. Zeta potential of protein microspheres.

Sample (size in µm)[a]	pH of ambient fluid[b]	Mobility of microspheres $\times 10^8$ ($m^2 s^{-1} v^{-1}$)[c]	Potential at 25 °C $\times 10^2$ (V)
Empty casein microspheres (16 µm)	6·5	2·1	2·7
Drug-loaded casein microspheres (36, 27 µm)	6·4, 5·5	1·9, 1·3	2·4, 1·7
Drug-loaded casein/polyaspartic acid microspheres (27 µm)	6·6	1·9	2·4
Empty albumin microspheres (15, 27, 20 µm)	7·0, 5·8, 5·8	2·1, 1·4, 0·9	2·8, 1·8, 1·2
Drug-loaded albumin microspheres (25, 14 µm)	6·0 5·9	0·7, 0·6	0·9, 0·8

[a] Microsphere size is expressed as 50% weight average.
[b] Microspheres suspended in water.
[c] The velocity of at least 20 microspheres was measured on a microelectrophoresis apparatus with electrodes at normal and reversed polarity. Electrophoretic mobility and zeta potentials were calculated from standard equations. For all samples, the surface charge was negative.

Microelectrophoresis

The surface charge of microspheres could have an influence on their biological fate. Consequently, we have determined surface charge, electrophoretic mobility and Zeta potential of drug-loaded casein and albumin microspheres; in addition, we compared those values with values obtained for empty microspheres and casein microspheres with a high content of drug by virtue of incorporation of polyaspartic acid into the matrix.

As shown in Table 2, the surface charge of empty casein microspheres was negative at the pH values studied; moreover, at comparable pH, incorporation of positively charged doxorubicin made no substantial difference to this. Drug-loaded albumin microspheres exhibited a lesser negative charge than casein microspheres at a comparable pH. Unlike the casein system, the surface charge of the drug-loaded albumin microspheres was less negative than empty particles at comparable pH.

Antitumour effect of drug-loaded casein microspheres

The potential of doxorubicin-loaded microspheres for loco-regional cancer chemotherapy was assessed by direct injection of the microspheres into subcutaneous growths of the tumour Sp107. Controls received the drug in solution administered in the same way. Fig. 4 shows that the drug in solution (50 and 25 µg) displayed significant antitumour activity although a smaller amount of drug in microspherical form (11 µg) was much more effective in inhibiting tumour growth. Empty microspheres were without effect in this system (data not shown).

DISCUSSION

Since the initial descriptions (Kramer 1974; Lee et al 1981) of the preparation of protein microspheres, albumin has been most frequently used as a drug carrier. However, whether its properties are optimal for the role of carrier matrix is unknown; indeed, the precise requirements for a carrier matrix are still in the process of being defined. As a first principle, high drug content and slow drug release rate are the objectives generally sought, but how these alter drug activity in-vivo is unclear in most systems. Thus, there is an urgent need for comparative data on the characteristics of different types of microsphere to allow correlation with in-vivo performance. The drug-loaded casein microspheres described here are one of a series of protein microspheres we have prepared, characterized, and evaluated in-vivo to elucidate factors affecting the antitumour action of the system.

Assessment of the antitumour effect of doxorubicin (11 µg) incorporated into casein microspheres revealed that the time taken for treated tumours to reach a weight of 12 g was 39·7 days whereas the

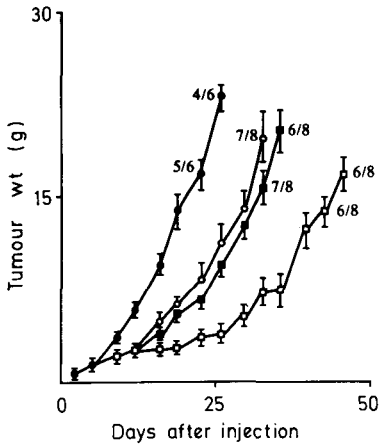

FIG. 4. Antitumour effect of doxorubicin-loaded casein microspheres (CMS). Subcutaneously growing Sp 107 tumours (approx. 1 g) were injected intratumourally in a volume of 0·5 mL with either drug in solution or in microspherical form (36 μm diam. prepared with 1·6% glutaraldehyde). At intervals, two tumour diameters were measured at right angles and a weight in g calculated. Error bars represent mean ± s.e. Animals were killed when the mean tumour diameter >4 cm. Number of curves are animals remaining/animals in group. Key: ●, no treatment; ○, 25 μg doxorubicin solution; ■, 50 μg doxorubicin solution; □ 11 μg doxorubicin CMS.

value for untreated tumours was 19·0 days. This represents a growth delay of 20·7 days (Fig. 4). Interestingly, a much greater amount of drug (85 μg) incorporated into albumin microspheres gave a similar growth delay of 18·6 days (Willmott & Cummings 1987). Clearly, the casein system conferred greater potency per unit amount of drug than did the albumin system.

The surface charge of microspheres could affect the potency of incorporated drug through its influence on the in-vivo fate of particles. For example, after intravenous injection negatively charged polystyrene particles were taken up at high efficiency by the liver, presumably via phagocytosis by Kupffer cells, whereas particles with a positive charge accumulated initially in the lung (Wilkins & Myers 1966). With particles >7 μm injected systemically, the surface charge exerts little influence on anatomical distribution and localization is essentially restricted to the first capillary bed encountered (Kanke et al 1980). However, the surface charge may affect interactions in-vivo between injected particles and surrounding cells involved in the localization and biodegradation of matrix material.

Changes in surface charge were modest and of doubtful in-vivo significance, however, the direction of change was consistent with our other results. From Table 2 it can be seen that surface charge of microspheres, as expected, depended on the pH at which it was examined. At comparable pH (6·5–7), the surface charge of casein and of albumin microspheres was similar ($2·7–2·8 \times 10^{-2}$ V), which suggests that the contribution of the matrix to the surface charge of the system is the same for both proteins. Interestingly, whilst incorporation of positively charged doxorubicin into casein microspheres did not influence surface charge, incorporation of the drug into the albumin system resulted in a less negative zeta potential. This suggests that in the albumin system a proportion of drug is situated near the microsphere surface where it influences surface charge. Being in a superficial location the drug would be expected to be rapidly released, which is consistent with results in Fig. 3. The case for compartmentalization of doxorubicin in microspherical systems has been cogently presented elsewhere (Gupta et al 1986).

In addition to compartmentalization of the drug, its loading characteristics reveal other contrasts between casein and albumin as matrix protein. Thus, the drug content of casein microspheres was approximately half that of albumin microspheres; however, evidence was obtained that the doxorubicin was not all present in its initial condition in the microspheres. Thus, for both systems, a consistent observation was the discrepancy between the drug content of microspheres as measured by total fluorescence and as measured by HPLC (Table 1). This may be an indication that chromatographically pure drug as measured by HPLC is not the only doxorubicin-derived species extant in these microspheres and that a fluorescent species, not detected by HPLC, exists. It is known that the drug can be covalently bound to protein by glutaraldehyde (Hurwitz et al 1975) and such a reaction could well take place during microsphere formation. Support for this scheme is supplied by thin layer chromatography of microspheres solubilized by trypsin digestion, which shows not only a spot identified as doxorubicin but also an, as yet, unidentified species derived from it (manuscript in preparation). The activity of such bound drug might only be apparent on the in-vivo biodegradation of matrix.

Interestingly, the in-vivo assessment showed that the drug-loaded casein microspheres, although containing only 11 μg of drug, exerted an antitumour effect (Fig. 4) that was comparable with that of the albumin system (Willmott & Cummings 1987) containing 85 μg of native drug. This raises the possibil-

ity that the drug present in complexed form within microspheres retains activity and contributes to the antitumour effect in our system.

Doxorubicin release rate from casein microspheres appeared slower than for albumin (Fig. 3). As reported previously (Willmott et al 1985a), particle size does not markedly influence the drug release rate from albumin microspheres. That was also the case in the present study and it also appeared to be true for casein; in the latter system the drug content was more important. The variation of drug release with particle size appears to depend on the system studied. Our results are in agreement with Kawashima et al (1984) who reported no relationship between those parameters for ethyl cellulose microcapsules containing doxorubicin, whereas Ishizaka & Koishi (1983) described an inverse relationship for release of phenacetin from albumin microspheres (i.e. the larger the particles the slower the drug release rate).

The finding that casein microspheres, containing only 11 μg of the drug, exhibited similar potency to the albumin system, containing 85 μg (Willmott & Cummings 1987) but with a more rapid release rate in-vitro (Fig. 3), is consistent with the lower release rate of the drug from the casein system contributing to antitumour effect in our studies.

Although the biological relevance is uncertain, it is worth recording that, as revealed by the electron microscope, the morphology of the casein microsphere is different from that of albumin (Willmott et al 1984), transferrin and haemoglobin microspheres (unpublished). In particular, the frequent occurrence of large, round, regular discontinuities in the microsphere matrix is notable (Fig. 1b). Whether these lacunae render casein microspheres more biodegradable than albumin is being investigated. An explanation for their presence might be entrapment of small air bubbles in the aqueous phase prior to emulsification. Buoyant density measurements on the different systems should be useful here. Certainly the mixture of doxorubicin and casein produced a viscous solution/suspension, which could also be the explanation for the insensitivity of microsphere size to the presence of the surfactant Span 80 in the oil phase (Fig. 2b).

In summary, comparison of the casein and albumin systems suggests a different distribution of incorporated drug in these microsphere types, leading to a different surface charge and drug release rate. In addition, there is evidence for the existence within microspheres of a species derived from, but different to, pure doxorubicin. Whilst the antitumour effect of both systems was similar, there was less drug in the casein microspheres. The rate of its release (i.e. rate of its delivery to the tumour) from casein microspheres was slower than from the albumin system. Taken together, these data are consistent with the proposition that the slow rate at which the drug is made available to the biological system, by diffusion and biodegradation, is sufficient to compensate for the lower dose of native drug administered from casein microspheres.

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REFERENCES

Artursson, P., Edman, P., Laakso, T., Sjoholm, I. (1984) J. Pharm. Sci. 73: 1507–1513

Chien, Y. W. (1982) in: Chien, Y. W. (ed.) Novel Drug Delivery Systems. Marcel Dekker, New York, pp 465–573

Cummings, J., Stuart, J. F. B., Calman, K. C. (1984) J. Chromatog. 311: 125–133

Dalgleish, S. G. (1982) in: Fox, P. F., Corelon, J. J. (eds) Food Proteins. Applied Science Publishers, London and New York, pp 155–178

DeLuca, P. P., Kanke, M., Sato, J. (1984) in: Davis, S. S., Illum, L., McVie, J. G., Tomlinson, E. (eds) Microspheres and Drug Therapy. Elsevier, Amsterdam, pp 343–344

Desoize, B., Jardillier, J. C., Kanoun, K., Guerin, D., Levy, M. C. (1986) J. Pharm. Pharmacol. 38: 8–13

Fox, P. F., Mulvihill, J. (1982) J. Dairy Research 49: 679–693

Glauert, A. M. (1967) in: Kay, D. (ed.) Techniques in Electron Microscopy. Blackwell Scientific Publications, London, p. 160

Gupta, P. K., Hung, C. T., Perrier, G. (1986) Int. J. Pharm. 33: 137–146

Hurwitz, E., Levy, R., Maron, R., Wilcheck, M., Arnon, R., Sela, M. (1975) Cancer Res. 35: 1175–1181

Ishizaka, T., Koishi, M. (1983) J. Pharm. Sci. 72: 1057–1061

Ishizaka, T., Endo, K., Koishi, M. (1981) Ibid. 70: 358–363

Juni, V., Ogate, J., Nakano, M., Ichihera, T., Mori, K., Akagi, M. (1985) Chem. Pharm. Bull. 33: 313–318

Kanke, M., Simmons, G. H., Weiss, D. L., Bivans, B. A., de Luca, P. P. (1980) J. Pharm. Sci. 69: 755–762

Kawashima, Y., Lin, S. Y., Kasai, A., Takenaka, H., Matsumani, K., Nochida, Y., Hirose, H. (1984) Drug Development and Industrial Pharmacy 10: 467–479

Kramer, P. A. (1974) J. Pharm. Sci. 63: 1646–1647

Lee, T. K., Sokoloski, T. D., Royer, G. P. (1981) Science 213: 233–235

Longo, W. E., Iwata, H., Lindheimer, T. A., Goldberg, E. P. (1982) J. Pharm. Sci. 71: 1323–1328

Morimoto, Y., Akimoto, M., Sugibayashi, K., Nadai, T., Kato, Y. (1980) Chem. Pharm. Bull. 28: 3087–3092

Tokes, Z. A., Ross, K. L., Rogers, R. E. (1984) in Davis, S. S., Illum, L., McVie, J. G., Tomlinson, E. (eds) Microspheres and Drug Therapy. Elsevier, Amsterdam, pp 139–150

Tomlinson, E. (1983) Int. J. Pharm. Tech. Prod. Mfr. 4: 49–57

Tomlinson, E., Burger J. J., Schoonderwoerd, E. M. A., McVie, J. G. (1984) in: Davis, S. S., Illum, L., McVie, J. G., Tomlinson, E. (eds) Microspheres and Drug Therapy. Elsevier, Amsterdam, pp 75–90

Widder, K. J., Senyei, A. E., Ranney, D. F. (1979) in: Advances in Pharmacology and Chemotherapy. Academic Press, New York, pp 213–271

Wilkins, D. J., Myers, P. A. (1966) Br. J. Exp. Pathol. 47: 565–576

Willmott, N., Cummings, J. (1987) Biochem. Pharmacol. 36: 521–526

Willmott, N., Kamel, H. M. H., Cummings, J., Stuart, J. F. B., Florence, A. T. (1984) in: Davis, S. S., Illum, L., McVie, J. G., Tomlinson, E. (eds) Microspheres and Drug Therapy. Elsevier, Amsterdam, pp 205–216

Willmott, N., Cummings, J., Florence, A. T. (1985a) J. Microencapsulation 2: 293–304

Willmott, N., Cummings, J., Stuart, J. F. B., Florence, A. T. (1985b) Biopharm. Drug Dispos. 6: 91–104