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Evaluation of Ion-exchange Microspheres as Carriers for the Anticancer Drug Doxorubicin: In-vitro Studies

YAN CHEN, MARK A. BURTON, JAMES P. CODDE, SYLVIA NAPOLI, JAN J. MARTINS* AND BRUCE N. GRAY

*Department of Surgery, University of Western Australia, Royal Perth Hospital, Perth 6001, Australia and *Department of Physiology, University of Western Australia, Nedlands 6009, Australia*

Abstract—A comparison study of doxorubicin loading, release characteristics and stability within sodium and hydrogen forms of ion-exchange resin microspheres has been performed. It was demonstrated that resins in the Na^+ form, although having lower drug loading capacity, showed similar release profiles to resins in the H^+ form but still maintain all the drug activity. Resins in the H^+ form, despite having high drug loading capacity, caused drug degradation within microspheres due to their strong acidic nature. Therefore, in comparison with the H^+ form, resins in the Na^+ form can be considered as better carriers for doxorubicin in terms of sustaining the release of drug and maintaining drug activity. Other factors such as the degree of resin cross-linkage and drug/resin mixing time have also been examined in relation to drug loading and release characteristics. Overall, this study demonstrated the significance of the characteristics of matrix materials and their influence on the drug activity and microsphere performance in-vitro.

Chemotherapy with doxorubicin is limited by its chronic cardiotoxicity, immunosuppressive activity and necrotic reaction at the injection site. Althouth regional therapy has been used to reduce these systemic toxicities, the high local toxicity is still problematic (Goldberg et al 1984). Utilization of a drug delivery system, such as microspheres, to transport the drug has been pursued and is believed to have great potential for improving the current doxorubicin chemotherapy (Tomlinson et al 1984; McArdle et al 1988; Burton et al 1990; Willmott et al 1990).

This technology is based on the chemoembolization of the drug-loaded microspheres via the tumour arterial supply. Because of their physical size, microspheres can be entrapped in capillary beds along with their load of cytotoxic drug after intra-arterial administration. As a result of this chemoembolization, the microspheres containing the cytotoxic drug can be delivered to the well vascularized tumour tissue. The drug is then released in a sustained manner into the tumour locale without being dispersed into the systemic circulation. This approach has demonstrated comparable and enhanced therapeutical response with suppressed systemic toxicity (Codde et al 1990; Burton et al 1990) of anticancer drugs in animal models.

The theory of using microspheres for targeted drug delivery is very attractive, but in practise a number of factors can influence the usefulness of microspheres as anticancer drug carriers. Drug loading is one of them. From a therapeutical and biological point of view, a drug delivery system with high drug loading capacity has many advantages. Firstly, this will allow an effective dose to be used to produce high levels of local drug concentration with consequent greater therapeutic efficacy. Secondly, administration of microspheres with high drug loading results in less of the matrix material being coadministered to the body, and biological reaction to the matrix material can be minimized.

A review of the literature reveals that the microspheres prepared using ion-exchange principles exhibit high doxorubicin loading capacity (> 30% w/w) (Goldberg et al 1984; Sawaya et al 1987; Jones et al 1989a; Cremers et al 1990), whereas those using chemical cross-linkage and physical entrapment approaches, display drug loading at levels less than 15% (Tokes et al 1982; Wingard et al 1985; Willmott et al 1985; Gupta et al 1986b; Chen et al 1987, 1988; Jones et al 1989a). This marked difference indicates that drug loading is affected by the techniques of drug incorporation. High levels of doxorubicin loading on microspheres can be achieved using ionic binding mechanisms.

High drug loading capacity in itself, however, cannot guarantee a drug delivery system to be an effective means of improving drug therapeutic value. It is also very important that a drug delivery system should maintain drug activity and provide sustained drug release profiles so that the exposure of drug to tumour cells can be maximized.

Although ionic binding principles have been widely used in the design of sustained release microspheres, further studies are required to reveal the underlying mechanisms which affect characteristics and performance of microspheres prepared using ionic binding principles. For this purpose, we conducted this research on the in-vitro characterization of sodium and hydrogen forms of resin microspheres with respect to drug loading, drug release characteristics and the state of the drug within microspheres. To investigate the mechanisms controlling these effects, other factors such as the degree of resin cross-linkage, pH, mixing time and type of resin have also been examined in relation to drug loading, release properties and drug stability within microspheres.

Materials and Methods

Doxorubicin was kindly provided by Farmitalia, Sydney. Ion-exchange resins Aminex A-6 with size of $17{\cdot}5 \pm 2{\cdot}0$ μm, 8% cross-linked, (resin A) and Aminex 50WX4 with size of $32{\cdot}5 \pm 2{\cdot}5$ μm, 4% cross-linked, (resin B) were purchased in Na^+ form from Bio-Rad. After being freeze-dried, they were used in the Na^+ form. Resin in the H^+ form was produced by slurrying 1 g resin with 3 M HCl for 1 h, followed by extensive washing with distilled water and subsequent freeze-drying.

Correspondence: M. A. Burton, Department of Surgery, University of Western Australia, Royal Perth Hospital, Perth 6001, Australia.

Resins were chosen on the basis of their size and potential physiological impact on embolization of the vasculature when used clinically. Both resin sizes chosen have been described in clinical studies using microspheres (Gray et al 1989).

Doxorubicin-loaded resin microspheres were prepared using the method previously described by Jones et al (1989b). Briefly, pre-dried cation ion-exchange resins were slurried with the concentrated drug solution in 1 : 1 ratio by weight at 4°C for 24 h. The loaded microspheres were then separated from the drug solution by centrifugation (2000 rev min^{-1}, 5 min) and washed twice with deionized water before being resuspended in a known volume of deionized water.

The amount of the drug loaded into the microspheres was determined by measuring the amount of doxorubicin remaining in the supernatant using UV-visible spectrophotometry and HPLC.

In-vitro release of doxorubicin from microspheres was assessed using a continuous flow-through system. A known amount of loaded microspheres (containing approximately 1 mg of drug) was immobilized in a glass column. This system was then subject to a constant flow (5·6 mL h^{-1}) of phosphate buffered saline containing 50 mM EDTA and 0·1% benzalkonium chloride as a preservative. The eluant was collected hourly and the amount of drug released was assayed by UV-visible spectrophotometry and HPLC.

To detect doxorubicin degradation products in the microspheres, the supernatant collected during the preparation of drug-loaded microspheres and the eluant from the release study were injected directly onto a reversed phase C18 column. The standards of doxorubicin and its metabolites, doxorubicinol and doxorubicinone, were used in the HPLC analysis to identify the degradation products. The method for the HPLC analysis is described in detail elsewhere (Cummings et al 1984).

Results and Discussion

Drug loading

Effects of resin ionic form and cross-linkage. It was found that resin in the H^+ form showed a higher drug loading capacity with a maximum of 57·2 and 99·8% for resin A and resin B, respectively, whilst resin in the Na^+ form contained a maximum of 29% (resin A) and 86·2% (resin B) (Table 1). It is believed that this apparent difference in drug loading of resin in different ionic forms is mainly due to two factors. First, the effect of pH on the ionization of the drug. Resin in H^+ and Na^+ forms possess different pH values, the former having pH < 2 but the latter with neutral pH. The drug, with pK_a (NH_2) of 8·2, is expected to be highly ionized at a low pH. Therefore, resin in the H^+ form allows a high degree of ion-exchange, resulting in high drug loading. Second, different counterions have different degrees of selectivity for resin. The H^+ counterion has a weak selectivity coefficient and is readily displaced by other cationic ions.

Table 1. Effect of ionic form of resins on drug loading.

Microspheres	Ionic form	Drug loading (% w/w)
Resin A	H^+	46·0–57·2
	Na^+	20·8–29·0
Resin B	H^+	98·0–99·8
	Na^+	67·3–86·2

The high drug loading associated with resin B is interpreted to be the result of a low degree of cross-linkage in the resin (resin A is cross-linked at 8% whereas resin B is 4% cross-linked). The less cross-linked network offers higher porosity of structure, therefore, more drug molecules can easily gain access to the ionic binding sites in the resin. A similar finding has been previously reported (Schacht 1983).

Effect of pH. Based on our observation that the resin in the H^+ form exhibited higher drug loading than that in the Na^+ form, we designed experiments to assess the effect of pH on the drug loading of resin A in the Na^+ form. The resin was partially converted to H^+ form by mixing vigorously with different concentrations of HCl solution for 2 h. After removal of the HCl solution, the resin was then loaded with doxorubicin. Table 2 shows the results of the experiments. It is evident that the change of pH (in the range of 3·8–7·4) did not affect drug loading of resin A. This indicates that the partial conversion of resin from the Na^+ form to the H^+ form does not significantly increase ion-exchange efficiency of resin A. A similar finding was observed with resin B.

Effect of mixing time. The effect of mixing time was examined by measuring drug loading of resins which had been mixed with the concentrated drug solution for different lengths of time. The results in Fig. 1 demonstrate that mixing time can affect drug loading. For resin A, a pronounced effect is observed in the initial 16 h of mixing. Afterwards, drug loading plateaus at a stable level of approximately 24%. For resin B, drug loading displays two stages of increase, in the initial 9 h and between 20 and 30 h of mixing. It is believed that the second increase in drug loading could be caused by mechanisms other than ionic bonding, e.g. by surface absorption phenomena (Borodkin & Yunker 1970; Gupta et al 1986a). In our further studies, resins were mixed with the drug for 24 h before use.

Release characterization

Effect of resin ionic form and cross-linkage. Results obtained in the release studies reveal that doxorubicin-loaded resin microspheres display a slow release profile in comparison with that of drug in solution. When comparing drug release from the same type (i.e. the same degree of cross-linkage) but different forms of resin (e.g. resin B in the H^+ and Na^+ form) the proportion of the total drug released, when measured by

Table 2. Effect of pH on drug loading of resin A microspheres.

HCl concentration	pH of the resulting microsphere suspension	Drug loading (% w/w)
0·01 M	2·6 (3·8*)	32·7
0·001 M	4·2	26·0–30·1
0·1 mM	6·6	25·2
0·0	7·4	20·8–29·0

* This is the pH value of the suspension after the resin A has been extensively washed with deionized water.

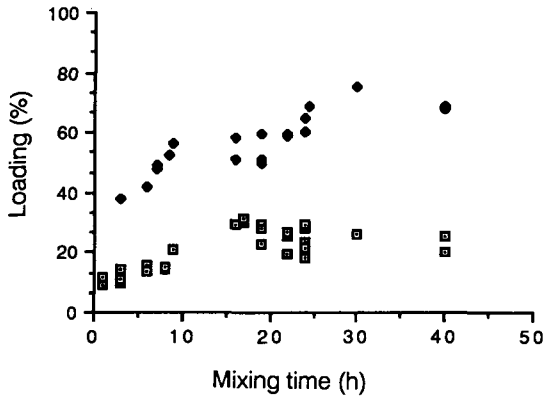

FIG. 1. Effect of mixing time on drug loading of resin microspheres in the Na^+ form, resin A (□) and resin B (◆).

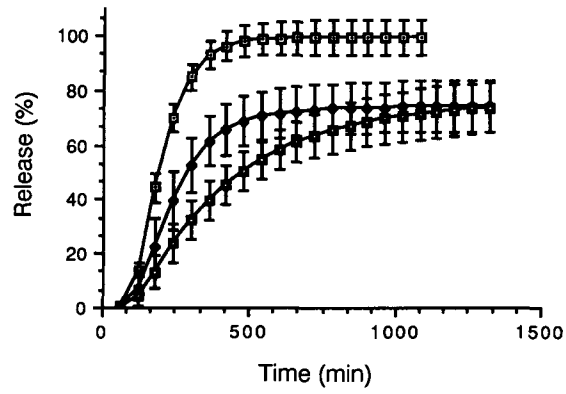

FIG. 3. Release profiles of doxorubicin in solution (□), n = 4, and resin microspheres in the Na^+ form, resin A (■), n = 4, and resin B (◆), n = 5.

UV-visible spectrophotometry, is similar irrespective of the difference in the ionic form of the resins used (Fig. 2). However, when the amount of doxorubicin released was assayed using the HPLC method, a different result was obtained. For resin in the H^+ form, the amount of drug released as measured by the HPLC method was half of that measured by UV-visible spectrophotometry, for the resin in the Na^+ form, both methods produced the same result.

Not surprisingly there is a difference in the drug release characteristics of the microspheres prepared from resins in Na^+ form with different degrees of cross-linkage (resins A and B) (Fig. 3). The former exhibited retarded release with 50% of the total drug released (T50) in 494 ± 90 min, n = 4, whereas, the latter released the drug more rapidly with T50 249 ± 33 min, n = 5 (Table 3). Both resin A and B released approximately 74% of the loaded drug in 22 h. The difference observed in the drug release rates between resin A and resin B results from the difference in their drug loading and the degree of resin cross-linkage. This is consistent with the results reported previously that drug is released faster when its loading in microspheres is high or when microspheres have a low degree of cross-linkage (Willmott et al 1985; Jones et al 1989a).

It is expected that the rate of drug release from ion-exchange resins is determined by the availability of competing ions and the diffusion rates of ions and drug into and out of the resins (Schacht 1983); controlling drug release rate include the size and concentration of competing ions, selectivity of the ions involved, and porosity of the resins. Our results suggest that the resin ionic form does not affect the total release of drug while the degree of resin cross-linkage does affect the release rate of drug.

Effect of drug loading. The influence of drug loading on the release rate of doxorubicin is shown in Table 4. With higher loading, the release is faster. This is believed to be due to high drug loading of microspheres resulting in a burst release of drug weakly attached to the resin through mechanisms other than ionic binding.

Although the above results indicate that high loading leads to an increased rate of release of drug, this situation can be

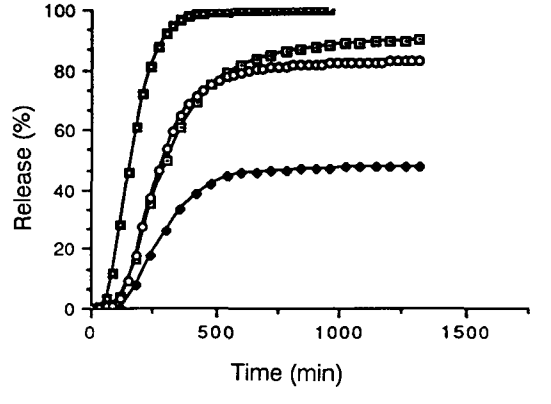

FIG. 2. Cumulative release of the drug from microspheres and drug solution. Elution of doxorubicin from the drug solution measured by UV and HPLC (□); doxorubicin released from resin B in the Na^+ form measured by UV and HPLC (○); total drug released from resin B in the H^+ form (including doxorubicin and degradation products) measured by UV (⊡); doxorubicin released from resin B in the H^+ form measured by HPLC (◆).

Table 3. Comparison of characteristics of resin A and resin B microspheres.

Microspheres (Na^+ form)	Loading (% w/w)	T50 (min)
Resin A	25·1 ± 3·2 (n = 4)	494 ± 90 (n = 4)
Resin B	75·0 ± 4·9 (n = 5)	249 ± 33 (n = 5)

Statistical analysis using a two-sample *t*-test indicates that resin A and resin B are significantly different in both drug loading and drug release ($P < 0.05$).

Table 4. Effect of drug loading on doxorubicin release.

Drug loading (% w/w)	T50 (min)
38·0	380
59·8	341
68·4	355
72·5	271
86·2	251

Microspheres described here were prepared from resin B in the Na^+ form. The different loading was achieved by mixing drug with resin for different periods of time. Spearman rank correlation coefficient $r_s = -0.9$, the correlation is significant at $P = 0.05$.

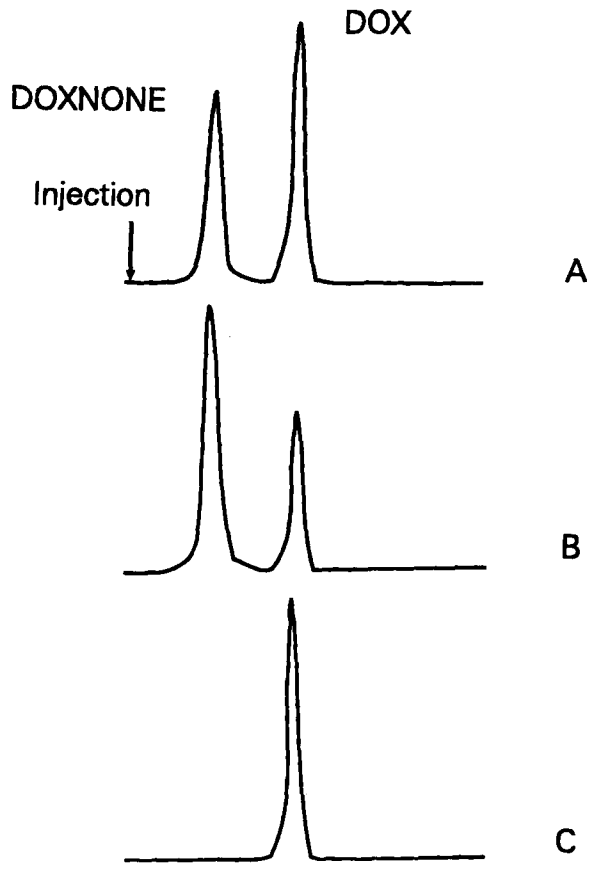

FIG. 4. HPLC of released doxorubicin from resin microspheres in the H^+ form and Na^+ form. A: doxorubicinone (DOXNONE) and doxorubicin (DOX) standards; B: doxorubicin released from resin microspheres in the H^+ form; C: doxorubicin released from resin microspheres in the Na^+ form.

readily modified by using techniques such as coating or microencapsulation of drug-loaded resins. Irwin et al (1988) demonstrated that coating treatment reduced the release rate of the drug. Currently our group is utilizing a similar approach to further slow down drug release from resin microspheres.

Doxorubicin stability within microspheres

To obtain a therapeutically effective drug delivery system it is important to produce a system with optimum drug loading and release rate while maintaining drug activity within the system. HPLC was employed to identify any chemical changes occurring to the drug in resin microspheres. Samples collected from the supernatant, washes and eluant of the release studies were analysed.

The analyses showed that doxorubicinone, a non-biologically active degradation product of doxorubicin, was present in resin of the H^+ form but not in the Na^+ form (Fig. 4). This was observed in all the samples examined. It is interesting to note that the percentage of doxorubicinone in the supernatant is very low for resin A in the H^+ form ($< 5\%$) but high for resin B in the same ionic form ($> 80\%$). The explanation for this difference is seen in the loading capacity of the resins. Resin A has relatively low drug loading, therefore, a substantial amount of native doxorubicin remains in the supernatant. Resin B in the H^+ form displayed an extremely high drug loading with almost no native doxorubicin left in the supernatant and, as a result, the percentage of degradation product was relatively high. A further point of interest which emerged from the HPLC analysis was that the level of doxorubicin diminished with each wash. There was no detectable doxorubicin in the second wash but doxorubicinone was detected in all the washes, although the level was not high ($2\ \mu g\ mL^{-1}$). This suggests that doxorubicinone was produced within the H^+ form microspheres.

To further investigate the state and level of doxorubicin in different ionic forms of microspheres, we conducted an HPLC analysis of the eluant. The results reveal that nearly half the amount of the drug released from resin A and more than a third from resin B microspheres in the H^+ form was doxorubicinone (Table 5). However, all the drug released from resin in the Na^+ form was pure doxorubicin. This also explains the discrepancy observed between UV and HPLC results (Fig. 2).

To examine the cause of the degradation of doxorubicin within ion-exchange microspheres the effect of pH was assessed. The low pH (about 2·0) in all resins in the H^+ form correlate with a high level of doxorubicinone in the microspheres, whereas the neutral Na^+ form showed no detectable doxorubicinone in the microspheres. To confirm the effect of low pH other Na^+ resins were partially converted to the H^+ form before they were used to absorb doxorubicin. The pH of resultant microspheres was 3·8 and only 5% doxorubicinone was detected in the release fractions. These findings are consistent with the known acid hydrolysis of doxorubicin (Beijnen et al 1985).

Monitoring the level of doxorubicin after 12 days of storage at $-20°C$ indicated that the ratio of doxorubicinone to doxorubicin had increased from 0·6 to 0·9. This suggests that the doxorubicin degradation within the resin in the H^+ form is an on-going process. In addition, it should be pointed out that HPLC results can not be used to predict the pure

Table 5. Effect of ionic form of resins on the characteristics of doxorubicin-carrying microspheres.

Microspheres	Ionic form	pH	% of total doxorubicin released in 22 h measured by UV	% of doxorubicin detected in the release measured by HPLC
Resin A	H^+	2·1	66	48
Resin B	H^+	2·2	89	36
Resin A	Na^+	7·4	60–86	none
Resin B	Na^+	7·8	67–96	none
Resin A	Na^+/H^+	3·8	60	5

doxorubicin loading in the H^+ form microspheres, as it is possible that the proportion of degraded drug detected in the eluant may not be the same as that within the microspheres.

In conclusion, this study shows that the resins in the Na^+ form, although having lower drug loading, displayed similar sustained release profiles as resins in the H^+ form but still maintain all the drug activity. Resins in the H^+ form, despite having high drug loading, caused drug degradation due to its strong acidic nature. It is anticipated that resins in the Na^+ form may offer a better biological response since all the drug remains in the active form. This work has clearly demonstrated the significance of the matrix material characteristics and their influence on the performance of microspheres. Achieving high drug loading and maintaining the activity of therapeutic agents are equally important considerations in the development of a drug delivery system.

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