*International Journal of Pharmaceutics*, 59 (1990) 57–67
Elsevier



IJP 01992

# Albumin microspheres. V. Evaluation of parameters controlling the efficacy of magnetic microspheres in the targeted delivery of adriamycin in rats

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(Received 12 January 1989)
(Modified version received 27 July 1989)
(Accepted 19 September 1989)

*Key words*: Magnetic albumin microsphere; Adriamycin content; Magnetic field characteristics; Drug targeting

## Summary

Two studies have been carried out to investigate the effect of drug content of magnetic albumin microspheres, and the feasibility of using a low-strength magnetic field (i.e. 1000 G) in the targeted delivery of adriamycin in rats. As a part of the first study, two types of magnetic microspheres, with ~ 1 and 3% w/w of adriamycin HCl, were administered via ventral caudal artery to two groups of rats (0.4 mg/kg), with the center of their tail (the target site) exposed to an 8000 G magnet for 30 min post-dose-administration. In both groups, the animals were killed over a 48 h period after dosing and the relevant tissues analyzed for adriamycin concentration using HPLC. The increase in drug content of the microspheres did not influence their efficacy in the selective delivery of drug. In the second study, two groups of rats received 0.4 mg/kg adriamycin via magnetic microspheres with the tail target site exposed to a 1000 G magnet for 30 and 60 min respectively, a third group receiving an equivalent dose of microsphere-delivered drug in the absence of the magnet (control). The use of the 1000 G magnet for 30 and 60 min increased the maximum drug concentration at the target site by ~ 3 and 4-fold respectively. In addition, the magnet increased the targeting efficiency of the magnetic microspheres, vs. non-target tissues, by a factor of at least 2. The implications of altered drug content of magnetic microspheres, and variations in the magnetic field characteristics, on the drug targeting potentials of magnetic delivery systems are discussed.

## Introduction

Albumin microspheres have been investigated for passive as well as active targeting of drugs (Widder et al., 1978; Sugibayashi et al., 1979; Morimoto et al., 1980a; Willmott et al., 1984; Fujimoto et al., 1985; Akasaka et al., 1988). Whereas the passive delivery of drug(s) to specific organs via these particles has been based on the size of the microspheres, efforts aimed at active drug delivery have mainly concentrated on incorporating magnetite ($Fe_3O_4$) into the microsphere matrix, so that their in vivo distribution may be controlled with the aid of an external magnet (Widder et al., 1978; Morimoto et al., 1981; Bartlett et al., 1984; Morris et al., 1984; Gupta et al., 1986a, 1989a; Gupta and Hung, 1989a).

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The success of a targeted drug delivery system depends upon several factors, e.g. effective localization of drug carrier at the diseased site, drug content and drug release characteristics of the carrier, and the bioacceptability of the carrier (Poznansky and Juliano, 1984; Thies and Bissery, 1984; Gupta and Hung, 1989b). Although albumin microspheres meet most of these 'ideal' requirements, some workers have stressed the need for improving the drug content of these particles (Oppenheim et al., 1984; Willmott et al., 1984, 1985). Theoretically, the increase in drug content of the microspheres should not only ensure its delivery in high concentrations at the target cells, but also alleviate toxic effects, if any, by reducing the total amount of carrier administered per dose.

Previous studies investigating the efficacy of magnetic albumin microspheres have considered the application of magnets with field strengths $\geq 3000$ G (Widder et al., 1978; Morimoto et al., 1980b, 1981). It is realized that the feasibility of using magnetic drug delivery systems may increase considerably if their distribution in the body could be controlled via a low-strength magnetic field. However, to date, the lower limit of magnetic field strength for the effective retention of a magnetic delivery device has not been defined.

This work was undertaken to investigate the effect of drug content of magnetic albumin microspheres, and the effect of a 1000 G magnetic field, on the targeting efficiency of microspheres in rats. In the first study, the target vs. non-target tissue drug distribution characteristics of two types of magnetic albumin microspheres, with ~ 1 and 3% w/w of adriamycin hydrochloride respectively, and administered in the presence of an 8000 G magnet, have been evaluated in two groups of healthy rats. In the second study, the effect of application of a 1000 G magnetic field on the targeting efficiency of magnetic microspheres has been determined and the results compared with data obtained in the absence of a magnetic field.

## Materials and Methods

*Apparatus and materials*

All apparatus, chemicals and reagents for the formulation and evaluation of adriamycin-loaded magnetic albumin microspheres, and in vivo experiments, were obtained as mentioned previously (Gallo et al., 1984, 1986, 1989; Gupta et al., 1986b, 1988). Adriamycin hydrochloride was kindly donated by Farmitalia Carlo Erba, Milan (Italy). Water was double-distilled and MilliQ ® filtered, and all glassware was silanised using Aquasil ® from Pierce Chemical (Rockford, IL).

*Synthesis of adriamycin-loaded magnetic albumin microspheres*

*Type A microspheres with ~ 1% w/w of adriamycin* These were essentially prepared using a method reported earlier (Gupta et al., 1988). To 250 $\mu$l of 40% w/v aqueous solution of bovine serum albumin, 200 $\mu$l of 5% w/v aqueous solution of adriamycin hydrochloride and 100 mg of magnetite slurry (30% w/v $Fe_3O_4$ in water) were added. The mixture was ultrasonicated in 30 ml of cottonseed oil (4°C) at 125 W for 2 min to produce a magnetic drug emulsion, which was subsequently heat-stabilized by adding ($100 \pm 10$ drops/min) to 100 ml of cottonseed oil maintained at 120°C and a stirring rate of 1500 rpm. After 10 min of stabilization, the system was ice-cooled to 20°C. Thereafter, the microspheres were recovered by adding 60 ml of anhydrous ether and washing off the oil by centrifugation at $3000 \times g$ for 15 min. After a total of four washings with ether, the microspheres were stored at −15°C until used for characterization and/or dosing the animals.

*Type B microspheres with ~ 3% w/w of adriamycin* The method used for the synthesis of type A microspheres was slightly modified to obtain microspheres with higher drug content. Essentially the whole procedure was identical except that a 30% w/v aqueous solution of albumin was used, the magnetic drug emulsion was added to the pre-heated oil (120°C) at a faster rate (i.e. $250 \pm 25$ drops/min) and a stabilization time of 2.5 min was considered.

*Characterization of microspheres*

The size and surface characteristics of both types of microspheres were monitored using a scanning electron microscope (SEM). Their adriamycin content was determined by digesting a

known amount of microspheres in 0.5 M acetic acid and analysing the supernatant using a reversed-phase ion-pair HPLC (Gallo et al., 1986; Gupta et al., 1987). The iron content of the microspheres was determined using atomic absorption spectroscopy, after their digestion in concentrated hydrochloric acid (Gupta et al., 1988).

*In vivo experiments*

*Study 1: effect of drug content of magnetic albumin microspheres on their targeting efficiency in rats* Two groups of adult female Wistar rats, each group comprising 21 animals (weight 225–250 g), were anesthetized with halothane and their tail demarcated into three parts: Tail-1, the dose-administration site, measuring 3 cm from the base; Tail-2, the target-site, measuring 4 cm from Tail-1; and Tail-3, the remaining tail length. The ventral caudal artery at Tail-1 was cannulated, and Tail-2 placed between two poles of an electromagnet (field strength 8000 G; field gradient 400 G/cm) (Gupta et al., 1986a, 1989a; Gallo et al., 1989).

The animals in one group received 0.4 mg/kg adriamycin hydrochloride via type A microspheres suspended in saline, with magnetic field directed on Tail-2 for 30 min. The animals in the other group received an equivalent amount of adriamycin hydrochloride via type B microspheres, under similar conditions of magnetic field. However before their administration, both type A and type B microspheres were washed four times with normal saline to remove the surface-associated drug (Gupta et al., 1986c, 1987). Both groups of animals were stored individually in metabolic cages and fed ad libitum, until being killed in triplicates, by exsanguination, over 48 h after dose administration. From each animal Tail-1, Tail-2, Tail-3 and liver were removed, washed with cold saline, blotted and homogenized in normal saline and/or acetic acid so as to determine their adriamycin level using HPLC (Gallo et al., 1986).

*Study 2: effect of 1000 G magnet on the targeting efficiency of magnetic albumin microspheres in rats* Three groups of female Wistar rats, each with 21 animals, were cannulated for the delivery of magnetic albumin microspheres as discussed above. In two groups, the animals received 0.4 mg/kg adriamycin hydrochoride, via type B microspheres, with the target site (Tail-2) exposed to a 1000 G electromagnet for 30 and 60 min, respectively. The third group of animals received an equivalent dose of adriamycin, via type B microspheres, in the absence of a magnetic field (control). Before dosing, the microspheres were washed with saline in a manner similar to that discussed above. After dosing, the animals were killed over a 48 h period to isolate Tail-1, Tail-2, Tail-3 and liver and determine their adriamycin level chromatographically (Gallo et al., 1986).

*Analysis of data*

The mean ($n = 3$) observed adriamycin concentration-time data for each tissue, and for each group of rats in both studies, were transformed to estimate the total area under the corresponding curves ($AUC_0^\infty$). The area from time zero to any time $t$ in the post-distribution phase ($AUC_0^t$) was estimated using the trapezoidal rule. The area from time $t$ to infinity ($AUC_t^\infty$) was calculated as (Gibaldi and Perrier, 1982):

$$AUC_t^\infty = C_t/k \tag{1}$$

where $C_t$ is the concentration of adriamycin at time $t$ (in $\mu$g/ml) and $k$ is the disposition rate constant (in $h^{-1}$). The value of $k$ for Eqn. 1 was calculated as a slope of the regression line fitted to a log concentration-time plot in the post-distribution phase (Gibaldi and Perrier, 1982). The two areas were then added to obtain $AUC_0^\infty$.

The targeting efficiency of the two types of microspheres, for the target-tissue Tail-2, was determined as $t_e$ (Gallo et al., 1989):

$$t_e = \left[ \frac{(AUC_0^\infty)_{\text{Tail-2}}}{(AUC_0^\infty)_{\text{Non-target tissue}}} \right]_i \tag{2}$$

where the subscript i refers to either type A or B microspheres. Here the value of $t_e$ equal to unity denotes comparable targeting efficiency of the microspheres towards the target as well as the non-target tissue(s). However values of $t_e$ more than unity refer to higher targeting efficiency for the target tissue, and vice versa.

## Results and Discussion

The physico-chemical characteristics of the two types of adriamycin-loaded magnetic albumin microspheres are summarized in Table 1. Essentially both types of microspheres were spherical in shape, with mean diameter $< 1$ $\mu$m. Whereas the magnetite content of these microspheres was comparable, their total adriamycin content differed by almost 100%, with $8.6 \pm 2.3\%$ w/w being the maximum drug loading observed under the optimum formulation conditions. The difference in drug content of the two types of microspheres can be attributed to changes in the drug/protein concentration ratio, as well as the stabilization time during the formulation of the two types of microspheres (Gupta et al., 1989b). Since fresh microspheres are known to be associated with a large fraction of surface-adsorbed drug, the microspheres were washed with normal saline before administration to rats. At the time of dosing type A and B microspheres contained $\sim 1$ and 3% w/w of adriamycin hydrochloride, respectively. Analysis of the microsphere dosing suspension revealed that despite washing $10.1 \pm 3.1$ and $18.1 \pm 4.2\%$ drug was released from type A and B microspheres, respectively, in the suspending medium. Hence the observed tissue drug concentrations at various time points were a result of the distribution of the free fraction of drug, as well as the microsphere-entrapped drug.

Previous studies in our laboratory have revealed that following the intra-arterial administration of 2.0 mg/kg adriamycin hydrochloride as a solution and via magnetic albumin microspheres, in the presence of an 8000 G magnetic field applied at Tail-2 for 30 min, the microspheres increased drug delivery to Tail-2 by 60%. In addition, a 100% increase in drug delivery to the liver was recorded. However, drug delivery to other major tissues, including heart, kidney, lung and spleen, was reduced by 40–60% (Gupta et al., 1986b; Gallo et al., 1989). Subsequent studies utilizing lower dose of microsphere-delivered drug, in the presence of an 8000 G magnet, improved drug delivery to Tail-2, at the expense of drug distribution to the non-target tissues (Gupta and Hung, 1989c). These studies, in general, revealed liver to be the most efficient organ as an indicator of non-target drug distribution. Tail-1 and Tail-3 comprise tissues segments adjacent to the target-site, and hence the determination of drug levels in these tissues, as a function of time, may provide an indication of the specificity of a magnet in controlling the distribution of magnetic drug carrier at the intended site. For these reasons, Tail-1, Tail-3 and liver were monitored for drug concentrations, along with the target-site Tail-2.

*Effect of drug content of magnetic albumin microspheres on their targeting efficiency in rats*

Fig. 1 displays the mean drug concentration vs. time profiles for Tail-1, Tail-2, Tail-3 and liver of rats, following the administration of 0.4 mg/kg adriamycin hydrochloride via the two types of magnetic microspheres. The mean maximum drug concentration ($C_{max}$) at Tail-1, with both types of microspheres, was comparable. However, compared to type A microspheres, those of type B increased the $C_{max}$ at Tail-2 and Tail-3 by almost

TABLE 1

*Physico-chemical characteristics of adriamycin hydrochloride loaded magnetic albumin microspheres*

| Variables | Type A microspheres [a] | Type B microspheres [b] |
|---|---|---|
| Size ($\mu$m) | $0.75 \pm 0.44$ ($n = 200$) | $0.87 \pm 0.36$ ($n = 200$) |
| $Fe_3O_4$ content (% w/w) | $21.2 \pm 2.4$ ($n = 4$) | $18.2 \pm 3.9$ ($n = 4$) |
| Adriamycin content (% w/w) [c] | $3.9 \pm 0.6$ ($n = 3$) | $8.6 \pm 2.3$ ($n = 3$) |
| [d] | $0.9 \pm 0.2$ ($n = 4$) | $2.8 \pm 0.9$ ($n = 4$) |

[a] Microspheres were prepared using 40% w/v aqueous albumin; stabilization conditions: 120 °C for 10 min.
[b] Microspheres were prepared using 30% w/v aqueous albumin; stabilization conditions: 120 °C for 2.5 min.
[c] Refers to drug content of fresh microspheres.
[d] Refers to drug content of microspheres washed four times.

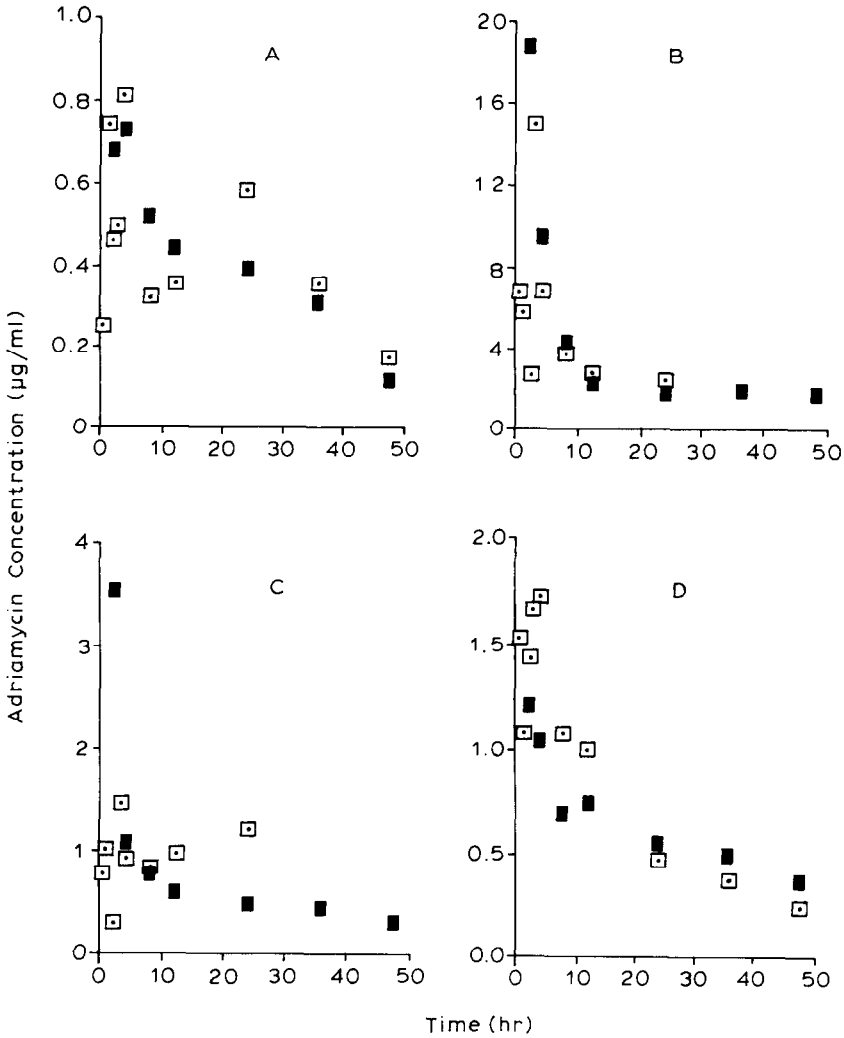


Fig. 1. Mean adriamycin concentration-time profiles at the target and non-target tissues of rat, following the administration of 0.4 mg/kg adriamycin via magnetic albumin microspheres of type A (⊡) and type B (■), in the presence of an 8000 G magnetic field applied at Tail-2 for 30 min. Each point represents the mean of three animals. Panels: (A) Tail-1, (B) Tail-2, (C) Tail-3, (D) liver.

25 and 150%, respectively, and decreased the $C_{max}$ at liver by 30%. These figures do not suggest any general trend in terms of the effect of the drug content of the two types of microspheres on the $C_{max}$ in various tissues monitored in this study.

The results on the total area under the adriamycin concentration-time profiles ($AUC_0^\infty$) for each tissue and both types of microspheres are listed in Table 2. It can be seen from these data that irrespective of drug content, both types of microspheres generated a 5–15 times higher $AUC_0^\infty$ at Tail-2 than that observed for the other tissues. Compared to type A microspheres, those of type B increased the $AUC_0^\infty$ at Tail-2 by 11%. However, this was accompanied by $\geq 20\%$ increase in $AUC_0^\infty$ at Tail-3 and liver.

Table 3 summarizes the values for the targeting efficiency ($t_e$) of both types of microspheres in the delivery of adriamycin to Tail-2. The $t_e$ values $> 1$ vs. all three non-target tissues, and for both

TABLE 2

*Mean total area under the tissue adriamycin concentration-time curve ($AUC_0^\infty$) obtained following the administration of 0.4 mg/kg drug via type A magnetic albumin microspheres (with ~ 1% w/w drug) and via type B magnetic albumin microspheres (with 3% w/w drug) in the presence of an 8000 G magnetic field, applied for 30 min at Tail-2*

| Tissues | $AUC_0^\infty$ ($\mu$g h ml$^{-1}$) [a] | | $AUC_0^\infty$ ratio [b] |
|---|---|---|---|
| | Type A microspheres | Type B microspheres | |
| Tail-1 | 23.00 | 20.34 | 0.88 |
| Tail-2 | 283.79 | 315.99 | 1.11 |
| Tail-3 | 42.99 | 51.62 | 1.20 |
| Liver | 37.35 | 46.90 | 1.26 |

[a] Assuming a tissue density of 1 g/ml.
[b] Determined using the relationship:

$$AUC_0^\infty \text{ ratio} = \frac{(AUC_0^\infty) \text{ type B microspheres}}{(AUC_0^\infty) \text{ type A microspheres}}$$

microsphere types, indicate that they were capable of controlling the delivery of drug to the target site. The $t_e$ values of 7.60 and 6.73 vs. liver, for type A and B microspheres respectively, suggest that following its administration via the magnetic carrier, the distribution of adriamycin to the liver was considerably reduced. Hence, the magnetic field not only retained the drug-loaded magnetic microspheres at the target site but also hampered their localization in the reticuloendothelial system. Indeed, a magnetic field of 8000 G applied for 30 min at Tail-2 has been shown to evoke extravascular transfer of magnetic albumin microspheres (Gupta et al., 1989a), which may also explain the high drug levels monitored at the target site in the present study, even after 48 h of administration of magnetic drug carrier.

TABLE 3

*Drug targeting efficiency of type A magnetic albumin microspheres and type B magnetic albumin microspheres ($t_e$) in the delivery of 0.4 mg/kg adriamycin to Tail-2, administered in the presence of an 8000 G magnetic field applied at Tail-2 for 30 min*

| Tissues | $t_e$ [a] | | $^r t_e$ [b] |
|---|---|---|---|
| | Type A microspheres | Type B microspheres | |
| Tail-1 | 12.34 | 15.54 | 1.26 |
| Tail-2 | 1.00 | 1.00 | 1.00 |
| Tail-3 | 6.60 | 6.12 | 0.93 |
| Liver | 7.60 | 6.73 | 0.89 |

[a] Determined using Eqn. 2.
[b] Determined using the relationship:

$$^r t_e = \frac{(t_e) \text{ type B microspheres}}{(t_e) \text{ type A microspheres}}$$

Despite the fact that microspheres of type B had almost 3-times higher drug content than type A the data on the in vivo kinetics of the two types of microspheres provide little evidence regarding the superior targeting efficiency of type B microspheres over type A. A possible explanation of these unanticipated results concerns the association of type B microspheres with ~ 18% free drug at the time of dose administration, as opposed to ~ 10% free drug associated with type A microspheres. A greater fraction of free drug in the dosing suspension of type B microspheres is likely to have contributed to a larger extent to the $AUC_0^\infty$ of non-target tissues rather than the $AUC_0^\infty$ for the target tissue, thus offsetting any possible improvement in targeted drug delivery due to the initial higher drug content of the microspheres. It is probable that if this free drug were instead present as a fraction entrapped within the microspheres, the $AUC_0^\infty$ at the target site would have been higher, and that at the non-target sites lower, than those listed in Table 2.

The significance of extravascular transfer of carrier at the target site towards the overall targeting efficiency of a drug delivery system, and the fact that the extravasation of magnetic albumin microspheres in healthy target tissue is a slow process, have been discussed previously (Gupta et al., 1989a). The correlation of these observations with the present data, with particular attention being paid to the fact that type B microspheres were stabilized to a much smaller extent and hence would release their drug load at a much faster rate than type A microspheres (Gupta et al., 1986c), raises the possibility that compared to type A microspheres, those of type B may have released a greater proportion of their drug content before extravascular transfer. This in turn may have compensated any functional difference in drug content between the two types of microspheres.

Apart from both of these possibilities, it is also probable that the magnetic field may have modulated the release behaviour of the two types of microspheres to differing extents, both in the vascular as well as extravascular compartment. Indeed, magnetic fields have been successfully employed in controlling the release of pharmaceuticals from magnetically responsive devices (Langer et al., 1980; McCarthy et al., 1984; Saslawski et al., 1988).

*Effect of 1000 G magnet on targeting efficiency of magnetic albumin microspheres in rats*

Fig. 2 displays the mean drug concentration-time profiles for Tail-1, Tail-2, Tail-3 and liver of rats, following the intra-arterial administration of 0.4 mg/kg adriamycin hydrochloride via type B magnetic microspheres in the presence and absence of a 1000 G magnet. The values of the maximum drug concentration ($C_{max}$) in various tissues of rat, for each group of animal, are com-

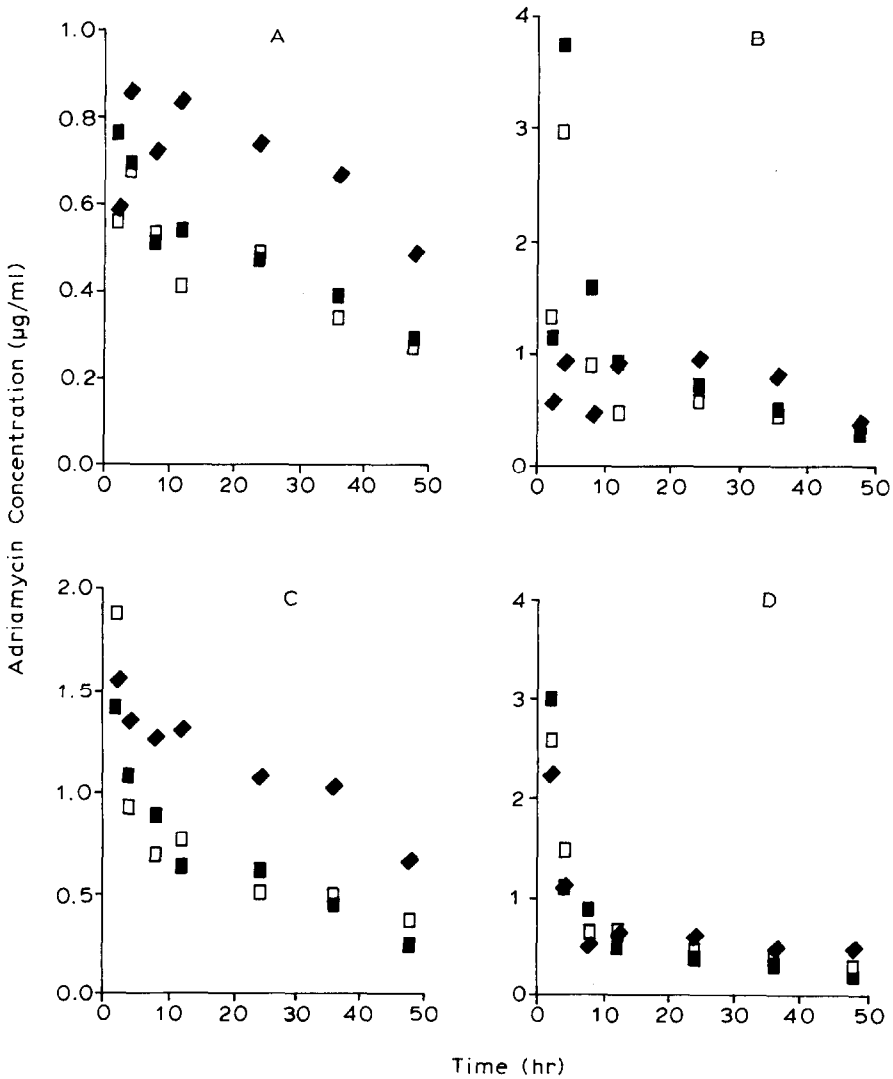


Fig. 2. Mean adriamycin concentration-time profiles at the target and non-target tissues of rat, following the administration of 0.4 mg/kg adriamycin via type B magnetic albumin microspheres in the absence (◆) and presence of a 1000 G magnetic field, applied at Tail-2 for 30 min (□) or 60 min (■). Each point represents the mean of three animals. Panels designated as in Fig. 1.

pared in Table 4. It can be seen from these data that following the administration of drug-loaded magnetic microspheres in the presence of the magnet, the $C_{max}$ values at Tail-2 increased 3–4-fold vs. the control group. However, the $C_{max}$ values for other tissues were comparable. In addition, the increase in duration of magnetic field application from 30 to 60 min increased the $C_{max}$ at Tail-2 by almost 25%. These observations suggest that (a) the use of a magnetic field of as low as 1000 G can increase the delivery of drug via magnetic microspheres to the target site, and (b) increasing the time of application of the magnetic field may increase the efficacy of magnetically controlled drug targeting. Similar results have been reported previously by others in studies where magnets with field strength $\geq$ 3000 G were employed and the drug concentration or radioactivity levels at the target and non-target sites were monitored only at two or three time points after administration of the magnetic microspheres (Widder et al., 1978; Morimoto et al., 1980b, 1981).

Table 5 lists the data on the mean total area under the adriamycin concentration-time profiles ($AUC_0^\infty$) for various tissues of rat, following administration via magnetic albumin microspheres in the presence and absence of a 1000 G magnetic field. The data indicate that the application of the magnetic field for 30 or 60 min gave rise to no significant alteration in the $AUC_0^\infty$ at the target site. These results are at variance with those concluded solely on the basis of $C_{max}$ values (Table 4), and hence a direct comparison of drug concentrations, in the target and non-target tissues, at limited time points, may not necessarily reveal accurate information regarding the efficacy of a delivery device. At times, such comparisons may lead to erroneous interpretation of the in vivo performance of the delivery device (Gupta and Hung, 1989d).

TABLE 4

*Maximum concentration of adriamycin ($C_{max}$) in the target and non-target tissues of rat, following administration of 0.4 mg/kg drug via magnetic albumin microspheres in the absence (control) and presence of a 1000 G electromagnet applied for 30 or 60 min at Tail-2 (experimental)*

| Tissues | $C_{max}$ ($\mu$g/ml) (mean $\pm$ S.D.) [a] | | |
|---|---|---|---|
| | Control | Experimental | |
| | | 30 min [b] | 60 min [b] |
| Tail-1 | 0.86 $\pm$ 0.28 | 0.68 $\pm$ 0.08 | 0.76 $\pm$ 0.20 |
| Tail-2 | 0.94 $\pm$ 0.66 | 2.97 $\pm$ 0.25 | 3.74 $\pm$ 0.24 |
| Tail-3 | 1.56 $\pm$ 0.75 | 1.87 $\pm$ 0.31 | 1.42 $\pm$ 0.20 |
| Liver | 2.23 $\pm$ 0.54 | 2.59 $\pm$ 0.23 | 3.01 $\pm$ 1.08 |

[a] $n = 3$.

[b] Refers to the time for which the 1000 G magnet was applied at Tail-2.

TABLE 5

*Mean total area under the tissue adriamycin concentration-time curve ($AUC_0^\infty$) following administration of 0.4 mg/kg drug via magnetic albumin microspheres in the absence (control) and presence of a 1000 G magnetic field applied for 30 or 60 min at Tail-2 (experimental)*

| Tissues | $AUC_0^\infty$ ($\mu$g h ml$^{-1}$) [a] | | |
|---|---|---|---|
| | Control | Experimental | |
| | | 30 min [b] | 60 min [b] |
| Tail-1 | 69.67 | 30.23 | 35.93 |
| Tail-2 | 43.17 | 41.71 | 49.86 |
| Tail-3 | 99.93 | 47.56 | 34.61 |
| Liver | 83.93 | 42.28 | 31.78 |

[a] Assuming a tissue density of 1 g/ml.

[b] Refers to the time for which the 1000 G magnet was applied at Tail-2.

Despite the fact that in the presence of the 1000 G magnet the $AUC_0^\infty$ at Tail-2 remained largely unchanged, the $AUC_0^\infty$ at Tail-1, Tail-3 and liver was reduced by 50–70%. Furthermore, the increase in duration of magnetic field application from 30 to 60 min decreased the $AUC_0^\infty$ for liver by 25%. The effect of altered tissue $AUC_0^\infty$ is further reflected in calculations of the targeting efficiency ($t_e$) of magnetic microspheres (Table 6). In the presence of a magnetic field, the $t_e$ vs. non-target tissues increased 2–3-fold. This increase was particularly noticeable against liver, which in the absence of a magnetic field displayed almost 2-fold greater drug exposure than the target-site Tail-2 (Table 5). However, in the presence of a magnetic field, drug delivery to this organ was considerably reduced.

Based on the present results, it is evident that the application of a 1000 G magnet for 30 or 60 min did not improve exposure of the target tissue

TABLE 6

*Targeting efficiency of magnetic albumin microspheres ($t_e$) in the delivery of adriamycin to Tail-2, following administration in the absence (control) and presence of a 1000 G magnetic field applied at Tail-2 for 30 or 60 min (experimental)*

| Tissues | $t_e$ [a] | | |
|---|---|---|---|
| | Control | Experimental | |
| | | 30 min [b] | 60 min [b] |
| Tail-1 | 0.62 | 1.38 | 1.39 |
| Tail-2 | 1.00 | 1.00 | 1.00 |
| Tail-3 | 0.43 | 0.88 | 1.44 |
| Liver | 0.51 | 0.99 | 1.57 |

[a] Determined using Eqn. 2.

[b] Refers to the time for which the 1000 G magnet was applied at Tail-2.

to the drug. Nonetheless, the reduction in exposure of liver, Tail-1 and Tail-3 to the drug suggests that drug toxicity to these tissues can be reduced.

*Effect of magnet strength on the targeting efficiency of magnetic albumin microspheres in rats*

Comparison of the data in column 3 of Table 2 with those listed in column 3 of Table 5 allows assessment of the effect of the magnetic field strength on the target and non-target tissue(s) disposition of adriamycin (0.4 mg/kg) delivered via type B magnetic albumin microspheres. Whereas non-target tissues (i.e. Tail-1, Tail-3 and liver) demonstrated $\leq 50\%$ variation in exposure to drug as a result of alteration in the magnetic field strength, the target-tissue Tail-2 displayed an approx. 8-fold decrease in $AUC_0^\infty$ following reduction in the magnet field strength from 8000 to 1000 G. These results suggest that the efficiency of magnetically controlled drug targeting is highly dependent upon the strength of the applied magnet.

## Conclusions

Our results indicate that micro-carrier systems with an apparently higher drug load may not necessarily guarantee improvement in the target/non-target tissue distribution of drug, as opposed to those with comparatively lower drug load. However, if drug content could be increased without affecting the physico-chemical characteristics of the carrier, e.g. size, stability, and drug release characteristics, the carrier may allow greater delivery of drug to the target tissue. The results indicate a general possibility that systems with adequate in vitro stability, but with poor in vivo stability, may not be ideal candidates for the targeted delivery of drugs with a low therapeutic index. This may be particularly true during the application of liposomal delivery systems in cancer chemotherapy, which exhibit low pharmaceutical stability and follow rapid release or leakage of drug in vivo (Allen and Cleland, 1980; Kirby and Gregoriadis, 1981; Senior and Gregoriadis, 1982). Hence, it appears important that in future emphasis be laid upon protocols optimizing physico-chemical factors which are responsible for the in vivo performance of the targeted drug delivery systems.

The other results of this work have shown that the use of a 1000 G magnet did increase $C_{max}$ at the target site, and also reduced the delivery of drug to the non-target tissues. However, the data do not provide evidence regarding the increase in $AUC_0^\infty$ at the target site. All these observations suggest that the 1000 G magnet was only partly able to control the distribution of adriamycin delivered via magnetic albumin microspheres.

## Acknowledgments

This project was supported by the grants from the Medical Research Council of New Zealand. The gift of adriamycin HCl from Farmitalia Carlo Erba, Milan (Italy), the technical assistance of Mrs. Charlotte Morris and the facilities provided by the Department of Pathology, University of Otago, are gratefully acknowledged.

## References

Akasaka, Y., Ueda, H., Takayama, K., Machida, Y. and Nagai, T., Preparation and evaluation of bovine serum albumin nanospheres coated with monoclonal antibodies. *Drug Design Del.*, 3 (1988) 85–97.

Allen, T.M. and Cleland, L.G., Serum-induced leakage of liposome contents. *Biochim. Biophys. Acta*, 597 (1980) 418–426.

Bartlett, J.M., Richardson, R.C., Elliot, G.S. and Blevins, W.E., Localization of magnetic microspheres in 36 canine osteogenic sarcomas. In Davis, S.S., Illum, L., McVie, J.G. and Tomlinson, E. (Eds.), *Microspheres and Drug Therapy. Pharmaceutical, Immunological and Medical Aspects*, Elsevier, Amsterdam, 1984, pp. 413–426.

Fujimoto, S., Miyazaki, M., Endoh, F., Takahashi, O., Okui, K. and Morimoto, Y., Biodegradable mitomycin C microspheres given intra-arterially for inoperable hepatic cancer with particular reference to a comparison with continuous infusion of mitomycin C and 5-fluorouracil. *Cancer*, 56 (1985) 2404–2410.

Gallo, J.M., Gupta, P.K., Hung, C.T. and Perrier, D.G., Evaluation of drug delivery following the administration of magnetic albumin microspheres containing adriamycin to the rat. *J. Pharm. Sci.*, 78 (1989) 190–194.

Gallo, J.M., Hung, C.T. and Perrier, D.G., Analysis of albumin microsphere preparation. *Int. J. Pharm.*, 22 (1984) 63–74.

Gallo, J.M., Hung, C.T. and Perrier, D.G., Reversed-phase ion-pair HPLC of adriamycin and adriamycinol in rat serum and tissues. *J. Pharm. Biomed. Anal.*, 4 (1986) 483–490.

Gibaldi, M. and Perrier, D., *Pharmacokinetics*, 2nd edn., Dekker, New York, 1982.

Gupta, P.K. and Hung, C.T., Magnetically controlled targeted micro-carrier systems. *Life Sci.*, 44 (1989a) 175–186.

Gupta, P.K. and Hung, C.T., Albumin microspheres. II. A review of its application in drug delivery. *J. Microencap.*, 6 (1989b) 463–472.

Gupta, P.K. and Hung, C.T., Effect of carrier dose on the multiple tissue disposition of doxorubicin hydrochloride administered via magnetic albumin microspheres in rats. *J. Pharm. Sci.*, 78 (1989c) 745–748.

Gupta, P.K. and Hung, C.T., Quantitative evaluation of targeted drug delivery systems. *Int. J. Pharm.*, 56 (1989d) 217–226.

Gupta, P.K., Gallo, J.M., Hung, C.T. and Perrier, D.G., Influence of stabilization temperature on the entrapment of adriamycin in albumin microspheres. *Drug Dev. Ind. Pharm.*, 13 (1987) 1471–1482.

Gupta, P.K., Hung, C.T. and Lam, F.C., Factorial design based optimization of the formulation of albumin microspheres containing adriamycin. *J. Microencap.*, 6 (1989b) 147–160.

Gupta, P.K., Hung, C.T., Lam, F.C. and Perrier, D.G., Albumin microspheres. III. Synthesis and characterization of microspheres containing adriamycin and magnetite. *Int. J. Pharm.*, 43 (1988) 167–177.

Gupta, P.K., Hung, C.T. and Perrier, D.G., Albumin microspheres. I. Release characteristics of adriamycin. *Int. J. Pharm.*, 33 (1986b) 137–146.

Gupta, P.K., Hung, C.T. and Perrier, D.G., Albumin microspheres. II. Effect of stabilization temperature on the release of adriamycin. *Int. J. Pharm.*, 33 (1986c) 147–153.

Gupta, P.K., Hung, C.T. and Rao, N.S., Ultrastructural disposition of adriamycin-associated magnetic albumin microspheres in rats. *J. Pharm. Sci.*, 78 (1989a) 290–294.

Gupta, P.K., Morris, C. and Hung, C.T., Evaluation of magnetic albumin microspheres for site-specific delivery of adriamycin. *Proc. Univ. Otago Med. Sch.*, 64 (1986a) 63–64.

Kirby, C. and Gregoriadis, G., Plasma-induced release of solutes from unilamellar liposomes is associated with pore formation in the bilayers. *Biochem. J.*, 199 (1981) 251–254.

Langer, R., Rhine, W., Hsieh, D.S.T. and Folkman, J., Control of release kinetics of macromolecules from polymers. *J. Membrane Sci.*, 7 (1980) 333–350.

McCarthy, M., Soong, D. and Edelman, E., Control of drug release from polymer matrices impregnated with magnetic beads. A proposed mechanism and model for enhanced release. *J. Control. Rel.*, 1 (1984) 143–147.

Morimoto, Y., Akimoto, M., Sugibayashi, K., Nadai, T. and Kato, Y., Drug carrier property of albumin microspheres in chemotherapy. IV. Antitumour effect of single shot or multiple shot administration of microspheres entrapped 5-FU on Ehrlich ascites or solid tumour in mice. *Chem. Pharm. Bull.*, 28 (1980a) 3087–3092.

Morimoto, Y., Okumura, M., Sugibayashi, K. and Kato, Y., Biomedical applications of magnetic fluids. II. Preparation and magnetic guidance of magnetic albumin microspheres for site-specific delivery in vivo. *J. Pharm. Dyn.*, 4 (1981) 624–631.

Morimoto, Y., Sugibayashi, K., Okumura, M. and Kato, Y., Biomedical applications of magnetic fluids. I. Magnetic guidance of ferro-colloid entrapped albumin microspheres for site-specific drug delivery in vivo. *J. Pharm. Dyn.*, 3 (1980b) 264–267.

Morris, R.M., Poore, G.A., Howard, D.P. and Sefranka, J.A., Selective targeting of magnetic albumin microspheres containing vindesine sulphate: Total remission in yoshida sarcoma-bearing rats. In Davis, S.S., Illum, L., McVie, J.G. and Tomlinson, E. (Eds.), *Microspheres and Drug Therapy. Pharmaceutical, Immunological and Medical Aspects*, Elsevier, Amsterdam, 1984, pp. 439–440.

Oppenheim, R.C., Gipps, E.M., Forbes, J.F. and Whitehead, R.H., Development and testing of proteinaceous nanoparticles containing cytotoxics. In Davis, S.S., Illum, L., McVie, J.G. and Tomlinson, E. (Eds.), *Microspheres and Drug Therapy. Pharmaceutical, Immunological and Medical Aspects*, Elsevier, Amsterdam, 1984, pp. 117–128.

Poznansky, M.J. and Juliano, R.L., Biological approaches to the controlled delivery of drugs: a critical review. *Pharmacol. Rev.*, 36 (1984) 277–336.

Saslawski, O., Couvreur, P. and Peppas, N.A., Alginate magnetic release systems: Cross-linked structure, swelling and release studies. *Proc. Int. Sym. Control. Rel. Bioact. Mat.*, 15 (1988) 26–27.

Senior, J. and Gregoriadis, G., Stability of small unilamellar liposomes in serum and clearance from the circulation: the effect of the phospholipid and cholesterol components. *Life Sci.*, 30 (1982) 2123–2125.

Sokoloski, T.D. and Royer, G.P., Drug entrapment within

native albumin beads. In Davis, S.S., Illum, L., McVie, J.G. and Tomlinson, E. (Eds.), *Microspheres and Drug Therapy. Pharmaceutical, Immunological and Medical Aspects*, Elsevier, Amsterdam, 1984, pp. 295–308.

Sugibayashi, K., Akimoto, M., Morimoto, Y., Nadai, T. and Kato, Y., Drug-carrier property of albumin microspheres in chemotherapy. III. Effect of microsphere-entrapped 5-fluorouracil on Ehrlich ascites carcinoma in mice. *J. Pharm. Dyn.*, 2 (1979) 350–355.

Thies, C. and Bissery, M., Biodegradable microspheres for parenteral administration. In Lim, F., (Ed.) *Biomedical Application of Microencapsulation*, CRC Press, Boca Raton, FL, 1984, pp. 53–74.

Widder, K.J., Senyei, A.E. and Scarpelli, D.G., Magnetic microspheres: a model system for site specific drug delivery in vivo. *Proc. Soc. Exp. Biol. Med.*, 58 (1978) 141–146.

Willmott, N., Cummings, J., Stuart, J.F.B. and Florence, A.T., Adriamycin-loaded albumin microspheres: preparation, in vivo distribution and release in the rat. *Biopharm. Drug Disp.*, 6 (1985) 91–104.

Willmott, N., Kamel, H.M.H., Cummings, J., Stuart, J.F.B. and Florence, A.T., Adriamycin-loaded albumin microspheres: Lung entrapment and fate in the rat. In Davis, S.S., Illum, L., McVie, J.G. and Tomlinson, E. (Eds.) *Microspheres and Drug Therapy. Pharmaceutical, Immunological and Medical Aspects,* Elsevier, Amsterdam, 1984, pp. 205–216.