ELSEVIER

International Journal of Pharmaceutics 147 (1997) 119–125

international journal of pharmaceutics

Preparation of magnetic granules containing bleomycin and its evaluation using model esophageal cancer

Hiromi Nagano[a], Yoshiharu Machida[b,*], Masanori Iwata[a], Toshio Imada[c], Yoshikazu Noguchi[c], Akihiko Matsumoto[c], Tsuneji Nagai[a]

[a]*Department of Pharmaceutics, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142, Japan*
[b]*Department of Clinical Pharmacy, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142, Japan*
[c]*Department of First Surgery, Yokohama City University Hospital, Fukuura 3-9, Kanazawa-ku, Yokohama-shi, Kanagawa 236, Japan*

Received 1 August 1996; revised 12 October 1996; accepted 12 November 1996

Abstract

Presuming a clinical application to targeting therapy for esophageal cancer, magnetic granules containing bleomycin (BLM) as an anticancer drug with bioadhesive polymers (hydroxypropyl cellulose-H/Carbopol 934® mixture, 3:2 w/w) and ultrafine ferrite (γ-Fe_2O_3) in weight ratio of 2:5:3 were prepared based on preliminary experiments in vitro using brilliant blue FCF (B.B.) as a model of anticancer drug. A high holding ratio on a target site was obtained when the granules were administered to normal rabbits using a mouth holder under magnetic guidance for the initial 2 min. However, residence time of the granules due to their bioadhesiveness after removal of the magnetic circuit was not sufficient. The BLM granules were administered once a day for 2 weeks to esophageal cancer model in rabbits prepared by transplantation of VX_2 cancer fragment. In spite of the granules being held at the targeting site, no difference in cancer growth was observed between the rabbits with and without administration of the granules. The results suggest that targeting drug delivery to esophageal mucosa by oral administration of the magnetic granules containing more drastic anticancer drug than BLM is promising if the bioadhesive property and release property of the granules could be improved. © 1997 Elsevier Science B.V.

Keywords: Targeting drug delivery; Magnetic granules; Ferrite; Bleomycin; Bioadhesive polymer; Experimental esophageal cancer; Local chemotherapy

1. Introduction

Cytotoxic agents have been extensively employed in cancer chemotherapy. However, the

* Corresponding author.

PII S0378-5173(96)04804-1

non-specificity of these drugs towards cancer in the body has curtailed their effective use in chemotherapy. Several modalities have been tried in order to improve the therapeutic efficacy of cytotoxic agents. Recently, several types of magnetically guided drug carriers for targeting therapy have been proposed, such as albumin microspheres (Gupta et al., 1986a,b, 1988, 1989a,b, 1990; Gallo et al., 1989), emulsions (Akimoto and Morimoto, 1983) and liposomes (Kiwada et al., 1986). The unique feature of these delivery systems over other drug targeting techniques is their ability to minimize the carrier uptake by RES.

With the intention of improving the topical effects of anticancer drugs, the investigation of magnetic guidance applied for local targeting chemotherapy of esophageal cancer by oral administration that is a new route in the field of magnetic guidance, was initiated by our group (Ito et al., 1990). In the study, the magnetic granules containing brilliant blue FCF as a model of anticancer drug were prepared and the effect of some conditions, e.g. viscosity of the solution used for administration of the granules, premagnetization time of granules, strength of the magnetic field applied and ferrite content in the granules, on the holding ability were reported. Moreover, good targeting efficiency and mucoadhesiveness of the granules in rabbit esophagus have been suggested.

As an expansion of the previous study, we carried out in vitro experiments presuming the clinical application of granules containing bleomycin (BLM). The holding ability of the magnetic granules containing BLM prepared on the basis of the results in vitro were examined in rabbit esophagus. Subsequently, the granules were applied to experimental esophageal cancer of rabbits produced by transplantation of VX_2 cancer.

2. Materials and methods

2.1. Materials

Ultrafine ferrite (γ-Fe_2O_3, needles of 0.01–0.05 μm diameter and 0.1–0.5 μm length) and bleomycin hydrochloride (BLM) were kindly supplied by Dainichi Seika Color and Chemicals and Nippon Kayaku, respectively. Brilliant blue FCF (B.B.) was purchased from Tokyo Chemical Industry. Agar powder was purchased from Wako Pure Chemical Industries. Hydroxypropylcellulose-H (HPC) and carboxyvinyl polymer (Carbopol 934®, CP) were obtained from Nippon Soda and B.F. Goodrich, respectively. Pentobarbital sodium injection (Nembutal® injection) and 1% lidocaine injection (Xylocaine® injection) were purchased from Dainabot and Fujisawa Pharmaceutical, respectively. All other reagents were of special reagent grade.

2.2. Preparation of magnetic granules

Brilliant blue FCF or bleomycin, ultrafine ferrite, and polymers (HPC/CP mixture of 3:2 w/w) were mixed well and kneaded in mortar with ethanol and passed through a 20-mesh sieve. The obtained granules were dried overnight in vacuum and the portion which passed through a 20-mesh sieve and remained on a 50-mesh sieve was used as the sample.

2.3. Determination of brilliant blue FCF and BLM contents in granules

Brilliant blue FCF was extracted from granules through stirring in purified water for 2 h followed by centrifugation at 3000 rpm for 10 min. The amount of B.B. in the supernatant was determined spectrophotometrically at 630 nm using a Ubest-30 spectrophotometer (Japan Spectroscopic).

The amount of BLM in granules was determined using the similar method at 292 nm.

2.4. Measurement of drug release from granules in vitro

Release of B.B. from granules having different particle size and compositions was measured according to agar-gel bed method (Machida et al., 1979). Release of BLM from granules was also measured by the same method.

2.5. Measurement of holding ratio of granules in vitro using model esophagus

The holding ratio of granules having different compositions was measured according to the tubular agar-gel method (Ito et al., 1990) using a pair of magnets (neodymium-iron-boron) generating a magnetic field of about 1700 G on the internal surface of the gel tube.

2.6. Evaluation of targeting ability of granules in vivo using rabbits

Male Japanese white rabbits ($\approx$ 2.5 kg body weight) were used in all experiments. Fig. 1 shows the permanent magnet circuit (Sumitsu and Co.) used in the experiments that generated a magnetic field of about 2400 G at point *P*. Rabbits were anesthetized with pentobarbital sodium (20 mg/kg, i.v.) after fasting for about 20 h and fixed in a magnetic circuit generating a magnetic field at the targeting site, as shown in Fig. 2. A catheter with a 5 mm o.d. was inserted through a mouth holder about 10 cm into the esophagus and 5 mg or 25 mg of granules were administered through the catheter with 0.65% HPC solution. At a predefined time after administration, the rabbits were immediately sacrificed and their esophagus excised. Each esophagus was divided into two segments, i.e. the lower part near the stomach where

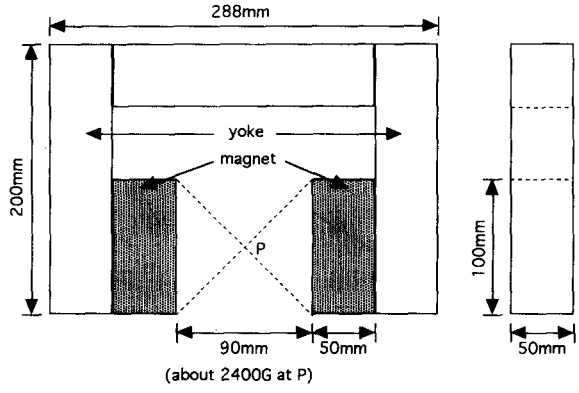

Fig. 1. Illustration of permanent magnetic circuit used for the in vivo study.

Fig. 2. Administration of granules for the study in vivo using rabbit.

the magnets were applied and the upper half. Potencies of BLM in the mucosa and remaining ferrite were determined using the microbiological method of the Japan Antibiotics Drugs Standards and a modified procedure of the *O*-phenanthroline method described in the literature (Ito et al., 1990), respectively.

2.7. Evaluation of granules containing BLM using esophageal cancer model in rabbits

Male Japanese white rabbits ($\approx$ 2 kg body weight) were anesthetized with pentobarbital sodium (20 mg/kg, i.v.) and excised their esophagus by cervical incision. Approximately 1 mm^3 of VX_2 cancer fragments were transplanted into esophageal submucosa of the rabbits from the serous membrane side.

2.7.1. Effect of mouth holder and premagnetization on holding ratio of granules

At 2 weeks after transplantation, 10 mg of BLM granules was admistered to the rabbits in a similar manner, as already mentioned, or in a non-anesthetized state without mouth holder. The granules held on the target site were collected and remaining ferrite was determined as previously mentioned.

Effect of premagnetization was examined using the BLM granules magnetized for more than 3 min in a magnetic circuit of 2400 G.

2.7.2. Effect of BLM magnetic granules on growth of transplanted cancer

From the day of transplantation, 10 mg of granules ($\approx$ 3.72 mg potency of BLM) was administered once a day to the rabbits in a similar manner as already described. The administration was done also in the non-anesthetized state without a mouth holder. The first administration was done within 5 h after transplantation and administration was repeated for 7–9 times. At 2 weeks after the transplantation, the rabbits were sacrificed and their esophagus immediately excised. The esophageal cancer was measured across the long diameter and short diameter and the cancer volume was calculated assuming a thickness as equal to the short diameter.

3. Results and discussion

3.1. Decision of formula of granules containing BLM

Magnetic granules containing B.B. in six different ratio combinations were prepared for the purpose of determining a formula to ensure the proper controlled release and fine holding property.

At first, the effect of particle size on the release property was examined by the agar-gel bed method. The B.B. release profiles of granules (B.B.:polymer:ferrite = 1:9:10 w/w) classified using each sieve (297 355, 425 500, 600 710 and 840 μm) are shown in Fig. 3. As a result, granules of 600–710 μm in particle size were chosen because it indicated a proper sustained release and high yield.

On the basis of the knowledge regarding ferrite content and magnetic force on the holding tendencies of granules (Ito et al., 1990), six different granule combinations (polymer and B.B.) in which the ferrite content was fixed at 30% were prepared. The release profiles of B.B. from granules prepared according to the six formulas and relationship between the 50% released time (T_{50}) and polymer content in the granules are shown in Fig. 4. Consequently, polymer contents above 40% seemed proper because of their sustained release property and T_{50} above 30 min.

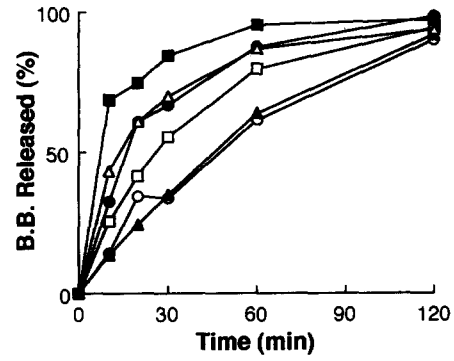

Fig. 3. Release profiles of B.B. from granules with different diameters obtained by the agar-gel bed method. ■, 297–355 μm; △, 355–425 μm; ●, 425–500 μm; □, 500–600 μm; ▲, 600–710 μm; ○, 710–840 μm.

The effect of polymer content on holding tendencies of the granules is shown in Fig. 5. It was considered that the effect of polymer content was slightly recognized because the holding property just after flushing was measured in the holding test in vitro using a model esophagus.

According to the results, the composition of BLM granules was fixed at BLM:polymer:ferrite = 2:5:3 w/w. The release profile of BLM from the granules obtained by the agar-gel bed method is shown in Fig. 6. The quantity of released BLM at 2 h after the beginning of the test was less than B.B. granules. The granules prepared were used in the following studies in vivo. However, the drug release characteristics of the granules should influence the inhibitory effect of the drug on cancer growth. So, an opti-

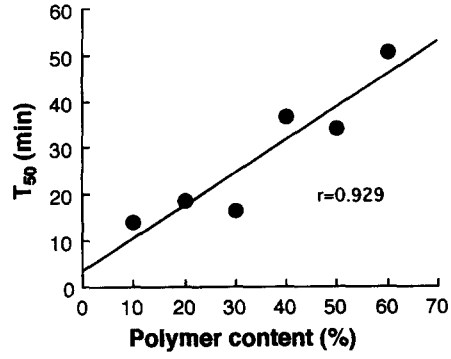

Fig. 4. Relation between 50% released time (T_{50}) and polymer content of granules. Magnetite content was fixed at 30%.

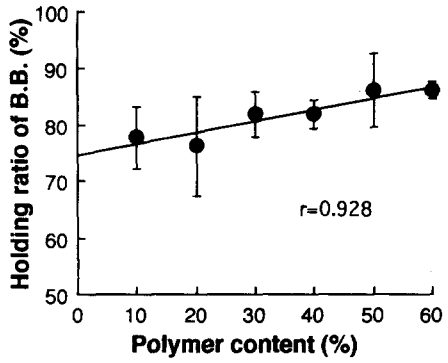

Fig. 5. Relation between holding ratio of B.B. and polymer content of granules containing 30% of magnetite. Each point represents the mean ± S.D. ($n = 3$).

mum release profile of the drug might be found later, referring to the therapeutic effect of the granules.

3.2. *In vivo evaluation of magnetic granules containing BLM as targeting drug delivery system*

Repeated administration was required for evaluation of the therapeutic efficacy of the BLM magnetic granules on cancer. Therefore, the administration was carried out using a larger magnetic circuit than in the previous study (Ito et al., 1990) and fixing cervicodorsalis of the rabbits, as shown in Fig. 2.

The esophagus, excised after administration of the magnetic granules containing BLM under application of the magnetic circuit was compared with that after administration without the circuit.

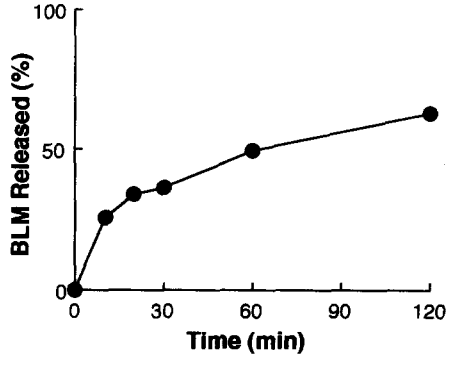

Fig. 6. Release profile of BLM from granules obtained by the agargel bed method. Drug:polymer:ferrite = 2:5:3 in weight.

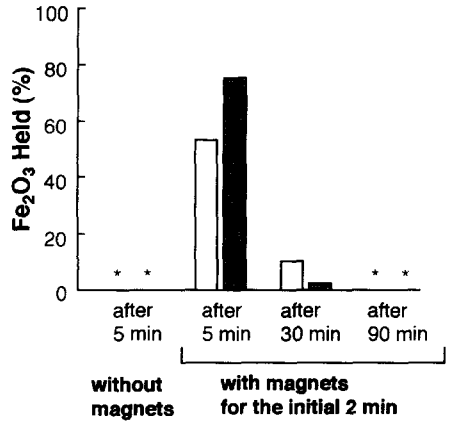

Fig. 7. Effect of magnetic field on holding ratio of ferrite at the targeting mucosa. Dose: □, 5 mg; ■ 25 mg. *, not detected.

As a result, granules were observed on the esophagus only in the region where the circuit was applied. This result demonstrates that the granules containing 30% ferrite could be guided under the influence of the magnetic field to the region where the circuit was applied. However, residence time of the granules after removal of the magnetic field was not sufficient, obviously because of the low mucoadhesiveness of the granules.

Ferrite in the granules recovered from targeting mucosa was measured as Fe^{2+}, yielding the results shown in Fig. 7. Ferrite was not detected in the esophagus excised at 90 min after administration of the magnetic granules with the application of the magnetic circuit and at 5 min after administration without the circuit. As for the esophagus excised at 5 and 30 min after administration with application of the circuit, ferrite was detected but there was no significant difference between the values obtained by administration of 5 or 25 mg granules.

BLM concentration in each segment of the esophagus at a predefined interval after administration of the magnetic granules is illustrated in Fig. 8. BLM concentration in targeting mucosa at 5 min after administration with the magnetic circuit was greater than those of others in accordance with the results for ferrite as previously described. Therefore, it is presumable that administration of 5 mg of magnetic granules containing BLM under magnetic guidance would be effica-

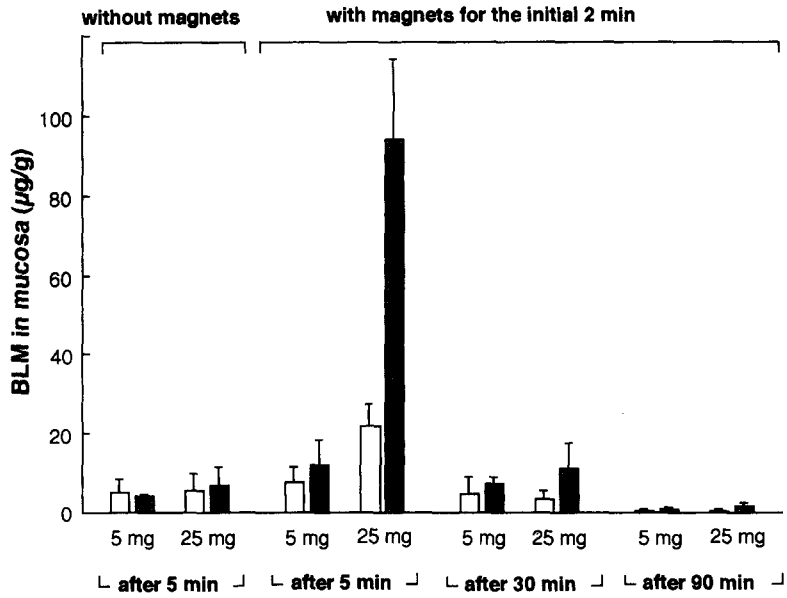

Fig. 8. Effect of magnetic field on BLM concentration in esophageal mucosa. □, non target; ■, target. Each value is the mean ± S.D. ($n = 3$).

cious against esophageal cancer if the residence time of the granules could be prolonged.

3.3. Evaluation of the magnetic granules containing BLM using experimental esophageal cancer of rabbits

Experimental esophageal cancer in rabbit was successfully prepared by transplantation of the VX_2 cancer. However, volume of cancer varied widely, as shown in Table 1. The large variation of cancer growth can be attributed to the transplantation of cancer fragment with different viability and also in unequal volume. Therefore, usage of the experimental esophageal cancer prepared in this study shuld be limited.

At 2 weeks after transplantation of the VX_2 cancer, experimental esophageal cancer grew to a diameter of about 10 mm and often gave rise to an ulcer.

As shown in Fig. 9, when the granules were administered to cancer loaded rabbits in non-anesthetized state without a mouth holder, they were not held on the esophageal mucosa and it was determined that the holding ratio was improved using a mouth holder, obviously due to the inhibited swallowing movement of the esophagus. Moreover, premagnetization of the granules for 3 min did not increase the holding ratio in vivo, in contrast with the results in vitro (Ito et al., 1990).

Cancer growth in administration of BLM magnetic granules under different situations was also shown in Table 1. No meaningful effect of gran-

Table 1
Effect of magnetic granules containing BLM on growth of VX_2 cancer transplanted into esophageal submucosa of rabbits

	Cancer volume (mm^3)	n
Control		
Non-anesthetized	404.3 ± 203.1	11
Anesthetized	246.8 ± 108.7	3
Administered		
Without magnets	200.9 ± 73.4	5
With magnets		
Non-magnetized granules[a]	180.6 ± 123.0	10
Magnetized granules[a]	116.9 ± 47.2	5
Magnetized granules[b]	293.5 ± 149.0	4

[a]Granules were administered under non-anesthetized condition without using mouth holder.
[b]Granules were administered under anesthesia with mouth holder.

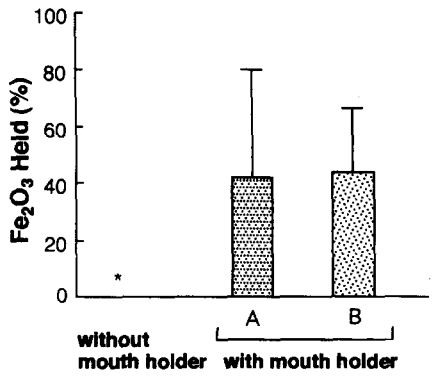

Fig. 9. Effect of mouth holder and premagnetization of granules on holding ratio of ferrite at the targeting mucosa. A, with premagnetization; B, without premagnetization. Each column represents the mean ± S.D. ($n = 4$). *, not detected.

ules was observed. Even in the animals where the granules should be held at the targeting site by administration using a mouth holder under anesthesia, no difference was observed in cancer growth between the animals receiving no granules. Mainly, this may be due to the large variation in cancer growth and to the fact that the cancer had been covered by normal mucosa which can disturb the permeation of BLM. Moreover, the retaining period of the drug on the target site could be estimated as insufficient. Therefore, further investigation should be done using a more powerful anticancer drug such as doxorubicin or cisplatin and a more reproducible model of cancer, considering the bioadhesive property and drug release profile of the granules.

4. Conclusion

Magnetic guidance of the magnetic granules containing BLM (BLM:polymer:ferrite = 2:5:3 w/w) for local chemotherapy of esophageal cancer via an oral route of administration was achieved in rabbits. However, residence time of the BLM granules on the esophageal mucosa after removal of the magnetic circuit was not sufficient. Further effort should be concentrated on improving the bioadhesiveness of the granules by employing other bioadhesive polymers.

Acknowledgements

This investigation was supported in part by the Mochida Memorial Foundation for Medical and Pharmaceutical Research. The authors are very grateful to Mr M. Hasuike, Sumitsu and Co., for valuable advice in designing the magnetic circuit, and to Dr J. Fujimori, Ms K. Takeyama and Ms Y. Inaba for their kind assistance during the experimental work.

References

Akimoto, M. and Morimoto, Y., Use of magnetic emulsion as a novel drug carrier for chemotherapeutic agents. *Biomaterials*, 4 (1983) 49–51.

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.

Gupta, P.K., Hung, C.T. and Perrier, D.G., Albumin microspheres. I. Release characteristics of adriamycin. *Int. J. Pharm.*, 33 (1986a) 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 (1986b) 147–153.

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

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 (1989b) 745–748.

Gupta, P.K. and Hung, C.T., Albumin microspheres. V. Evaluation of parameters controlling the efficacy of magnetic microspheres in the targeted delivery of adriamycin in rats. *Int. J. Pharm.*, 59 (1990) 57–67.

Ito, R., Machida, Y., Sannan, T. and Nagai, T., Magnetic granules: a novel system for specific drug delivery to esophageal mucosa in oral administration. *Int. J. Pharm.*, 61 (1990) 109–117.

Kiwada, H., Sato, J., Yamada, S. and Kato, Y., Feasibility of magnetic liposomes as a targeting device for drugs. *Chem. Pharm. Bull*, 34 (1986) 4253–4258.

Machida, Y., Masuda, H., Fujiyama, N., Ito, S., Iwata, M. and Nagai, T., Preparation and Phase II clinical examination of topical dosage form for treatment of carcinoma colli containing bleomycin with hydroxypropyl cellulose. *Chem. Pharm. Bull*, 27 (1979) 93–100.