Local drug delivery system using ceramics: vacuum method for impregnating a chemotherapeutic agent into a porous hydroxyapatite block

M. ITOKAZU*, M. ESAKI, K. YAMAMOTO, T. TANEMORI, T. KASAI
Department of Orthopaedic Surgery, Gifu University School of Medicine, Gifu, 500–8705, Japan E-mail: itohan@cc.gifu-u.ac.jp

We performed an experimental study on a new drug delivery system that employs a porous hydroxyapatite block (HAb) (composition: $Ca_{10}(PO_4)_6(OH)_2$) to conduct sustained release of a chemotherapeutic agent. To confirm penetration of the agent into the HAb (2 cm^3), an aqueous solution containing eosin dye was used at various vacuum pressures. To estimate the storage capacity of the HAb, blocks were weighed before and after being impregnated with the aqueous dye solutions, and the capacity of the block was calculated from the increase in weight after vacuum. In this slow-release study using vacuum, the anti-cancer drug methotrexate (MTX) was used *in vitro*. Four HAb (1 cm^3) containing different concentrations of MTX, ranging from 1.22 to 2.38 mg per block, were studied. All were found to release the drug, maintaining a mean concentration of 0.22 to 0.32 μg/ml even after twelve days. This concentration is high enough to be effective against tumor cells. The results suggest that HAb impregnated with a chemotherapeutic agent using a simple vacuum system may serve as a valuable new method of administering local chemotherapy, primarily when used as a strut graft for bone defects. This new drug delivery system can also be used as an adjuvant material in extended curettage, which can also discourage recurrence of benign tumors without any risk of systemic toxicity.
© 1999 Kluwer Academic Publishers

1. Introduction

An important consideration when treating bone tumors and soft-tissue tumors with chemotherapeutic agents is maintaining a long-acting, localized, yet effectively high concentration of a drug at the site of the tumor cells while producing minimum systemic side effects. Localized chemotherapy, if effective, could reduce the risk of local recurrence of bone tumors following curettage, while limiting the toxicity problems associated with traditional chemotherapy. Porous hydroxyapatite block (HAb) has demonstrated excellent biocompatibility and is structurally similar to bone. HAb has been employed as a suitable material for filling bone defects or dead space created through the surgical excision of bony foci [1]. Therefore, it was postulated that HAb block impregnated with a chemotherapeutic agent might be useful for filling grafts after the curettage of bone tumors. To date, we have employed such a delivery system to treat osteomyelitis in an *in-vitro* study using centrifuge methods [2] and to treat experimental osteogenic sarcoma in mice dorsal skin in an *in vivo* study [3]. The study demonstrated the effectiveness of this system under conditions of clinical use when HAb was implanted in infected bone lesions [4]. In the present study, HAb blocks were impregnated with the chemotherapeutic agent methotrexate (MTX) using a vacuum method, and the drug-releasing capacity of the blocks was examined *in vitro*.

2. Materials and methods

2.1. Evidence of antibiotic penetration into HAb pores

The study used a porous hydroxyapatite block with a composition of $Ca_{10}(PO_4)_6(OH)_2$ and an amount of porosity of 50% (Fig. 1); this block was a product developed by Asahi Optical Co. Ltd (Tokyo, Japan). Further, this block was used to fabricate $20 \times 20 \times 20$ mm cubic blocks that would be used as an ordinary bone graft (Fig. 2a). An eosin dye solution was used to demonstrate the ability of the antibiotic to penetrate the HA block. Blocks were immersed in the eosin dye solution under varying levels of vacuum 10 in. (254 mm) Hg, 5 in. (127 mm) Hg, 2.5 in. (63.5 mm)Hg, and 0 mmHg) for 20 min with a Miniature Vacuum/ Pressure Pump OM036 (Nihon Millipore Limited Tokyo,

*Author to whom all correspondence should be addressed.

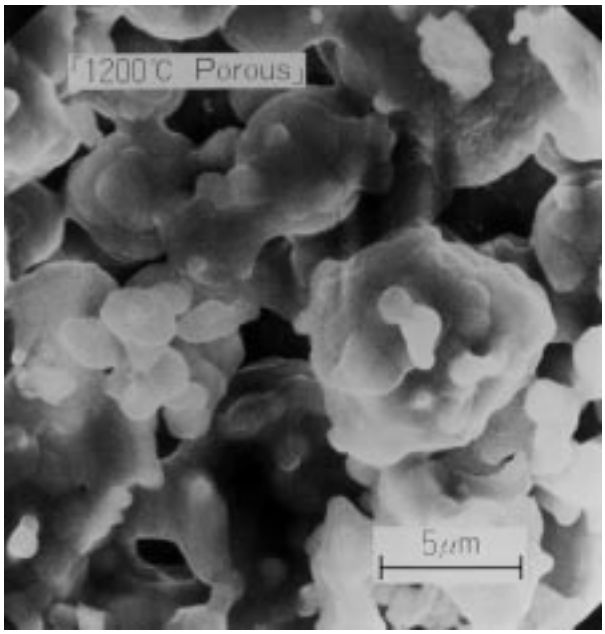

Figure 1 SEM photograph of the structure of interporous hydroxyapatite.

Japan) (Fig. 3). After vacuuming, the HAbs were cut with a dental bar to determine whether the dye solution fully penetrated to the center (Fig. 2b).

2.2. Experimental study of the drug delivery system using HAb impregnated with MTX

Aqueous solutions of MTX were used as the chemotherapeutic agent, which was loaded into four fabricated HAbs (10 mm × 10 mm × 10 mm). The concentrations of the MTX solutions were adjusted by dissolving 50 mg of the antibiotic in 8 ml and 16 ml of PBS (pH: 7.4) for concentrations of 6.25 mg/ml (HAb/8) and 3.13 mg/ml (HAb/16), respectively. The HAbs were placed in these solutions and decompressed to 10 in. (254 mm)Hg for 15 min, a level that the previous study had shown was sufficient to produce an MTX/HAb composite. To estimate the concentration of MTX released, the blocks were stored in 3 ml of phosphate-buffered saline (PBS) at a temperature of 37 °C. The PBS was replaced every 48 h. The PBS samples containing released MTX were stored at a temperature of –45 °C until the assay. *In vitro* elution studies were then performed by assaying the MTX using high-performance liquid chromatography (HPLC).

3. Results

In the study of the penetration of antibiotics into HAb pores using a dye solution, it was discovered that in the experimental study the dye penetrated to the center of the HAb after 15 min at vacuum levels of 10 in. (254 mm)Hg and after 20 min at 5 in. (127 mm)Hg because the increasing rate of HAb weight maintained equilibrium (Fig. 4). Based on the increase in HAb weight, the study indicated that the content of MTX in a 1 cm^3 block was as follows: [HAb1/8] = 2.38 mg, [HAb2/8] = 1.68 mg, [HAb1/16] = 1.26 mg, and [HAb2/16] = 1.22 mg. Evaluation of the slow-release properties of HAb indicated that the MTX concentration remained at a high concentration of 0.22–0.32 μg/ml even after 12 days, or 6 exchanges of the PBS *in vitro* (Table I).

4. Discussion

The effectiveness of chemotherapy has improved in recent years; however, side effects associated with the administration of anti-cancer chemotherapeutic agents may necessitate suspending this treatment. For this reason, a number of sustained-release drug delivery systems have been developed, employing carriers such as activated carbon particle [5] and ethyl ester of iodized poppy-seed oil fatty acids [6, 7], with the goal of minimizing systemic side effects while permitting long-term localized delivery of anti-cancer chemotherapeutic agents at higher concentrations. In an investigation of localized chemotherapy treatment of bone metastasis, Greco *et al*. [8] reported that doxorubicine and cisplatinum released from polymethylmethacrylate were effective *in vitro* against normal human fibroblasts and colon and breast carcinoma cell lines.

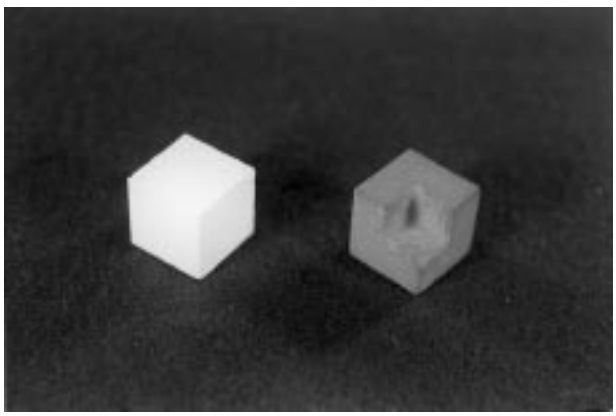

Figure 2 20 × 20 × 20 mm blocks were fabricated of interporous hydroxyapatite ceramic $[Ca_{10}(PO_4)_6(OH)_2]$ (a); after vacuum, the HAbs were cut with a dental bar: dye solution penetrated to the center of the HAb (b).

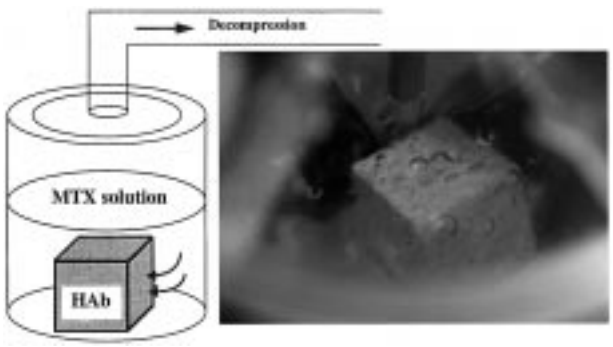

Figure 3 Blocks were immersed in the dye or MTX solution under varying levels of vacuum: 10 in.(254 mm)Hg, 5 in.(127 mm)Hg, 2.5 in.(63.5 mm)Hg, and 0 mmHg to 20 min with miniature vacuum/pressure pump. Air bubbles are seen from HAb by vacuum decompression.

TABLE I Release of MTX from the MTX/HAb (1 cm^3)

HAb/8 (aqueous MTX is adjusted to 6.25 mg/ml by 8 ml PBS and loaded)

	MTX concentration (μg/ml)		
Day	No. 1 (HAb1/8)	No. 2 (HAb2/8)	Mean
2	1241.14	1237.60	1293.50
4	92.50	95.62	94.06
6	19.20	20.40	19.80
8	2.02	2.22	2.12
10	0.92	0.70	0.81
12	0.32	0.30	0.31

HAb/16 (aqueous MTX is adjusted to 3.13 mg/ml by 16 ml PBS and loaded)

	MTX concentration (μg/ml)		
Day	No. 1 (HAb1/16)	No. 2 (HAb2/16)	Mean
2	874.80	835.44	855.12
4	89.54	86.42	87.98
6	9.88	9.76	9.82
8	1.64	1.54	1.59
10	0.72	0.60	0.66
12	0.22	0.24	0.23

HAb, porous hydroxyapatite block
Content of MTX indicate 2.38 mg/No. 1 (HAb1/8), 1.68 mg/No. 2 (HAb2/8), 1.26 mg/No. 1 (HAb1/16), and 1.22 mg/No. 2 (HAb2/16).

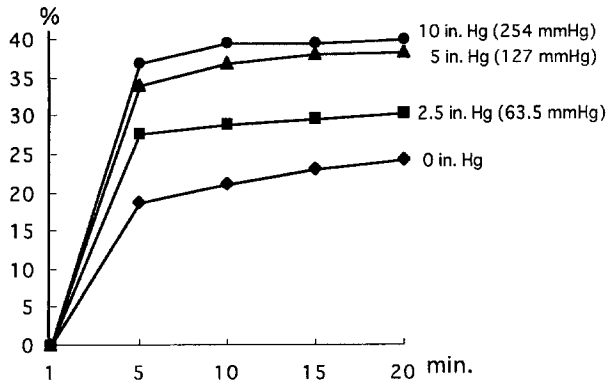

Figure 4 In the vacuum study, 20 × 20 × 20 mm cubic blocks were fabricated of HAb (amount of porosity: 50%). In the study of the penetration into HAb pores using a dye solution, the dye penetrated to the center of the HAb after 15 min at vacuum levels of 10 in.(254 mm)Hg and after 20 min at 5 in.(127 mm)Hg. The maximum loaded ratio of the dye solution was indicated to be about 40% per one-block volume.

Bajpai and Benghuzzi [9] and Uchida *et al*. [10] also reported on the slow release of anti-cancer drugs from PAC. However, for these methods to be applied, the blocks must be manufactured to enclose the drugs.

In 1982, Amino [11] reported on the tissue concentrations of methotrexate used in high-dose intravenous infusions of 100 mg/kg to treat osteosarcoma. Primary tumors were removed in five cases and metastatic tumors in two cases after 12 days of MTX administration. The concentrations of MTX in osteosarcoma tissues were higher than those in normal tissues and serum, ranging from 15.0 to 168 ng/g. Our experimental study on the slow release of MTX from HAb revealed that the concentration of MTX remained at a high level of 0.22–0.32 μg/ml even after 12 days and six exchanges of PBS. However, there were no statistical differences among the four experimental blocks; this result is explained by the fact that more than 90% of the MTX was released in the first two exchanges of PBS. Practical application of this method using MTX/HAb would be valuable in treating metastatic bone tumors or for minimizing the chance of recurrence of localized benign bone tumors such as giant cell tumor (GCT). In the treatment of GCT, the use of polymethylmethacrylate has become commonplace [12, 13]. This method, however, carries with it an approximately 20% chance of local recurrence [14]. Some surgeons recommend curettage and cryosurgery using liquid nitrogen as a way of reducing the rate of local recurrence [15, 16]. The method we developed, using HAb impregnated with a chemotherapeutic agent prepared by vacuum, may prove to be a valuable new form of localized chemotherapy, primarily when used as a strut graft to the bone defect or as an adjuvant to extended curettage. In these applications, it could provide a means to reduce the recurrence of the tumor while minimizing the systemic side effects.

References

1. M. ITOKAZU, T. MATSUNAGA, M. ISHII and W. YANG, *Arch. Orthop. Trauma. Surg.* **115** (1996) 115.
2. M. ITOKAZU, T. MATSUNAGA, S. KUMAZAWA and W. YANG, *J. Appl. Biomater.* **6** (1995) 167.
3. M. ITOKAZU, S. KUMAZAWA, E. WADA and W. YANG, *Cancer Lett.* **107** (1996) 11.
4. M. ITOKAZU, T. MATSUNAGA, S. KUMAZAWA and M. OKA, *Clinical Materials* **17** (1994) 173.
5. A. GOTOH, S. MAEDA, A. TAKENAKA, M. HORIO, K. GOHJI and S. KAMI DONO, *Nippon Hinyokika Gakkai Zasshi—Japanese J. of Urology* **81** (1990) 1337.
6. S. BHATTACHARYA, J. R. NOVELL, M. C. WINSLET and K. E. HOBBS, *Brit. J. of Surg.* **81** (1994) 1563.
7. J. S. LEE, T. TAKAHASHI, A. HAGIWARA, C. YONEYAMA, M. ITOH, T. SASABE, S. MURANISHI and S. TASHIMA, *Cancer Chemotherapy & Pharmacology* **36** (1995) 211.

8. F. GRECO, L. DE PALMA, N. SPECCHIA, S. JACOBELLI and C. GAGGINI, *Orthopedics* **15** (1992) 189.
9. P. K. BAJPAI and H. A. BENGHUZZI, *J. Biomed. Maters. Res.* **22** (1988) 1245.
10. A. UCHIDA, Y. SHINTO, N. AKAKI and K. ONO, *J. Orthop. Res.* **10** (1992) 440.
11. K. AMINO, *Jpn. J. Orthop. Assoc* **56** (1982) 765.
12. J. J. ECKARDT and T. S. GROGAN, *Clin. Orthop.* **202** (1986) 45.
13. B. M. PERSSON and H. W. WOUTERS, *Clin. Orthop.* **120** (1976) 125.
14. D. J. MCDONALD, F. H. SIM, R. A. MCLEOD and D. C. DAHLIN, *J. Bone Joint Surg.* **68** (1986) 235.
15. R. C. MARCOVE, L. D. WEIS, M. R. VAGHAIWALLA and R. PEARSON, *Clin. Orthop.* **134** (1978) 275.
16. R. C. MARCOVE, *Clin. Orthop.* **163** (1982) 231.

Received 24 April
and accepted 29 June 1998