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Notes

Collagen-poly (HEMA) hydrogels for the controlled delivery of methotrexate and cisplatin

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Abstract

Collagen-poly (HEMA) hydrogels containing methotrexate (HGM) and cisplatin (HGC) were prepared and characterised for their drug content. The in vitro release profiles of surface crosslinked HGM (S-HGM), and deeper crosslinked HGM (D-HGM) were studied in phosphate buffer at pH 6.8 and 7.4 and compared with those of uncrosslinked HGM (U-HGM), and uncrosslinked HGC (U-HGC). All types of hydrogels showed controlled and near zero order release of the two anticancer drugs. Crosslinked HGM released MTX at a slower rate than uncrosslinked HGM. Between the two types of crosslinking studied, surface crosslinked hydrogels showed a faster release rate than deeper crosslinked HGM.

Keywords: Collagen-poly (HEMA) hydrogels; Methotrexate; Cisplatin; Crosslinked deeper crosslinked and uncrosslinked HGM; Uncrosslinked HGC; In vitro release

1. Introduction

Among the host of biomedical applications of hydrogels, controlled delivery of anticancer drugs to mitigate their toxic side effects has been recognised as a vital area of research (Jeyanthi and Panduranga Rao, 1990; Domb et al., 1991; Derrissagnet and Piusienx, 1995). Studies on the development of hydrogels which combine favourable properties such as the mechanical strength of synthetic polymers and biological acceptability of natural polymers like collagen-poly (HEMA) composites have been reported (Stol, 1991; Grazia Cascone et al., 1995). In this investigation we sought to extend the application of collagen-poly (HEMA) hydrogels for the controlled delivery of two widely used anticancer drugs namely methotrexate (MTX) and cisplatin. In this paper, work on the preparation of colla-

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Table 1
Anticancer drug content of collagen-poly (HEMA) hydrogels

Type of hydrogel	Anticancer drug	Drug loading (mg/g) dry wt. hydrogel	% entrapment
HGM	Methotrexate	6.50	92.86
HGC	Cisplatin	3.00	60.00

gen-poly (HEMA) hydrogels containing MTX and cisplatin, their characterisation for anticancer drug content and in vitro evaluation of drug release profiles from uncrosslinked and crosslinked hydrogels is reported.

Soluble collagen was extracted from fetal calf skin purified and made non-antigenic by a procedure standardized in our laboratory which is a slight modification of the method reported by Weiss and Elstow (1983). The pepsin treated telopeptide poor collagen was lyophilized and stored at 20°C for further use. Preparation of all hydrogels was carried out in glass tubes. Hydrogels containing MTX (HGM) (MTX was a gift sample from Tamilnadu Dadha Pharmaceuticals Limited, India) and hydrogels containing cisplatin (HGC) were prepared by adding an aqueous solution of the anticancer drug (25 mg MTX/1 ml phosphate buffer, (PB), pH 7.4; 20 mg cisplatin/1 ml water) to 6 ml of 2% collagen solution in 1 mM HCl, followed by the addition of 2.4 ml of HEMA and mixing thoroughly (Collagen/HEMA w/v was 1:20). Ethylene glycol (3 ml) was added to the collagen solution followed by the addition of 0.3 ml of 6% (w/v) ammonium persulphate and 0.3 ml of 12% (w/v) sodium metabisulphite. The contents were thoroughly mixed and the polymerisation reaction was allowed to proceed for 3 h at 37°C. The smooth, cylindrical and opaque gels formed were washed exhaustively with PB, pH 7.4 in the case of HGM and distilled water in the case of HGC. The hydrogels were then subjected to repeated acetone extractions to remove the low molecular weight homopolymer not involved in the graft co-polymerisation reactions. The gels were finally washed quickly with water and stored at 4°C until further use.

The phosphate buffer and water washings of the hydrogels were filtered through 0.45-μm Millipore filters and assayed using a Shimadzo 2100-s UV spectrophotometer at 370 nm for MTX content and at 300 nm for cisplatin content. From the amount of anticancer drug added and the amount of drug present in the washings, the amount of entrapped drug was calculated. The amount of MTX and cisplatin incorporated in HGM and HGC respectively are given in Table 1. Percentage entrapment of 92.86% and drug loading of 6.5 mg/g dry weight hydrogel was obtained in the case of MTX in HGM. In the case of HGC the amount of cisplatin incorporated in the hydrogel was 3 mg/g dry weight hydrogel with 60% drug entrapment. When compared to cisplatin a higher amount of drug loading and percentage entrapment of methotrexate in collagen-poly (HEMA) hydrogels was achieved. The higher incorporation efficiency obtained in the case of MTX is due to higher solubility of MTX in phosphate buffer, pH 7.4, when compared to the solubility of cisplatin in water. It is proposed to increase the amount of cisplatin that can be entrapped in hydrogels by adopting more suitable and efficient methods of drug incorporation in our future studies.

The HGM were subjected to two types of crosslinking. One set of HGM were surface crosslinked by placing them in 25% glutaraldehyde for 1 min. Another set of HGM were deeper crosslinked by leaving them in 0.08% glutaraldehyde for 4 days. The surface crosslinked HGM (S-HGM) and deeper crosslinked HGM (D-HGM) as well as the uncrosslinked HGM (U-HGM) and HGC (U-HGC) were evaluated in vitro for their drug release characteristics. All the in vitro release experiments were carried out at 37°C, by placing known weights of hydrogels containing the anticancer drugs in known volumes of 0.01 M PB. The release studies for HGM, S-HGM and D-HGM were carried out in PB at pH 6.8 and 7.4, whereas the release profiles of

U-HGC were studied at pH 7.4. At stipulated time intervals, aliquots from the release media were pipetted out and filtered through 0.45-μm Millipore filter and assayed spectrophotometrically.

Fig. 1 shows the in vitro release profiles of MTX from U-HGM, S-HGM and D-HGM at pH 6.8. It was seen that about 98% of MTX was released from U-HGM in a controlled manner for 11 days, whereas only 58% and 55% of MTX were released from S-HGM and D-HGM respectively, in 11 days. Fig. 2 shows the in vitro release profiles of MTX from U-HGM, S-HGM, and D-HGM at pH 7.4. The U-HGM released about 98% of MTX in 11 days in a controlled manner. On the other hand S-HGM released about 50% of MTX and D-HGM released about 45% of MTX in the same time period (11 days). It is evident from the results of the in vitro release studies at pH 6.8 and pH 7.4 that the crosslinked hydrogels released MTX at a slower rate than uncrosslinked hydrogels. Between surface crosslinked and deeper crosslinked hydrogels, it can be seen that the rate of MTX release was faster from the former when compared to the latter type at pH 6.8 as well as pH 7 (about 58% and 50% from S-HGM and 55% and 45% from D-HGM in 11 days). It was also evident that although the release profiles of MTX from U-HGM, D-HGM and D-HGM at pH 6.8 and pH 7.4 were more or less similar, a higher amount of MTX was released in the initial 2 days from the hydrogels at pH 6.8 when compared to pH 7.4.

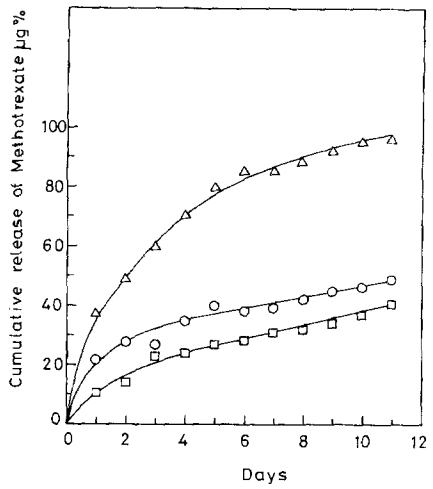

Fig. 2. In vitro release profiles of MTX from (△) U-HGM; (○) S-HGM and (□) D-HGM in PB, pH 7.4 at 37°C.

The in vitro release profile of cisplatin from U-HGC in PB, pH 7.4 is shown in Fig. 3. The release of cisplatin from U-HGC showed zero order release behaviour. U-HGC released about 98% of cisplatin within 7 days in a controlled manner. When compared to the rate of MTX

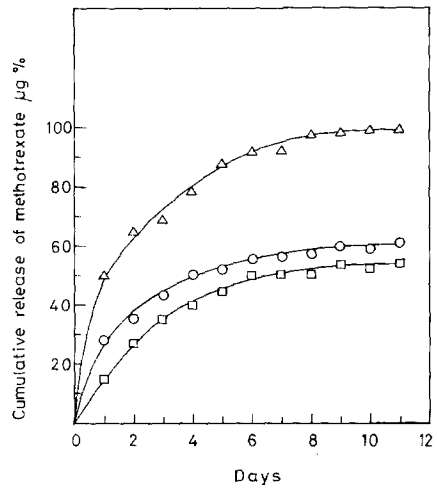

Fig. 1. In vitro release profiles of MTX from (△) U-HGM; (○) S-HGM and (□) D-HGM in PB, pH 6.8 at 37°C.

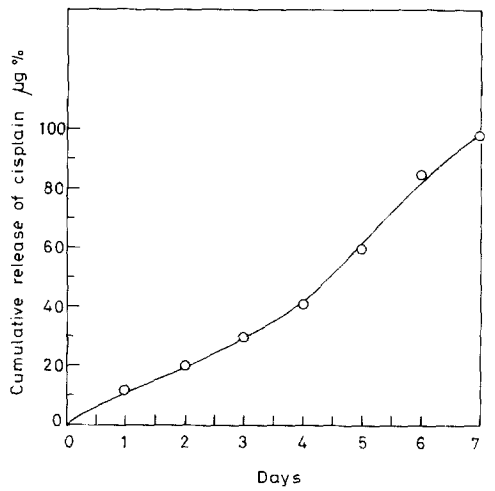

Fig. 3. In vitro release profiles of cisplatin from (○) U-HGM in PB, pH 7.4 at 37°C.

release from uncrosslinked hydrogels the rate of release of cisplatin from uncrosslinked hydrogels was faster. Uncrosslinked collagen-poly (HEMA) hydrogels released about 98% of MTX in 11 days, whereas the same amount of cisplatin was released within 7 days. The molecular weight of the entrapped drugs seems to influence their release rates from hydrogels. The molecular weights of the drugs entrapped were in the order of 454.46 for MTX and 300.1 for cisplatin. The rate of release followed a pattern in that the release rate of lower molecular weight cisplatin was higher than that for MTX.

Control of the release rates of anticancer drugs through the hydrogel network is of great importance, during the fabrication of the hydrogel as a drug delivery system. Various factors can affect the rate of drug release from the hydrogel matrices such as degree of crystallinity and crosslink density. A considerable decrease in the diffusion co-efficient is reported to have occurred when the amount of crosslinking agent was increased (Burczak et al., 1994). This report corroborated with the result obtained in the present investigation which demonstrated that crosslinked hydrogels released MTX at a slower rate than uncrosslinked hydrogels. Between the two types of crosslinking applied, it was evident that deeper crosslinking of hydrogels was more effective in retarding the drug release when compared to surface crosslinking of hydrogels. It can be concluded from this investigation that collagen-poly (HEMA) hydrogels show good potential as delivery systems for the controlled release of the low molecular weight anticancer drugs namely methotrexate and cisplatin. The rate of drug release can be further retarded by crosslinking the hydrogels. Uncrosslinked or crosslinked hydrogels can be used for drug delivery depending on the time period over which the release is desired. It is possible to modulate the release of anticancer drugs over a protracted period of time by applying different types of crosslinking to suit the requirements of cancer chemotherapy.

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