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Gelatin microsphere cocktails of different sizes for the controlled release of anticancer drugs

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Abstract

One of the factors which influences the rate of drug release is the particle size of microspheres. In this investigation three individual sets of gelatin microspheres containing 5-fluorouracil (I-GF) in the mean particle size range of 1–5 μm (I-G_aF), 5–10 μm (I-G_bF) and 10–15 μm (I-G_cF) and three individual sets of gelatin microspheres containing methotrexate (I-GM) in the mean particle size ranges of 20–25 μm (I-G_xM), 25–30 μm (I-G_yM) and 30–35 μm (I-G_zM) were prepared and characterised for drug contents. The in vitro release profiles of each individual set was determined in 0.01 M phosphate buffer, pH 7.4, and compared with that from cocktails consisting of a combination of two or three individual sets of microspheres containing 5-fluorouracil (5-Fu) namely, 1–5 + 5–10 μm (C-G_{a+b}F), 1–5 + 10–15 μm (C-G_{a+c}F), 5–10 + 10–15 μm (C-G_{b+c}F), 1–5 + 5–10 + 10–15 μm (C-G_{a+b+c}F) and cocktails of methotrexate containing microspheres namely, 20–25 + 25–30 μm (C-G_{x+y}M), 20–25 + 30–35 μm (C-G_{x+z}M), 25–30 + 30–35 μm (G_{y+z}M) and 20–25 + 25–30 + 30–35 μm (C-G_{x+y+z}M). The results showed that the controlled near zero order release profiles with more desirable features can be obtained by using cocktail of microspheres of various particle size ranges than an individual set consisting of microspheres in the same particle size range, the benefits of which can be applied in cancer chemotherapy.

Keywords: Gelatin microspheres; Cocktails; Methotrexate; 5-Fluorouracil; In vitro release

1. Introduction

Controlled drug release from delivery systems like microspheres, has been sought after with great vigour to combat the indiscriminate toxicity of anticancer drugs towards malignant as well as healthy cells for the last few years (Chek et al., 1994; Cremers et al., 1994; Balthasar and Fung, 1995). In our earlier papers we had reported that the rate of drug release decreased with the in-

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crease in the particle size of microspheres which corroborated with reports by other investigators (Narayani and Panduranga Rao, 1994; Narayani and Panduranga Rao, 1995). Therefore, drug release profiles of a cocktail containing microspheres of various size ranges will be different from any one of the individual set (forming the cocktail) containing microspheres of same size range.

The present work was geared towards the study of controlled release of anticancer drugs 5-Fu and methotrexate (MTX) from cocktails of two or more sets of microspheres where the particle size range of each individual set of microspheres was different from the other sets. The results obtained were compared with the drug release profiles of the same anticancer drug from each individual set which formed the cocktail and consisted of microspheres in same size range.

Gelatin microspheres were prepared by a novel polymer dispersion technique as reported earlier (Jeyanthi and Panduranga Rao, 1987). Gelatin microspheres containing 5-Fu (GF) and gelatin microspheres containing MTX (GM) were prepared in the same manner except that the drug solution (5-Fu in water/MTX in PB, pH 7.4) was added to gelatin solution before dispersing in polymethylmethacrylate (PMMA). During the addition of PMMA and the crosslinking agent, the drug containing gelatin solution was stirred using a vortex mixer (Remi, India) By varying the speed of mixing and concentration of PMMA and gelatin during preparation microspheres of various particle sizes were obtained.

Anticancer drug loaded gelatin microspheres (10 mg) were hydrolysed in 6 N HCl at 60°C for 20 min. The solution was filtered using a 0.45 μm Millipore filter and aliquots from the clear solution were read spectrophotometrically to determine the amount of entrapped drug in microspheres. The percentage entrapment and drug loading of the two anticancer drugs in gelatin microspheres of different sizes were calculated. Percentage entrapments of about 69 to 72% of 5-Fu and about 76 to 90% of MTX in microspheres of three different sizes were achieved.

The rate and duration of drug release is dependent on the surface area of particulate carriers. Hence particle size distribution of microspheres is a vital factor in the characterisation of microspheres. The particle sizes of GF and GM of various size ranges were determined using an optical microscope (Hertal Reuss, Germany) with the help of a micrometer scale fitted to it. The particle size distribution of gelatin microspheres containing the anticancer drugs namely 5-Fu and MTX ranged from 1 to 30 μm. The mean particle sizes for the various preparations of 5-Fu entrapped microspheres ranged from 1–5 μm, 5–10 μm and 10–15 μm. In the case of MTX entrapped microspheres the mean particle sizes for the three microsphere preparations varied between 20–25 μm, 25–30 μm and 30–35 μm.

The in vitro drug release profiles of individual sets containing microspheres of the same size as well as cocktails consisting of microspheres of different sizes were determined by carrying out the drug release studies in 0.01 M phosphate buffer (PB), pH 7.4, at 37°C. First, the drug release characteristics of I-GF and I-GM of three different mean particle size ranges (1–5 μm, 5–10 μm and 10–15 μm; 20–25 μm, 25–30 μm, and 30–35 μm respectively) were studied. Next drug release from cocktails containing GF of two different sizes and three different sizes, i.e. 1–5 + 5–10 μm, 1–5 + 10–15 μm, 5–10 + 10–15 μm and 1–5 + 5–10 + 10–15 μm, respectively, were determined. Similarly, the release profiles of cocktails containing GM of two different sizes and three different sizes, i.e. 20–25 + 25–30 μm, 20–25 + 30–35 μm, 25–30 + 30–35 μm and 20–25 + 25–30 + 30–35 μm were also established. In all release experiments a known weight of microspheres was placed in known volume of the release medium which was sampled at stipulated time intervals and assayed for the anticancer drug content spectrophotometrically.

From Fig. 1 it can be seen that 5-Fu was released in a near zero order controlled manner from the three sets of I-GF of different mean particle size ranges. I-GF of mean particle size

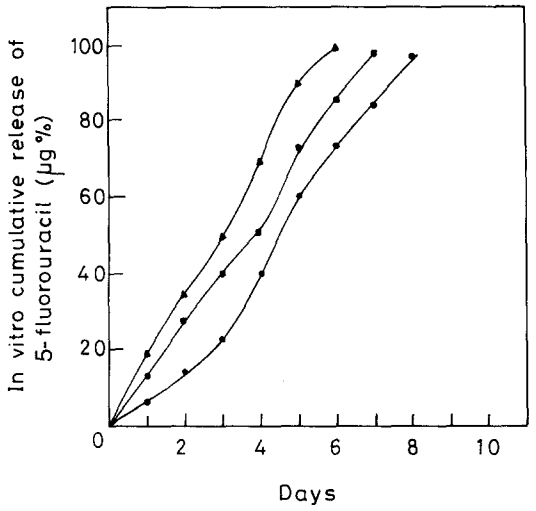

Fig. 1. In vitro release profiles of 5-Fu in 0.01 M PB, pH 7.4, from individual sets of gelatin microspheres of mean particle size ranges (▲) 1–5 μm, (■) 5–10 μm and (●) 10–15 μm.

ranges, i.e. 1–5 μm, 5–10 μm and 10–15 μm released about 97–99% of 5-Fu in 6, 7 and 8 days, respectively; whereas, in the case of studies using cocktails (C-GF) of various size ranges, i.e. C-G_{a+b}F, C-G_{a+c}F and C-G_{b+c}F, it can be seen that they released about 97–99% of 5-Fu in about

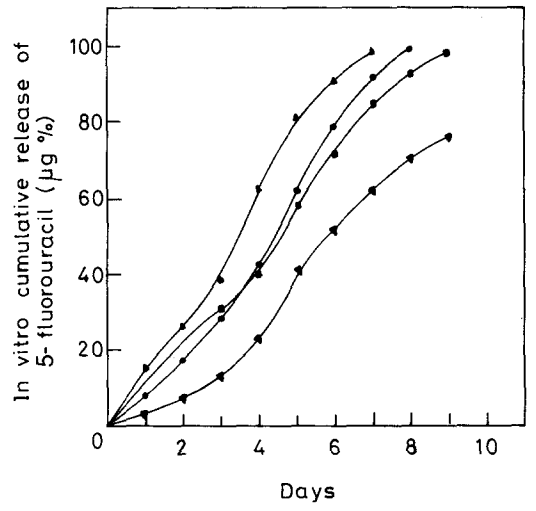

Fig. 2. In vitro release profiles of 5-Fu in 0.01 M PB, pH 7.4, from cocktails of gelatin microspheres of mean particle size ranges (▲) 1–5 and 5–10 μm, (■) 1–5 and 10–15 μm, (●) 5–10 and 10–15 μm, (▼) 1–5, 5–10 and 10–15 μm.

7, 8 and 9 days respectively, as shown in Fig. 2. The drug release from cocktails of microspheres was slower and more prolonged when compared to drug release profiles from any one of the individual sets which formed the cocktails. Fig. 1 shows that in the case of individual sets I-G_aF, I-G_bF and I-G_cF the release profiles of 5-Fu ascend steeply from day 4 onwards which correspond to a period of rapid drug release from microspheres, whereas the drug release profiles from combination cocktails, C-G_{a+b}F, C-G_{a+c}F and C-G_{b+c}F are relatively uniform throughout the experimental period. For instance, a steep increase was seen in the release curve of I-G_aF of mean particle size range 1–5 μm between days 4 and 5 (Fig. 1). But this was modified by mixing I-G_aF (1–5 μm) with I-G_bF (5–10 μm) as seen in the release profile of C-G_{a+b}F (Fig. 2). The cocktail C-G_{a+c}F formed by mixing microspheres in size ranges 1–5 and 10–15 μm showed a biphasic release profile in which the initial fast phase up to 3 days is attributed to drug release at a fast rate by smaller microspheres in the 1–5 μm size range which was augumentend by simultaneous drug release from microspheres of 10–15 size range. Moreover the multiple combination cocktail C-G_{a+b+c}F consisting of microspheres in three different size ranges provided a controlled release profile with a slower rate of drug release than any other cocktail consisting of two sets of microspheres, i.e. only 75% of the drug was released after 8 days (Fig. 2).

Fig. 3 shows the in vitro release profiles of MTX from individual sets of MTX containing microspheres which lie in the same particle size range, namely I-G_xM, I-G_yM and I-G_zM. All sets released MTX in a controlled fashion following near zero order kinetics. About 96–99% of MTX was released from I-G_xM, I-G_yM and I-G_zM for 9–10 days, whereas the cocktails C-G_{a+b}M, C-G_{a+c}M and C-G_{b+c}M released about 98% of MTX for 9.5, 10 and 10.5 days respectively, as seen in Fig. 4. The cocktail C-G_{x+y+z}M containing microspheres in three different size ranges showed the slowest and most prolonged release of about 80% of MTX up to 11 days. The drug release profiles obtained from cocktails consisting of 2 or 3 sets of microspheres of various particle

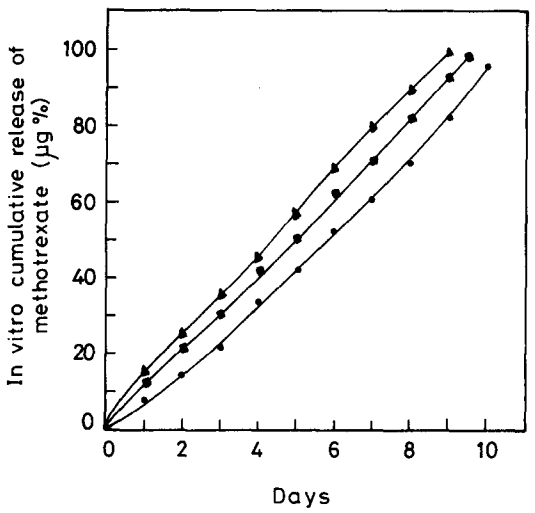

Fig. 3. In vitro release profiles of MTX in 0.01 M PB, pH 7.4, from individual sets of gelatin microspheres of mean particle size ranges (▲) 20–25 μm, (■) 25–30 μm and (●) 30–35μm.

size ranges were different from the release profile of each individual set which formed the cocktail. In the case of both 5-Fu containing microspheres as well as MTX containing microspheres, the multiple combination cocktail consisting of three sets of microspheres of different particle size ranges released drug at a slower rate when compared to cocktails consisting of two sets of microspheres in two different size ranges. This investigation also showed that, by mixing microspheres of smaller size with larger size microspheres, a release profile with intermediate drug release rate that was slower than the former but faster than that of the latter can be obtained. There are also possibilities that discrepancies such as the terminal 'lag phase' or initial 'burst phase' of drug release arising in the release curves can be modified by mixing microspheres of appropriate size ranges in the appropriate ratio.

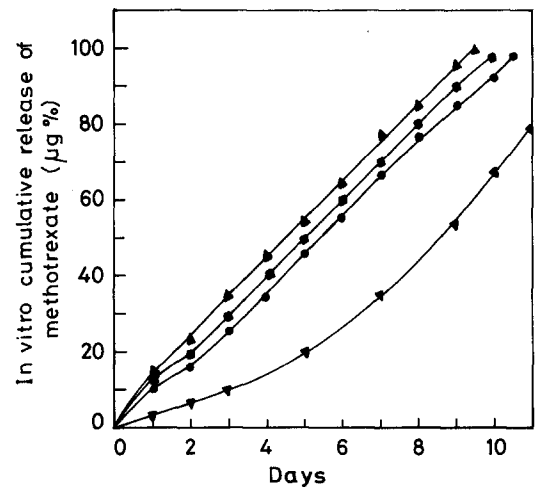

Fig. 4. In vitro release profiles of MTX in 0.01 M PB, pH 7.4, from cocktails of gelatin microspheres of mean particle size ranges (▲) 20–25 and 25–30 μm, (■) 20–25 and 30–35 μm, (●) 25–30 and 30–35 μm, (▼) 20–25, 25–30 and 30–35 μm.

It can be concluded that the results of the present investigations indicated possibilities for obtaining better drug release profiles which are devoid of undesirable features by applying multiple combination cocktails of gelatin microspheres containing 5-Fu/MTX of different size ranges. However, further elaborate investigations involving polymeric microspheres of various sizes entrapping different drugs will throw more light on this subject. Since particle size has a bearing on the release rates of microspheres, controlled delivery of drugs can be modulated by appropriate mixing of microspheres of different sizes to suit the requirements of cancer chemotherapy.

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