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Solid tumour chemotherapy using implantable collagen-poly(HEMA) hydrogel containing 5-fluorouracil

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Abstract—Implantable collagen-poly(HEMA) hydrogels containing the anticancer drug 5-fluorouracil (5-FU) have been prepared and evaluated for their efficacy towards a solid tumour fibrosarcoma in Wistar rats. The tumour was developed by inoculation of a 10% tumour-cell suspension in the anterior aspect of the hind limb. Four groups were studied—untreated control, intratumoural injection of free 5-FU, subcutaneous implantation of placebo and implantation of 5-FU-bearing hydrogel pellets (10 mm diameter × 1 mm thick) containing the drug in close proximity to the tumour. The hydrogel showed an improved antitumour activity over free 5-FU as evidenced by the gross tumour weight assessments and by [^{3}H]thymidine incorporation in-vitro. This was attributed to the controlled and slow release of 5-FU compared with free 5-FU over the same period of treatment. The implantation of hydrogel could thus be a potential alternative to free 5-FU therapy in the treatment of solid tumours such as fibrosarcoma.

The controlled release of drugs for their durable and safe use has been much studied (Fujimoto et al 1985; Miyazaki et al 1986; Davis & Illum 1988; Leucuta et al 1988), and we have carried out systematic investigations for the development of novel controlled release systems for cancer chemotherapy (Jeyanthi & Panduranga Rao 1987, 1988, 1989). These include gelatin microspheres as injectable delivery systems and collagen-poly(HEMA) hydrogels as implantable delivery systems. Entrapping the drug in polymeric hydrogels is an effective means of controlled release because the hydrogels have excellent shaping and moulding properties as well as good biocompatibility. Further, drug release from the hydrogels can be easily regulated by controlling water-swelling and crosslink density.

The collagen-poly(HEMA) hydrogels developed by us were found to be highly biocompatible and non-toxic (Jeyanthi & Panduranga Rao 1990b). In this paper, the antitumour activity of collagen-poly(HEMA) hydrogels containing 5-fluorouracil (5-FU) for the chemotherapy of a solid tumour fibrosarcoma in rats is discussed.

Materials and methods

Collagen. Calf-foetus (200 days) was obtained from a local slaughter-house and preserved in ice until extraction of soluble collagen.

Chemicals. Hydroxyethyl methacrylate (HEMA) (Fluka), ammonium persulphate and sodium metabisulphite (Loba, India) were used as received. [^{3}H]Thymidine (methyl-T) (High specific activity) was obtained from the Isotope Group, Bhabha Atomic Research Centre, Bombay, India. Scintillation grade naphthalene, dioxane, 2,5-diphenyl oxazole (PPO), 1,4-bis-(5-phenyloxazol-2-yl) benzene (POPOP), methanol and ethylene glycol were obtained from Sisco Research Laboratory, India. 5-Fluorouracil was also obtained from Sisco Research Laboratory, India. All other chemicals were of analytical reagent grade.

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Animals. Female, albino Wistar rats, 150–160 g, were maintained on a standard Hindustan Lever Rat Feed pellet diet with free access to water.

Tumour cell line. Methylcholanthrene-induced rat fibrosarcoma cell-line was obtained from the Cancer Institute, Madras. The solid tumour could be propagated by inoculating the cells subcutaneously after two weeks.

Preparation of collagen-poly(HEMA) hydrogels containing 5-FU. Collagen-poly(HEMA) hydrogels containing 5-FU were prepared by polymerizing monomeric HEMA in the presence of collagen using an ammonium persulphate-sodium metabisulphite redox initiation technique (Jeyanthi & Panduranga Rao 1990a). Briefly, soluble collagen was extracted from foetal calf skin. To a 2% collagen solution in 1 mM HCl was added an aqueous solution of 5-FU with thorough mixing. To this solution was added HEMA, followed by ethylene glycol, ammonium persulphate (6% w/v) and sodium metabisulphite (12% w/v). The contents were mixed and the polymerization reaction was allowed to proceed at 37°C for 3 h. The resulting smooth, cylindrical, opaque hydrogels were then exhaustively washed with Tris-HCl buffer, pH 7·0 to remove residual monomer and ethylene glycol. The homopolymer was removed by repeated washing with acetone and the gels were finally washed with distilled water and stored at 4°C.

In-vitro release studies.
The in-vitro release of the drug from the hydrogel matrices was measured after a single hydrogel pellet had been placed in a flask containing 25 mL 0·01 M phosphate buffer, pH 7·4, at 37°C in a shaking water bath. At intervals 0·5 mL samples were filtered through a 0·45 μm Millipore filter and assayed spectrophotometrically λ max for 5-FU: 266 nm. After each sampling, an equal volume of fresh buffer was added to the release medium. The cumulative release profile was then plotted as the mean of five experiments.

Induction of fibrosarcoma and evaluation of antitumour activity. Before treatment, rats were inoculated subcutaneously with 0·5 mL of a 10% tumour cell-suspension in sterile 0·9% NaCl (saline) in the anterior aspect of the hind limb. Treatment commenced after 8 days of inoculation when the tumour was palpable. In all four groups studied, animals were assessed for weight change and tumour size. The treatment protocol was as follows: (i) untreated control by intratumoural injection of saline on days 8, 10, 12; (ii) placebo hydrogel placed in proximity to the tumour on day 8; (iii) free drug given 15 mg intratumourally on days 8, 10 and 12; (iv) hydrogel with drug (45 mg), one pellet placed in proximity to the tumour on day 8 (n = 4 for each group).

Tumour size measurements. Tumour measurements were made using vernier calipers and two diameters at right angles to each other were recorded every day from the time of treatment (8th day) up to 15 days of tumour growth. Tumour weights (w) (g) were calculated using the formula (Geran et al 1972)

$$w = a \times b^2/2$$

where 'a' is the longest diameter (in cm) and 'b' is the shortest diameter (in cm).

Viability studies. Fifteen days after being inoculated, animals were killed and the tumours were excised and weighed. Treated tumours were tested (i) for their ability to grow after transplantation in-vivo and (ii) for their inhibition of DNA synthesis which was assessed by their uptake of [^{3}H]thymidine in-vitro.

(i) Transplantation studies. A piece of tumour was processed to give a 10% tumour cell-suspension in saline and 0·5 mL of this was transplanted subcutaneously into the respective syngeneic strains of rats. Tumour size was measured on days 8, 11 and 15 post-transplantation and weights were calculated as described above.

(ii) Study of the inhibition of [^{3}H]thymidine incorporation in-vitro. Another piece of tumour was washed thoroughly with cold phosphate-buffered saline (PBS) and processed to give a 10% single-cell suspension in PBS containing 5% rat serum. Two hundred μL of this suspension was incubated in triplicate with 2 μCi of [^{3}H]thymidine at 37°C for 3 h. The uptake of [^{3}H]thymidine was arrested after 3 h by the addition of cold PBS. The suspension was then vacuum-filtered through an 0·45 μm Millipore filter and proteins precipitated with cold 5% trichloroacetic acid. The filter circles were dried at 60°C, placed in 10 mL scintillation fluid and counted. Zero-time samples were taken as a measure of non-specific adsorption.

Results and discussion

Composites of collagen and poly(HEMA) have been prepared to combine mechanical stability and biological acceptability. The hydrogels prepared using 5% collagen content by the redox initiation technique were stable and resilient. Hydrogels containing 10% or more collagen were fluid though gelation occurred. (Jeyanthi & Panduranga 1990a). Hence, hydrogels containing 5% collagen only were evaluated for their efficacy in in-vitro drug release and in-vivo antitumour activity.

Fig. 1 shows the cumulative release profile of 5-FU from the hydrogels. The release followed a zero-order pattern behaviour lasting for about 10 days. The daily of release was 9–10% ($\sim$270 μg day^{-1}) of entrapped drug. The initial 'burst effect' observed with drug release from some copolymeric hydrogels with high HEMA content was not observed in this case (Korsmeyer & Peppas 1984).

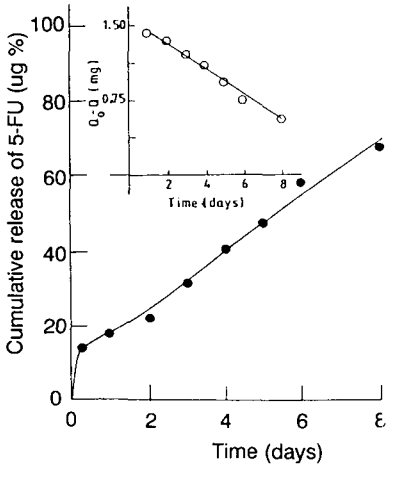

FIG. 1. In-vitro release of 5-fluorouracil from collagen-poly(HEMA) hydrogel matrix. Inset is a plot of the residual values versus time. Q_0 is the original amount of drug present in the hydrogel at zero time. Q is the amount of drug released at time, t. Q_0-Q is the residual amount of drug present in the hydrogel at time, t.

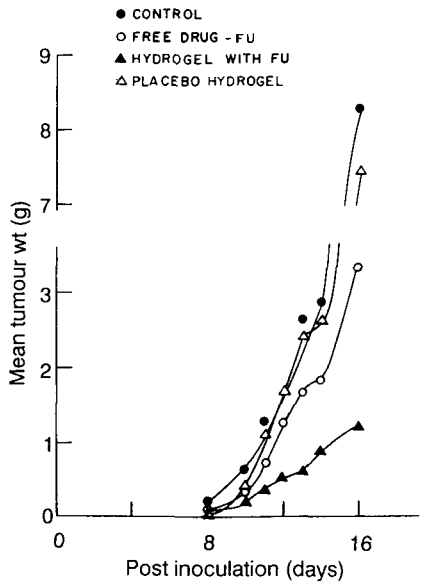

FIG. 2. Tumour growth curve of rat fibrosarcoma after treatment with 5-FU bearing collagen-poly(HEMA) hydrogel (between 8 and 15 days post-inoculation) (1°).

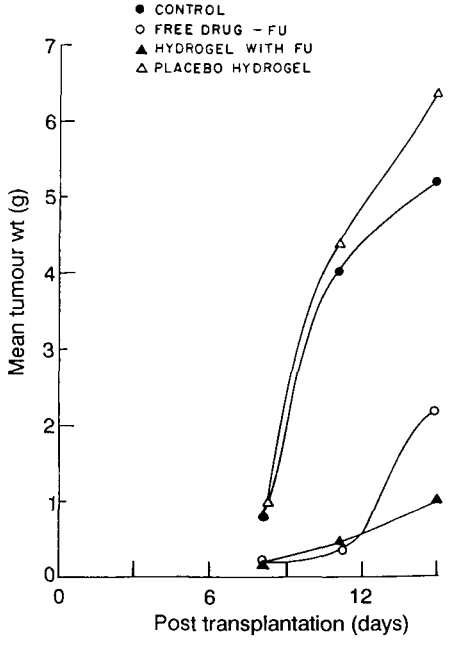

FIG. 3. Tumour growth curve of transplanted rat fibrosarcoma (treated tumours of 1°-excised and transplanted into recipient rats) between 8 and 15 days post-transplantation.

The biocompatibility of collagen-poly(HEMA) hydrogel copolymer has been established by Jeyanthi & Panduranga Rao (1990b). The antitumour activity of hydrogels containing 5-FU, a drug known to be effective for local chemotherapy, was evaluated against a solid fibrosarcoma in rats by subcutaneous implantation of the hydrogel pellet in close proximity to the tumour.

Table 1. Effect of 5-FU containing collagen-poly (HEMA) hydrogel on tumour growth and viability of rat fibrosarcoma.

Tumour growth post-inoculation (days)	Mean tumour weight (g)			
	Untreated control	Placebo hydrogel	Free 5-FU	5-FU containing hydrogel
8	0·20±0·01	0·10±0·01	0·14±0·02	0·12±0·02
10	0·62±0·06	0·38±0·08	0·33±0·02	0·23±0·02
11	1·24±0·02	1·07±0·09	0·73±0·06	0·38±0·08
12	1·32±0·01	1·65±0·15	1·26±0·12	0·51±0·06
13	2·64±0·09	2·42±0·54	1·67±0·14	0·55±0·03
14	2·84±0·14	2·60±0·09	1·81±0·14	0·88±0·08
15	8·53±0·13	7·45±0·06	3·38±0·01	1·20±0·20
[^{3}H]Thymidine incorporation (counts min^{-1} g^{-1})	2912·5	4082·5	2732·5	1967·5
Tumour growth post-transplantation (days)	Mean tumour weight of transplanted tumour in-vivo (g)			
	Untreated control	Placebo hydrogel	Free 5-FU	5-FU containing hydrogel
8	0·70±0·05	0·97±0·02	0·41±0·05	0·38±0·09
11	4·05±0·02	4·76±0·16	0·65±0·11	0·95±0·09
15	5·43±0·30	6·83±0·04	2·43±0·19	1·06±0·10

The curve showing mean tumour weights of the different groups studied between 8 and 15 days post-inoculation, is shown in Fig. 2. Though initially all rats showed a similar rate of tumour growth, significant differences were observed from the 11th day post-inoculation. Between 11 and 15 days, the untreated control and placebo tumours showed a rapid growth to about 8 g while the 5-FU hydrogel and free 5-FU treated rats on the 15th day, had mean tumour weights of 1·2±0·48 and 3·4±0·1 g, respectively.

Tumours from 5-FU-treated rats were tested for their ability to grow in-vivo by transplantation into their respective syngeneic strains of rats (recipient rats) and by the inhibition of [^{3}H]thymidine incorporation in-vitro. The results (Fig. 3) corroborated the earlier data.

Table 1 shows the mean tumour weights of the rats between 8 and 15 days post-inoculation (1°) and post-transplantation (2°).

The reduced viability of the 5-FU hydrogel-treated tumour cells was further confirmed by the data obtained from the in-vitro uptake of [^{3}H]thymidine (Table 1). The inhibition of DNA synthesis by the 5-FU-hydrogel treated tumour cells was much higher than the free 5-FU treated cells as evidenced by the lower uptake of [^{3}H]thymidine by 5-FU-hydrogel treated cells. The placebo and the untreated controls behaved similarly and showed a high uptake of labelled thymidine indicating a higher rate of DNA synthesis.

Analysis of variance (ANOVA) was applied to the weight data and the treatments were found to be significantly different ($P<0·01$). From the means of the tumour weights on the 15th day of each treatment (Table 1), it is evident that the 5-FU hydrogel treatment [mean tumour weights 1·20 g (1°) and 1·06 g (2°)] is superior to free 5-FU therapy [mean tumour weights 3·39 g (1°) and 2·43 g (2°)] by virtue of its ability to inhibit tumour growth more effectively, not only during treatment but also in the secondary spread of the tumour.

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