In-vivo Drug Delivery of 5-Fluorouracil using Poly(2-hydroxyethyl methacrylate-co-acrylamide) Hydrogels

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Abstract

Poly(2-hydroxyethyl methacrylate-co-acrylamide) hydrogels crosslinked with ethylen glycol dimethacrylate were used as devices for the in-vivo drug release of 5-fluorouracil (5-FU).

Drug-loaded hydrogels were subcutaneously implanted in the back of Wistar rats. All hydrogel discs reached an equilibrium swelling degree, which was slightly larger than that determined in-vitro. After 30 days of implantation, the hydrogel discs were transparent, and without fracture or apparent degradation. In addition, a fibrous capsule was not detected around the hydrogels that had greater hydration degrees. Release of 5-FU from these hydrogels allows the drug to remain in the plasma from 1 to 5 days, in spite of its short plasma half-life (15 min). This was an improvement of up to 98-times compared with the intraperitoneal drug administration.

Administration of 5-FU by implantation of 2-hydroxyethylmethacrylate-co-acrylamide copolymeric hydrogels seems to be a good candidate for 5-FU therapy, since the drug released results in a therapeutically suitable plasma concentration of 5-FU for an extended period of time, despite the short half-life of the drug.

The design of suitable polymeric matrices for the controlled release of a drug must include not only the chemical and physical factors involved in their synthesis, but also the effects of the biological environment on the diffusion behaviour of drugs from such polymeric devices. Often research data obtained in in-vitro experiments are not absolutely recreated in-vivo (Silver & Doillon 1989).

5-Fluorouracil (5-FU), a fluoropyrimidine, has been extensively used in the chemotherapy of many solid tumours, including breast and colorectal cancers. 5-FU is a fluorinated analogue of the pyrimidine uracil. It is converted by multiple alternative biochemical pathways to several cytotoxic forms, which interfere with DNA and, to a lesser extent, with RNA synthesis. As its oral absorption is incomplete and unpredictable, 5-FU is administered parenterally, however, it has a short biological half-life due to rapid metabolism. 5-FU causes toxic side-effects due to non-specific distribution of drug in tumour tissues as well as in normal tissues. Clinical side-effects include

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stomatitis, esophagopharyngitis, diarrhoea, dermatological changes, alopecia, haematopoietic depression, fever and death (Kennedy 1999).

An effective therapeutic dose of 5-FU produces high drug plasma levels by usual administration routes (Heggie et al 1987). Thus the inclusion of 5-FU in a polymeric matrix that would allow its controlled release giving suitably low plasma levels of the drug, together with the possibility of locating the drug-loaded device close to a solid tumour, would decrease side-effects and improve antineoplastic action. Poly(2-hydroxyethyl methacrylate-co-acrylamide) hydrogels loaded with 5-FU, showing different diffusion characteristics in in-vitro experiments (García et al 2000), were subcutaneously implanted in the back of Wistar rats to observe the effects of monomer composition of the copolymers and the degree of crosslinking on the release of 5-FU in in-vivo systems.

Materials and Methods

2-Hydroxyethyl methacrylate (HEMA) (Merck, Darmstadt, Germany) was purified by vacuum

distillation at 315–318 K and 3·7 mmHg (vacuum pump: Eduar 8). Acrylamide (A) (Merck), ethylen glycol dimethacrylate (EGDMA) (Merck), ammonium peroxodisulphate $((NH_4)_2S_2O_8)$ (Merck), dimethyldichlorosilane solution (BDH, Poole, UK), sodium chloride (Merck), and sodium hydroxide (Probus, Barcelona, Spain), were used as received. 5-FU $(C_4H_3N_2O_2F$; molecular mass 130·1 Da) was kindly supplied by Roche Laboratories as a crystalline powder.

Synthesis of poly(2-hydroxyethyl methacrylate-co-acrylamide)

The synthesis of copolymeric hydrogels of poly(2-hydroxyethyl methacrylate-co-acrylamide) (HEMA/A) was carried out in different monomeric proportions (wt/wt): 90HEMA/10A, 75HEMA/25A and 50HEMA/50A, and in turn each was crosslinked with different proportions of EGDMA, 5% for 90HEMA/10A and 50HEMA/50A gels and 5, 10 and 13% for 75HEMA/25A gels. The amount of crosslinker is expressed as a percentage of the total weight of the two comonomers present in the gel. The synthesis was conducted by bulk polymerization in small glass vials previously siliconized using ammonium peroxodisulphate $(0·05\,g\,mL^{-1})$ as an initiator of the reaction. A volume of 0·65 mL of the feed mixture, formed by the monomers and crosslinker (60 wt%) and aqueous initiator solution (40 wt%), was poured into small glass vials. After de-aeration by gaseous nitrogen for 5 min, the vials were sealed. Polymerization was carried out in an oven at 323 K for 3 h. The vials were then broken and the polymer discs removed and dried for one week to a constant weight. 5-FU was trapped in the gels by inclusion in the 40 wt% aqueous solution of the polymerization mixture. To incorporate 5-FU into the polymerization feed mixture, water solutions of 5-FU were used and neutralized with an equivalent amount of sodium hydroxide, to increase the solubility of the drug, since the sodium salt of the 5-FU is a pharmacologically active compound. In-vitro release studies of 5-FU from hydrogels showed that the amount of drug released was the same of that included in the polymerization feed mixture (García et al 2000). Thus, there are no strong interactions between 5-FU and the polymer matrices that hold the drug in the polymer network. Discs with 12·5 mg 5-FU per disc (2·7 wt% of the total formulation) were thus obtained. Discs were stored in an anhydrous atmosphere in darkness until use to prevent possible deterioration of the drug (García et al 2000).

In-vivo 5-FU administration

Male Wistar rats, 250 ± 20 g, were obtained from the Animal Department of the Universidad Complutense of Madrid which operates according to the requirements relating to animal experimentation regulations (DC 86/609/CEE; RD 223/1988; OM 13/X/1989). The rats were kept on a 12-h light–dark schedule, fed standard rat food and had free access to water.

In-vivo 5-FU release from five hydrogels was studied: 50HEMA/50A–5% EGDMA, 90HEMA/10A–5% EGDMA, 75HEMA/25A–5% EGDMA, 75HEMA/25A–10% EGDMA and 75HEMA/25A–13% EGDMA. For each hydrogel 15 rats, divided in three groups, were used. In the first group, rats were implanted with two hydrogel discs without 5-FU (control group). The rats were anaesthetized with diethyl ether and a single incision (1–2 cm) was made on their backs. Blunt-scissor dissection was used to create a lateral implant site immediately beneath the skin. The implants were inserted a distance from the incision which was then sutured.

In the second group, two hydrogel discs containing 5-FU were implanted subcutaneously in the backs of the rats. Each disc contained 12·5 mg 5-FU so the total amount of the drug was 25 mg, representing a dose of 100 mg kg^{-1}.

The third group of rats received daily intraperitoneal injections of a 5-FU solution neutralized with sodium hydroxide, prepared freshly each day. The total amount of drug administered was 25 mg (total dose 100 mg kg^{-1}), and the doses were distributed evenly over the total number of days of 5-FU release from the implanted gels. The intraperitoneal cavity presents a large surface for drug absorption and is a usual route of administration in the laboratory, although it is not a routine procedure in man (Benet & Sheiner 1986).

Plasma 5-FU determination

At predetermined times after drug administration, the rats were anaesthetized with diethyl ether. Blood (1 mL) was collected by puncture of the jugular vein in heparinized polypropylene tubes (75 units = 15 μL) (Leo Laboratories, Madrid, Spain) in an ice bath. The blood was centrifuged at 10 000 g for 5 min, in a Sigma 202M centrifuge, immediately after collection, and the plasma was stored at −20°C.

Plasma samples (500 μL) were incubated with 25 μL 2 M trichloroacetic acid and centrifuged to obtain protein precipitation. 5-FU was not modified by this treatment. The 5-FU plasma concentration was determined by HPLC (Spectra-Physics SP

8800 HPLC pump; SP 100 UV absorbance detector; and SP 4400 computing integrator). The stationary phase was Spherisorb ODS, $5\,\mu m$ (Teknokroma) (200×4.6 mm i.d.). The eluent was $1\,\text{M}\,\text{KH}_2\text{PO}_4/75\,\text{M}\,\text{Na}_2\text{HPO}_4$ buffer solution at pH 7.0 (Ouchi et al 1990). The flow rate was $1.0\,\text{mL}\,\text{min}^{-1}$ and the detection wavelength was 270 nm. 5-FU standards of $0.1-100\,\mu\text{g}\,\text{mL}^{-1}$ in saline solution (NaCl 0.9 wt%) were measured to construct a calibration curve (r = 0.999). Standards of plasma with known amounts of 5-FU were also chromatographed to confirm the retention time of the drug. The 5-FU retention time was 4.8 ± 0.1 min. The limit of 5-FU detection in plasma samples was $0.2\,\mu\text{g}\,\text{mL}^{-1}$.

Blood samples were taken from rats implanted with gels at 30 min and 4, 8 and 24 h after the implant and then at 24-h intervals thereafter. For 5-FU administered intraperitoneally, three samples were taken daily, 15, 30 and 120 min after drug administration.

The pharmacokinetic parameters were calculated using a non-compartment model (Doménech & Moreno 1997). The elimination constant (K) and the corresponding half-life ($t_{\frac{1}{2}} = \ln 2/K$) were derived from the terminal slope of semi logarithmic plots of plasma concentration against time. The area under the curve (AUC) was determined by the trapezoidal method. The total body clearance (CL) was derived from dose/AUC and the volume of distribution (Vd) from CL/K.

When 5-FU was not detected in plasma some of the rats were killed with diethyl ether and the implanted gel discs were removed. The discs were weighed to determine the degree of swelling equilibrium. These discs were placed in 100 mL saline solution, and one week later a sample was chromatographed to determine the total in-vivo 5-FU release from the hydrogel.

Hydrogel discs were left implanted for longer periods of time in some rats in the implanted groups, to observe, macroscopically, the effect on the hydrogels. These rats were killed at intervals of 15 and 30 days.

Results and Discussion

A dose of $100\,\text{mg}\,\text{kg}^{-1}$ 5-FU was administered via different hydrogel preparations as well as via intraperitoneal injection. Thus, the effect of the route of administration on 5-FU plasma concentration could be determined by comparing drug administration via subcutaneously implanted hydrogels with that via intraperitoneal injection. Different doses of 5-FU have been used in rats:

doses of $0.5-80\,\text{mg}\,\text{kg}^{-1}\,\text{h}^{-1}$ for 6 h administered by perfusion (Collins 1985), and intraperitoneal injections of $20\,\text{mg}\,\text{kg}^{-1}$ daily for 7 and 10 days have been used for the treatment of colorectal carcinoma in Wistar rats (Weiber et al 1994). A dose of $100\,\text{mg}\,\text{kg}^{-1}$ has been established as the maximum dose that is tolerated in rats (Spector et al 1995). The clinically recommended dose is between 12 and $15\,\text{mg}\,\text{kg}^{-1}$ daily for 9 days (Bjerkeset & Fjsne 1986). Thus, the dose used in this study was in accordance with that used clinically, although the models of total metabolic clearance of 5-FU are different in man and rats.

Usually, when a drug is administered, the plasma drug concentration increases quickly and then decreases exponentially as the drug is eliminated, metabolized or degraded (Vert 1986). This is particularly important when a drug has a short half-life since large doses must be administered, which can result in a greater number of side-effects. This is the case for 5-FU, which has a plasma half-life of approximately 15–20 min (Barberi-Heyob et al 1992), and its administration by usual routes at clinically effective doses can cause serious side-effects (Diasio et al 1988; Fata et al 1999). The in-vivo release of 5-FU from the hydrogels showed similar kinetics (Figures 1–3). The drug was initially quickly released and a high plasma concentration of 5-FU was detected. The plasma level of 5-FU then decreased and, in general, reached a concentration that was maintained almost constant up to the point when the drug was no longer detected.

The 5-FU release from 50HEMA/50A–5% EGDMA (Figure 1) took place over 2 days. The plasma drug concentration was $2\,\mu\text{g}\,\text{mL}^{-1}$ at
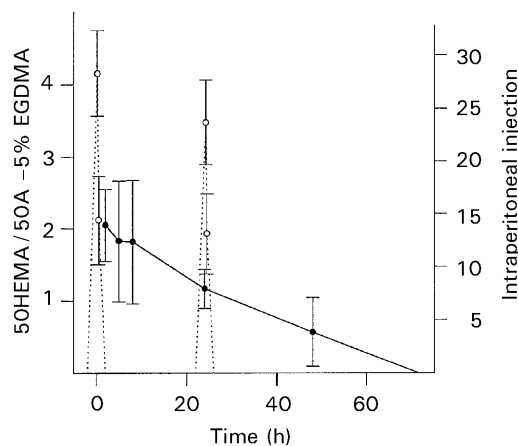
Figure 1. Plots of plasma concentrations of 5-FU ($\mu\text{g}\,\text{mL}^{-1}$) against time of treatment for rats implanted with 50HEMA/50A–5% EGDMA hydrogel discs (●) and for rats injected intraperitoneally (○).
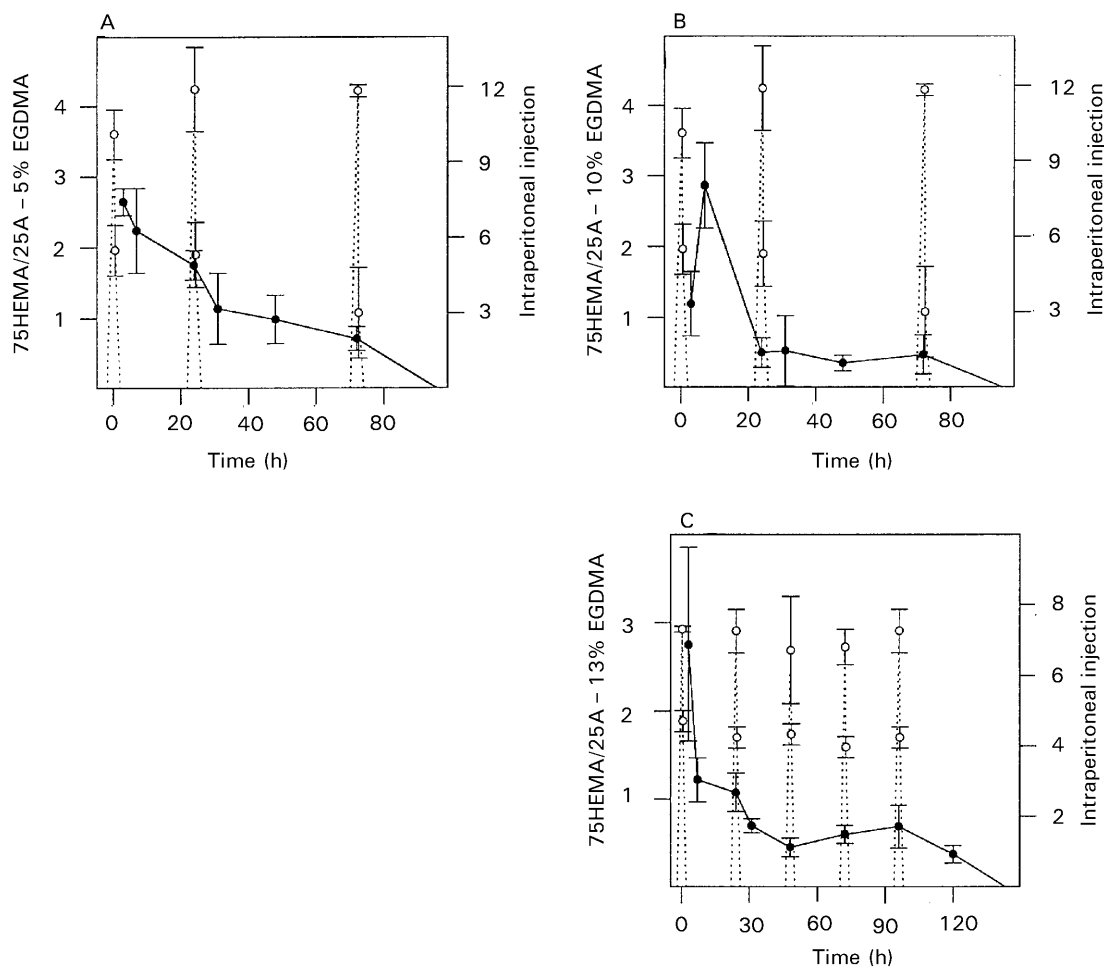
Figure 2. Plots of plasma concentrations of 5-FU (μg mL^{-1}) against time of treatment for rats implanted with 75HEMA/25A–5% EGDMA (A), 75HEMA/25A–10% EGDMA (B), 75HEMA/25A–13% EGDMA (C), hydrogel discs (●) and for rats injected intraperitoneally (○).
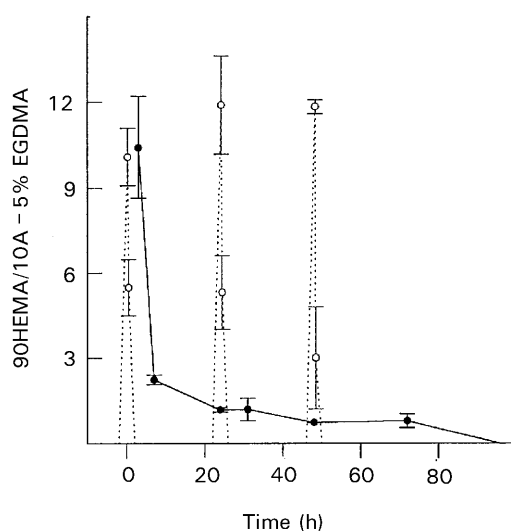

Figure 3. Plots of plasma concentrations of 5-FU (μg mL^{-1}) against time of treatment for rats implanted with 90HEMA/10A–5% EGDMA hydrogel discs (●) and for rats injected intraperitoneally (○).

2 h after implantation and it decreased to 0·55 μg mL^{-1} at 48 h, and was not detected 24 h later. When the total dose of 5-FU was administered over 2 days (50 mg kg^{-1} daily) by intraperitoneal injection, the drug plasma concentration was approximately 12-times higher than the maximum concentration released from the gels at 15 min, and no drug was detected 2 h after the injection. Thus there were periods without drug between the two consecutive injections. The equilibrium swelling degree of the 50HEMA/50A–5% EGDMA gels was 54·8±0·8 wt%. Although histological studies were not carried out, macroscopically the implantation of the gels did not cause alterations in the surrounding tissue. The presence of a fibrous capsule around implants of some hydrogels has been described (Yoshida et al 1987; Jeyanthi & Panduranga Rao 1990). Even after 30 days of implantation fibrous capsules were not formed around the polymeric discs (with or without drug) and there was no visually apparent inflam-

matory response of the surrounding tissues. When removed, the gel discs were transparent and had maintained their physical form with no apparent degradation.

Study of the release of 5-FU from 75HEMA/25A gels with different percentages of crosslinker showed the effect of the amount of EGDMA on the matrix structure and on the release kinetics of the drug (Figure 2). 5-FU release from 75HEMA/25A–5% EGDMA and 75HEMA/25A–10% EGDMA took place over 3 days. For the hydrogel with 10% EGDMA, a constant 5-FU plasma concentration ($0.5\,\mu g\,mL^{-1}$) was obtained 24 h after implantation and was maintained up to 72 h, the drug not being detected at 96 h. The hydrogel with 5% EGDMA produced sustained release of the drug, the plasma concentration of which decreased continuously from 2·7 to $0.7\,\mu g\,mL^{-1}$ at 72 h after implantation, after which the drug was not detected. This different drug release behaviour is closely related to the degree of hydration of the matrices. The in-vivo equilibrium swelling degree of 75HEMA/25A–5% EGDMA and 75HEMA/25A–10% EGDMA was 48.8 ± 0.3 and 35.4 ± 0.1 wt%, respectively, values slightly higher than those obtained in-vitro in saline solution (44.2 ± 0.2 and 32.5 ± 0.2 wt%, respectively) (García et al 2000). The degree of swelling of the hydrogels is defined by the average pore size of the matrices, which depends on the degree of cross-linker. When hydrogels were removed 15 days after implantation no fibrous capsule was detected around the discs. However, a thin fibrous capsule was detected around the polymeric matrix removed 30 days after implantation. This capsule closely adhered to 75HEMA/25A–5% EGDMA and 75HEMA/25A–10% EGDMA discs, in a similar manner as described by other workers using different hydrogels (Yoshida et al 1987; Jeyanthi & Panduranga Rao 1990). The hydrogel discs maintained their physical form, were transparent, and no degradation of the polymeric matrices is observed. Visually, any apparent inflammatory response of the surrounding tissues was not detected. The intraperitoneal administration of $33.33\,mg\,kg^{-1}$ 5-FU daily for 3 days gave plasma peaks of 5-FU, the concentrations of which were noticeably higher than those obtained when the drug was released from 75HEMA/25A–5% EGDMA or 75HEMA/25A–10% EGDMA hydrogels.

An increase in the amount of crosslinker in the polymeric matrix prolonged the presence of 5-FU in plasma (Figure 2). Thus, the 5-FU release from 75HEMA/25A–13% EGDMA took place over 5 days, and a constant plasma concentration of 5-FU (approx. $0.6\,\mu g\,mL^{-1}$) was observed between 24

and 120 h. These gels reached the equilibrium swelling degree in-vivo (33.5 ± 1.4 wt%), the value of which was slightly higher than that obtained in-vitro (30.7 ± 0.5 wt%) (García et al 2000). In this case a thin fibrous capsule around the gel disc was observed 15 days after implantation. Again, no degradation of the hydrogels was observed, they were transparent and maintained their physical form. There was no apparent inflammatory response of the surrounded tissues. 5-FU administration via intraperitoneal injection at a dose of $20\,mg\,kg^{-1}$ daily for 5 days gave peaks of 5-FU in plasma, the concentrations of which were higher than those obtained when the drug was released from 75HEMA/25A–13% EGDMA hydrogels. The drug was not detected in plasma for more than 20 h, between consecutive injections (Figure 2C).

When 90HEMA/10A–5% EGDMA hydrogels were used for the controlled release of 5-FU, total release was obtained over 3 days, with a constant 5-FU plasma concentration ($1\,\mu g\,mL^{-1}$) between 10 and 72 h after the implantation (Figure 3). This constant concentration was substantially lower than that obtained when the drug was administered by intraperitoneal injection for 3 days ($33.33\,mg\,kg^{-1}$ daily). The in-vivo equilibrium swelling degree of this hydrogel was 34.6 ± 1.2 wt% (in-vitro: 26.2 ± 1.4 wt%) (García et al 2000), and a thin fibrous capsule was detected around the discs 15 days after implantation. Visual examination of the surrounding tissues indicated good biocompatibility of the matrix and no degradation of the polymeric discs.

The in-vivo equilibrium swelling degree of hydrogels was slightly greater than that determined in-vitro in saline solution for all copolymers. This suggests that some of the swelling is probably a result of other physiological substances in the subcutaneous environment rather than to the contribution of the saline solution to swelling. The presence or not of a thin fibrous capsule around the hydrogel discs largely depends on their water content in the swelling equilibrium, and also on the period of time necessary to its formation. Similar to the 50HEMA/50A–5% EGDMA hydrogel, the aqueous content of which was approximately 55 wt%, hydrogels with a large swelling capacity are not included in a fibrous capsule when they are subcutaneously implanted. Thus, poly(acrylamide-co-monoethyl itaconate) or poly(acrylamide-co-monopropyl itaconate) hydrogels, whose equilibrium swelling degree in-vivo is approximately 80 wt%, are well tolerated, and a fibrous capsule was not detected around them when they were implanted in rats (Blanco et al 1996; Gómez et al 1998). A water content of less than 55 wt% causes

Table 1. Pharmacokinetic parameters of $100 \, \text{mg kg}^{-1}$ 5-fluorouracil (5-FU) after intraperitoneal injection and subcutaneous implantation of different 5-FU-loaded hydrogels.

Administration route	Elimination rate constant (h^{-1})	Half-life (h)	Total AUC $(\mu \text{g h mL}^{-1})$	Total body clearance		Volume of distribution (L)
				(L h^{-1})	$(\text{mL min}^{-1} \text{m}^{-2})$	
50HEMA/50A–5% EGDMA	0·026±0·001	25·11±2·46	89·06±27·99	0·301±0·099	758±249	11·63±3·84
Intraperitoneal injection (2 days)	2·695±0·416	0·257±0·04	5·06±3·02	3·383±2·424	8525±6108	1·26±0·89
90HEMA/10A–5% EGDMA	0·030±0·012	22·95±10·11	130·18±27·35	0·198±0·043	499±108	6·56±1·41
75HEMA/25A–5% EGDMA	0·027±0·006	25·86±4·62	64·46±13·29	0·339±0·084	854±212	14·90±3·14
75HEMA/25A–10% EGDMA	0·032±0·010	21·80±5·44	86·18±23·67	0·305±0·086	769±217	9·62±2·73
Intraperitoneal injection (3 days)	2·439±0·483	0·2842±0·06	3·33±2·57	4·083±3·718	10289±9369	2·03±1·14
75HEMA/25A–13% EGDMA	0·034±0·007	20·69±4·20	101·35±23·12	0·256±0·060	645±151	7·63±1·79
Intraperitoneal injection (5 days)	1·750±0·420	0·412±0·10	0·37±0·15	15·205±6·625	38316±16695	8·69±3·79

Data are mean±s.d., n = 3 AUC_i area under plasma concentration–time curve.

the formation of a fibrous capsule, as in the case of 75HEMA/25A–5% EGDMA, 75HEMA/25A–10% EGDMA, 75HEMA/25A–13% EGDMA and 90HEMA/10A–5% EGDMA hydrogels. The time needed for the capsule to form around the gels is influenced by their aqueous content; for values between 50 and 35 wt%, the presence of a fibrous capsule was detected approximately 30 days after the implantation, whereas gels with lower aqueous content favour encapsulation of the polymeric discs 15 days after the implantation.

The pharmacokinetic parameters of 5-FU after both intraperitoneal injection and subcutaneous implantation of drug-loaded hydrogels are showed in Table 1.

Administration of 5-FU hydrogels seemed to result in suitable plasma concentrations of 5-FU for a definite period of time. The half-life of 5-FU released from 50HEMA/50A–5% EGDMA hydrogels was increased up to 98-times compared with intraperitoneal administration. The AUC was increased by 18- and 274-times for 50HEMA/50A–5% EGDMA and 75HEMA/25A–13% EGDMA, respectively. The volume of distribution increased by 3- and 9-times for 90HEMA/10A–5% EGDMA and 50HEMA/50A–5% EGDMA, respectively. A noticeable decrease in total body clearance was also observed as shown in Table 1.

5-FU has a short plasma half-life (approx. 15 min) and it is predominantly toxic against cell cycling through the S-phase (i.e. undergoing DNA synthesis), which represents only a small proportion of the entire tumour cell population at any one given time. Thus, exposing tumours to 5-FU administered as a continuous infusion, by modulating 5-FU pharmacokinetics and pharmacodynamics or by drug delivery systems is advantageous. Attempts to modulate 5-FU with agents such as leucovorin, interferon, and N-(phosphonacetyl)-L-aspartate have met with limited success (Cao et al

1994; Kennedy 1999). Several clinical studies have confirmed that continuous infusion of 5-FU is superior to bolus intravenous injection, with improved anticancer activity and reduced toxicity (Rougier et al 1997). A pharmacokinetic study of 5-FU infusion in rats showed that over the range $3–480 \, \text{mg m}^{-3}/\text{h}^{-1}$, total body clearance of 5-FU decreased from 600 to less than $90 \, \text{mL min}^{-1} \text{m}^{-2}$ (Collins 1985). Continuous infusion chemotherapy requires an indwelling central venous catheter, with the associated risks of infection and thrombosis, and regular medical visits. This is inconvenient for the patient and reduces quality of life. From the results of this work, the administration of 5-FU by implantation of HEMA-co-acrylamide copolymeric hydrogels seems to be a good candidate for 5-FU therapy, since the drug released results in a therapeutically suitable plasma concentration of 5-FU for an extended period of time, despite the short half-life of the drug, and to have the drug delivery system would improve, in general, the patient quality of life.

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