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Organ distribution of 5-fluorouracil loaded gelatine microspheres in mice

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The aim of this study was to investigate the organ distribution characteristics of 5-fluorouracil (5-Fu) loaded crosslinked gelatine microspheres (5-Fu-MS) after intravenous (i.v.) injection compared to 5-Fu solution in mice, and to evaluate the targetability of 5-Fu-MS. The concentrations of 5-Fu in mice plasma, heart, liver, lung, kidney, spleen and brain were determined by HPLC. The parameters drug targeting index C_e, time-averaged relative drug exposure values r_e, drug targeting efficiency t_e, and drug distributed to target-tissue (%) were used to evaluate the targetability of the 5-Fu-MS delivery system. The results showed that the concentration of 5-Fu in the lung was significantly higher than after application of 5-Fu solution, and that the maximum concentration of 5-Fu, 72.8 $\mu g \cdot g\ organ^{-1}$, was reached in the lung at 15 min after i.v. administration. As for 5-Fu-MS, r_e was calculated to be 2.2, and t_e was considerably higher than after application of 5-Fu in solution. Furthermore, the percentage of drug distributed to the lung was 2 times as high as after application in solution. Accordingly, 5-Fu-MS were capable of effectively delivering 5-Fu to the lung and possessed specific targetability towards the lung compared to 5-Fu solution.

1. Introduction

5-Fluorouracil (5-Fu) is an antineoplastic agent widely employed in the treatment of many types of cancer, but it has a number of side effects due to its unspecific tissue distribution. To overcome these drawbacks and to increase the therapeutic index of this drug macromolecular carriers and microparticles were used. Many attempts have been made to deliver 5-Fu to target sites by means of advanced drug delivery systems, such as microspheres (Chandy et al. 2000, Chiang et al. 2001; Denkbas et al. 1999; Roullin et al. 2003; Sugibagashi et al. 1977), liposomes (El Maghraby et al. 2001; Jing et al. 1997; Joondeph et al. 1988), nanoparticles (McCarron et al. 2000; Mukherji et al. 1989; Mukherji et al. 1990) and macromolecular prodrugs (Nichifor et al. 1996; Nichifor et al. 1997; Ouchi et al. 1998), all of which exhibited specific-release properties or sustained release. Application of drug carriers may improve the drug's anti-cancer activity at a lower dose and concurrently reduce toxicity.

In a previous study, we have successfully prepared 5-Fu loaded crosslinked gelatine microspheres (5-Fu-MS). At present, in order to evaluate the organ targetability of 5-Fu-MS, the biodistribution of 5-Fu-MS in various organs of mice was investigated and compared to 5-Fu solution. The concentrations of 5-Fu in the organs were determined by HPLC.

Simply comparing the drug concentration between two dosage forms may lead to misinterpretation of the efficacy of a drug delivery system, and so the organ targetability was evaluated using the parameters drug targeting index C_e, time-averaged relative drug exposure values r_e, drug targeting efficiency t_e, and drug distributed to target-tissue (%). The combination of these parameters led to more accurate results, since each parameter selected represents a different pharmacokinetic point of view to evaluate drug delivery systems.

2. Investigations, results and discussion

The mean concentrations of 5-Fu in plasma and different organs after i.v. administration of 5-Fu solution are shown in Fig. 1. 5-Fu was rapidly distributed into different organs of mice. The concentrations of 5-Fu in kidney and lung 2 min after i.v. application were 68.8 $\mu g \cdot g\ organ^{-1}$, and 60.3 $\mu g \cdot g\ organ^{-1}$, respectively, which exceeded those of other organs, but the concentrations in brain and liver

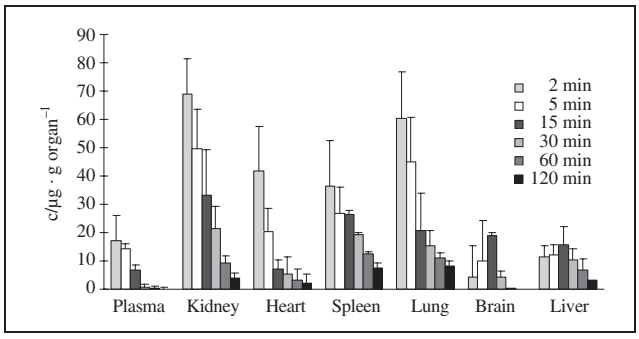

Fig. 1: Concentration-time profiles of 5-Fu in mice plasma and organs after i.v. administration of 5-Fu solution (n = 6)

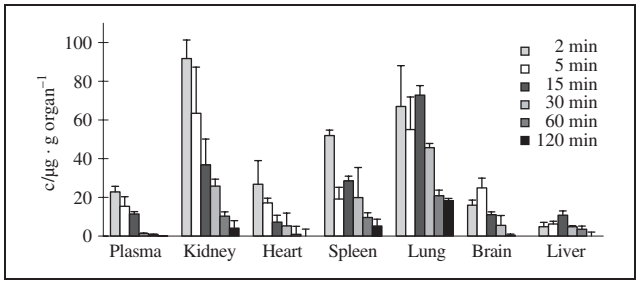

Fig. 2: Concentration-time profiles of 5-Fu in mice plasma and organs after i.v. administration of 5-Fu-MS (n = 6)

were lower. Figure 2 summarizes the distribution characteristics of 5-Fu-MS. The concentrations of 5-Fu in kidney and lung increased significantly compared to the solution. 5-Fu-MS exhibited selective accumulation of drug in the lung, reaching a maximum of about 72.8 μg · g organ^{-1} at 15 min after i.v. application, but the liver uptake decreased. So it could be concluded that 5-Fu-MS had a tendency to preferentially deliver the drug to the lung.

All the drug concentration data were analyzed using the trapezoidal rule to obtain the area under concentration-time curve between 0 min and 120 min (AUC_{0-120}). It can be seen in Fig. 3 that AUC_{0-120} of 5-Fu in plasma, kidney, lung and brain after i.v. infusion of 5-Fu-MS were higher than those achieved with the solution. In particular, there was a great difference of the two dosage forms in drug amount delivered to lung. Therefore, 5-Fu-MS could selectively transfer the drug to the lung as target organ.

The methods of evaluating targeted drug delivery systems have been reviewed by Gupta and Hung (1989). Simply comparing the drug concentration between two delivery systems may, however, lead to misinterpretation of the efficacy of a drug delivery system. Accordingly, all the parameters C_e, r_e, t_e and drug distributed to target-tissue (%) were chosen in this study to evaluate 5-Fu-MS delivery systems from different pharmacokinetic points of view.

The drug targeting index is defined as $C_e = \frac{C_P}{C_S}$, where C_P means drug concentration in tissue at time t after administration of the test drug delivery system; C_S means drug concentration in the tissue at time t after administration of the drug as a solution. With regard to 5-Fu-MS, the drug lung targeting index C_e at times 2, 5, 15, 30, 60, 120 min was 1.11, 1.22, 3.50, 2.97, 1.88, 2.23, respectively. Because of $C_e > 1$, the amount of 5-Fu delivered by microspheres was higher than that achieved with 5-Fu solution. Therefore, it could be concluded that 5-Fu-MS compared to 5-Fu solution had a preference to accumulate 5-Fu in the lung.

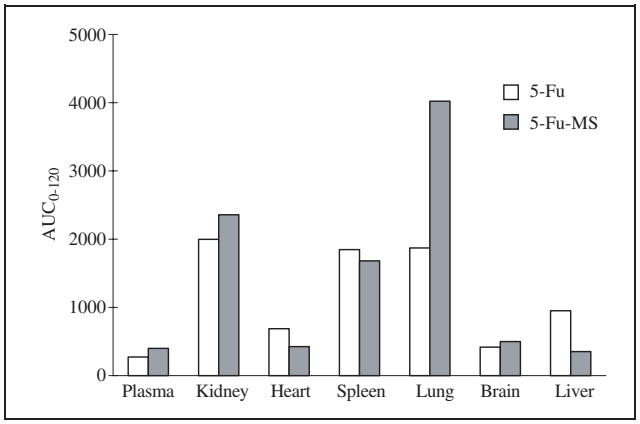

Fig. 3: AUC_{0-120} of 5-Fu in mice plasma and in various organs after i.v. administration of 5-Fu and 5-Fu-MS (n = 6)

Table 1: AUC_{0-120} in various organs of mice, following the i.v. administration of 5-Fu solution and 5-Fu-MS at a dose of 20 mg/kg

Organs	AUC_{0-120} (mg min L^{-1})		r_e
	Solution	Microspheres	
Heart	688	426	0.62
Liver	951	353	0.37
Spleen	1846	1682	0.91
Lung	1872	4021	2.15
Kidney	1997	2357	1.18
Brain	417	499	1.20

(n = 6)

AUC_{0-120} for different organs of mice following the i.v. of 5-Fu solution and 5-Fu-MS at a dose of 20 mg/kg, as well as the time-averaged relative drug exposure values (r_e), are summarized in Table 1. Parameter r_e was determined using the equation:

$$r_e = \frac{(AUC_{0-120})_P}{(AUC_{0-120})_S}$$

If the values of r_e is greater than one, the tissue is exposed to drug to a greater extent by the test drug delivery system. In our study, the lung targeting parameter r_e was 2.15, indicating that the exposure of the 5-Fu to lung was significantly increased by microspheres delivery system. Therefore, it could be concluded that 5-Fu-MS are more specific in the delivery of 5-Fu to lung than 5-Fu solution.

Drug targeting efficiency

$$t_e = \frac{(AUC_{0-120})\ \text{target-tissue}}{(AUC_{0-120})\ \text{non-target-tissue}}$$

refers to the drug targeting efficiency of a delivery system against non-target tissue. Taking the weight of organs into account, drug targeting efficiency could be more accurately expressed as t_e^*, i.e.

$$t_e^* = \frac{(AUC_{0-120})\ \text{target-tissue} \times (\text{weight or volume})}{(AUC_{0-120})\ \text{non-target-tissue} \times (\text{weight or volume})}$$

Table 2 compares the targeting efficiency of microspheres with solution dosage form. Here values $t_e > 1$ for 5-Fu solution indicated that the formulation had some selectivity in terms of drug distribution to the lung. However, the t_e value for the 5-Fu-MS delivery system was higher than that of the 5-Fu solution, which indicated that 5-Fu-MS further targeted the lung. The values of t_e for heart, liver and brain suggested that 5-Fu-MS exhibited a considerable

Table 2: Drug targeting efficiency (t_e) and weighted-average drug targeting efficiency (t_e^*) of 5-Fu solution and 5-Fu-MS at a dose of 20 mg/kg.

Organs	t_e		t_e^*	
	Solution	Microspheres	Solution	Microspheres
Heart	2.72	9.45	3.52	12.21
Liver	1.97	11.39	0.28	1.63
Spleen	1.01	2.39	1.47	3.46
Lung	1	1	1	1
Kidney	0.94	1.71	0.62	1.13
Brain	4.48	8.06	2.08	3.74

(n = 6)

Table 3: Percentage of drug distributed to various organs following i.v. administration of 5-Fu solution and 5-Fu-MS at a dose of 20 mg/kg

Organ	Solution	Microspheres	r
Heart	3.7	2.6	0.70
Liver	46.8	19.6	0.42
Spleen	8.9	9.2	1.03
Lung	13.1	31.9	2.43
Kidney	21.1	28.2	1.33
Brain	6.3	8.5	1.35

(n = 6)

discrimination between lung and the other three organs. But if the weight of the organs were taken into account, the values of t_e^* suggested that the microspheres possessed little selectivity towards the heart as opposed to the lung.

Drug distributed to target-tissue (%)

$$= \frac{(AUC_{0-120}) \text{ target-tissue} \times (\text{weight or volume})}{\sum_{i=1}^{n} (AUC_{0-120})_i \times (\text{weight or volume})} \times 100$$

The percentage of drug distributed to various organs following i.v. administration of 5-Fu solution and 5-Fu-MS listed in Table 3. Two times as much 5-Fu was distributed to the lung from 5-Fu-MS than from 5-Fu solution; however, just half the amount of drug was distributed to the liver. In conclusion, 5-Fu-MS were more specific and selective to the lung than 5-Fu solution.

From these results, 5-Fu loaded gelatine microspheres appeared to be an effective carrier system for lung targeting and to be a promising candidate for the treatment of lung cancer.

3. Experimental

3.1. Reagents and chemicals

5-Fu was obtained from Nantong General Pharmaceutical Factory; 5-bromouracil (5-Bru) was purchased from Shanghai No. 2 Chemical Reagent Company; gelatine (type A, bloom strength of 220 and pI = 8) was purchased from Hebei Dongguang Bee Manufacture Co. Ltd; soyabean oil was purchased from Yingkou Oil & Fats Industry Co. Ltd; Tween 80 was obtained from Shenyang Reagent Company; glutaraldehyde (50%, v/v) was of analytical grade from Tianjin Bo Di Chemicals Co. Ltd; acetonitrile was of HPLC grade from Shandong Yuwang Co. Ltd.

3.2. Preparations of 5-fluorouracil gelatine microspheres

A modified emulsification chemical-crosslinking method was used to prepare 5-Fu loaded gelatine microspheres (Li et al. 1998). 5-Fu was added to a 10% (w/v) gelatine solution. After dissolving, the gelatine solution was placed in a water bath at 40 °C then 0.5 mL of 5-Fu gelatine solution was added drop-wise to 50 mL of soyabean oil, preheated to 40 °C. Subsequently, the biphasic system was emulsified and homogenized at 19000 rpm for 10 min to form a w/o emulsion by using a FA-25 High-Shear Homogenizer (FLUKO Equipment Shanghai Co. Ltd). After the desired size was obtained, the emulsion was cooled to −20 °C in a refrigerator. When the temperature of the emulsion fell below the gelling point of gelatine, the emulsion was changed into a suspension. The suspension was agitated at 500 rpm at 0 °C, and 25 mL isopropyl alcohol were added. Stirring was continued for 10 min at 0 °C. The microspheres were filtered and washed with isopropyl alcohol. After being air-dried, the non-crosslinked 5-Fu-MS dispersed in 1 mL of glutaradehyde isopropyl alcohol solution (5%, v/v) at 25 °C for 12 h to crosslink. The mean diameter of microspheres used in this study was about 5 μm.

3.3. Particle size distribution of microspheres

Particle size distribution of microspheres (Fig. 4) was determined using a Coulter LS 230 counter (Coulter, USA). Before the size analysis, the microspheres were suspended in deionized water containing Tween® 80 (0.1% w/v) and then sonicated for 2 min.

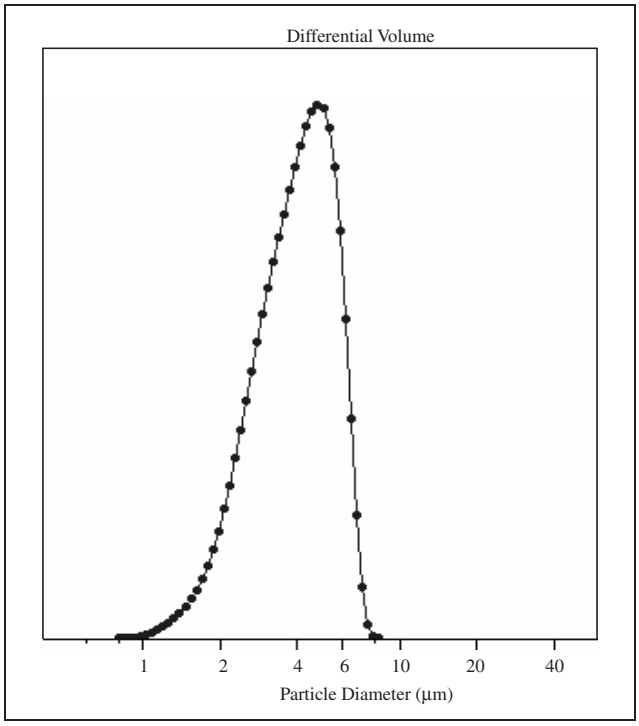

Fig. 4: Particle size distribution of 5-Fu gelatine microspheres

3.4. 5-Fu-MS in vitro release studies

Microspheres were placed in dialysis bags. *In vitro* 5-Fu release from the microspheres was determined in 250 ml of 0.1 M phosphate buffered saline (PBS, pH 7.4) at 37 °C and the stirring speed was set at 50 rpm. At designated times, a small amount of sample was withdrawn, and assayed for 5-Fu concentration using a UV spectrophotometer at 265 nm. An equal volume of PBS was added to the dissolution medium to maintain a constant volume. Because dialysis bags have a retarded release effect, 5-Fu solution was selected as the control. Each determination was carried out in triplicate and the release results were plotted as the cumulative release percentage versus time, as shown in Fig. 5.

3.5. In vivo experiment

Animal experiments conformed to the Guidelines for Animal Experimentation at Shenyang Pharmaceutical University.

Kunming mice (20–24 g, male and female, Certificate No. 2003-008) were purchased from the animal laboratory of Shenyang Pharmaceutical University (Shenyang, China). Seventy-two mice were divided into two groups at random. The control group received 5-Fu solution via the tail vein injection at a single dose of 20 mg/kg, and the test group received 5-Fu-MS suspension. The mice were sacrificed at 2, 5, 15, 30, 60, 120 min after i.v. administration, respectively. Plasma samples were collected and heart, liver, spleen, lung, kidney and brain were excised. The organ samples were homogenized 3 times in saline solution. All the samples were stored in a refrigerator at −20 °C before analysis.

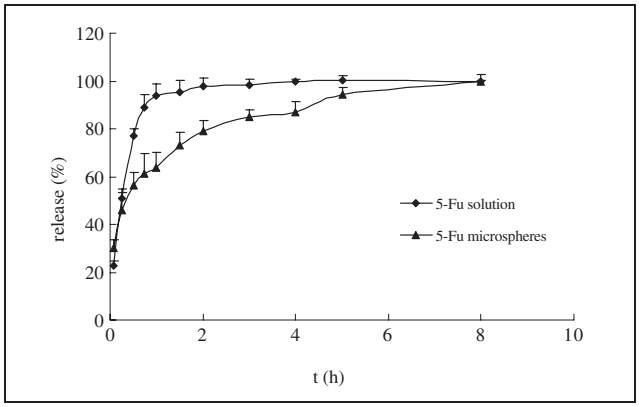

Fig. 5: *In vitro* release profiles of 5-Fu released from solution and gelatine microspheres in 0.1 M PBS (pH 7.4) at 37 °C

3.6. Drug assay for biological samples

Biological samples from plasma, heart, spleen, brain, lung, liver, kidney were analyzed by HPLC. Samples 100 μL of plasma, heart, spleen and 10 μL of internal standard (5-Bru, 50 μg/mL) were extracted with 2 mL of ethyl acetate. Subsequently, the sample was centrifuged at 3000 rpm for 10 min. Then 1.8 mL of the organic layer were evaporated to dryness under nitrogen gas flow at 40 °C. The residue was redissolved in 50 μL of water, and 20 μL was injected into the HPLC system. 200 μL sample of lung, liver, kidney, brain and 10 μL of internal standard (5-Bru, 50 μg/mL) were extracted with 3 mL ethyl acetate as mentioned above.

Chromatographic analysis was performed using a HPLC system consisting of a SHIMADZU LC-10AT pump, a SPD-10A UV detector, and a 7725i sample injector (Kyoto, Japan). The analytical column used was Kromasil C_{18} (4.6 mm × 200 mm, 5 μm) from Tianhe Corp. (China). The column temperature was maintained at room temperature. The UV detector was set at 265 nm. The mobile phase consisted of a mixture of acetonitrile-water (1 : 99, v/v), and the flow rate was 0.9 mL · min^{-1}.

References

Chandy T, Das GS, Rao GHR (2000) 5-Fluorouracil-loaded chitosan coated polylactic acid microspheres as biodegradable drug carriers for cerebral tumours. J Microencapsul 17: 625–638.

Chiang CH, Tung SM, Lu Dw, Yeh MK (2001) *In vitro* and *in vivo* evaluation of an ocular delivery system of 5-fluorouracil microspheres. J Ocular Pharmacol Thera 17: 545–553.

Denkbas EB, Seyyal M, Piskin E (1999) 5-Fluorouracil loaded chitosan microspheres for chemoembolization. J Microencapsul 16: 741–749.

El Maghraby GM, Williams AC, Barry BW (2001) Skin delivery of 5-fluorouracil from ultradeformable and standard liposomes in-vitro. J Pharm Pharmacol 53: 1069–1077.

Gupta PK, Hung CT (1989) Quantitative evaluation of targeted drug delivery systems. Int J Pharm 56: 217–226.

Jing M, Xi S, Chen R (1997) The inhibitory effect of tissue plasminogen activator combined with 5-fluorouracil polyphase liposome on the scar formation in experimental filtration surgery. Zhonghua Yan Ke Za Zhi 33: 376–380.Joondeph BC, Peyman GA, Khoobehi B, Yue BY (1988) Liposome-encapsulated 5-fluorouracil in the treatment of proliferative vitreoretinopathy. Ophthalmic Surg 19: 252–256.

Li JK, Wang N, Wu X.S (1998) Gelatine nanoencapsulation of protein/peptide drugs using an emulsifier-free emulsion method. J Microencapsul 15: 163–172.

McCarron PA, Woolfson AD, Keating SM (2000) Sustained release of 5-fluorouracil from polymeric nanoparticles. J Pharm Pharmacol 52: 1451–1459.

Mukherji G, Murthy RSR, Miglani BD (1989) Preparation and evaluation of polyglutaradehyde nanoparticles containing 5-fluorouracil. Int J Pharm 50: 15–19.

Mukherji G, Murthy RSR, Miglani BD (1990) Preparation and evaluation of cellose nanospheres containing 5-fluorouracil. Int J Pharm 65: 1–5.

Nichifor M, Schacht EH, Seymour LW (1996) Macromolecular prodrugs of 5-fluorouracil. 2: Enzymatic degradation. J Control Release 39: 79–92.

Nichifor M, Schacht EH, Seymour LW (1997) Polymeric prodrugs of 5-fluorouracil**.** J Control Release 48: 165–178.

Ouchi T, Tada M, Matsumoto M, Ohya Y, Hasegawa K, Arai Y, Kadowaki K, Akao S, Matsumoto T, Suzuki S, Suzuki M (1998) Design of macromolecular prodrug of 5-fluorouracil using N-acetylpolygalactosamine as a targeting carrier to hepatoma. React Funct Polym 37: 235–244.

Roullin VG, Lemaire L, Venier-Julienne MC, Faisant N, Franconi F, Benoit JP (2003) Release kinetics of 5-fluorouracil-loaded microspheres on an experimental rat glioma. Anticancer Res 23: 21–25.

Sugibagashi K, Morimoto Y, Nadai T, Kato Y (1997) Drug-carrier property of albumin microspheres in chemo-therapy I. Tissue distribution of microsphere-entrapped 5-fluorouracil in mice. Chem Pharm Bull 25: 3433–3438.