Carbohydrate Polymers 87 (2012) 2642–2647

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

# A novel 5-fluorouracil prodrug using hydroxyethyl starch as a macromolecular carrier for sustained release

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ARTICLE INFO

*Article history:*
Received 7 October 2011
Received in revised form 2 November 2011
Accepted 13 November 2011
Available online 22 November 2011

*Keywords:*
5-Fluorouracil-1-acetic acid
Hydroxyethyl starch
Conjugates
Hydrolysis
Enzymolysis
Pharmacokinetics

ABSTRACT

The objective of this study was to develop a sustained-release drug delivery system for 5-fluorouracil (5-FU) to improve its short half-life. 5-Fluorouracil-1-acetic acid (FUAC) was prepared and then conjugated to hydroxyethyl starch (HES) through ester bonds. The conjugates were relatively stable in acidic buffer solution at pH 5.8 and slowly released FUAC but became more sensitive to hydrolysis with an increase in the pH and temperature. The conjugates were degraded to FUAC both in human and rat plasma with half-time life of 20.4 h and 24.6 h, respectively. Both 5-FU and FUAC were released in a rat liver homogenate following a 12 h incubation of the conjugates. The pharmacokinetic behavior was evaluated in rats after intravenous injection of 5-FU, FUAC and the conjugates. The drug release data in vitro and in vivo indicated that HES is a promising carrier for the sustained-release of antitumor drugs.



## 1. Introduction

5-Fluorouracil (5-FU), a low molecular weight drug, is widely used in the treatment of various kinds of cancer, especially solid tumors (Brockman & Anderson, 1963). However, due to the fact that there is little selectivity in its pharmacological action, the administration of 5-FU is always accompanied by systemic toxicity (Macdonald, 1999). Moreover, the short in vivo half-life (Ouchi, Hagihara, Takahashi, Takano, & Igarashi, 1992) means that a sustained therapeutic effect cannot be achieved. To improve an unsuitable property like this, macromolecular prodrugs, which conjugate a low molecular weight drug and a macromolecular carrier through chemical coupling (Takakura & Hashida, 1995), have been developed. Several studies have linked polysaccharides to 5-FU at the 2-position through a chemical bond and the obtained conjugates have exhibited potent inhibition of cancer cell growth (Ouchi, Banba, Fujimoto, & Hamamoto, 1989). Since the vascular endothelium is not a barrier to the distribution of low molecular weight drugs into tissues except the brain, they distribute throughout the body. This makes it difficult to control the distribution of such drugs in vivo; also, the disposition of macromolecules in vivo depends on both their physicochemical and biological properties, and anatomical and physiological characteristics of the body (Hashida & Takakura, 1994; Takakura & Hashida, 1995). When antitumor drugs of low molecular weight are coupled with water soluble carriers of high molecular weight (Sezaki, Takakura, & Hashida, 1989), the uptake of the formed conjugates is blocked by the capillary endothelium in most tissues except most solid tumors which have a high permeability because of neovascularization (Jain, 1987; Petrak & Goddard, 1989; Takakura & Hashida, 1995). Therefore, using macromolecules as a drug carrier allows the drugs to be transported selectively to the tumor.

To be suitable for such use, a macromolecular carrier must be biodegradable and biocompatible, lack any antigenic and toxic effects, without any accumulation in the body, and contain suitable functional groups to allow chemical conjugation (Sezaki & Hashida, 1984). Hydroxyethyl starch (HES), used as a blood plasma substitute, has been studied over the years and shown to be safe and biocompatible in vivo (Hershenson, 1970; Westphal et al., 2009). Previous reports have shown that, there is no relevant accumulation in plasma after repetitive infusion of a solution of medium molecular weight HES (130/0.4) which exhibits a renal excretion rate over 10 days, and good local and systemic tolerability following 10 repeated doses (Waitzinger, Bepperling, Pabst, & Opitz, 2003). HES has been conjugated with deferoxamine mesylate, and the conjugates exhibited no acute toxicity. In addition, the drug residence time in plasma was significantly prolonged with an initial half-life of 22–33 h (Dragsten et al., 2000).

However, little work has been carried out on the conjugation of HES and 5-FU. It is known that HES has numerous hydroxyl groups in the glycosyl unit which act as functional groups for drug

*Abbreviations:* 5-FU, 5-fluorouracil; FUAC, 5-fluorouracil-1-acetic acid; HES, hydroxyethyl starch; FUAC/HES conjugates, the conjugates of 5-fluorouracil-1-acetic acid and hydroxyethyl starch.

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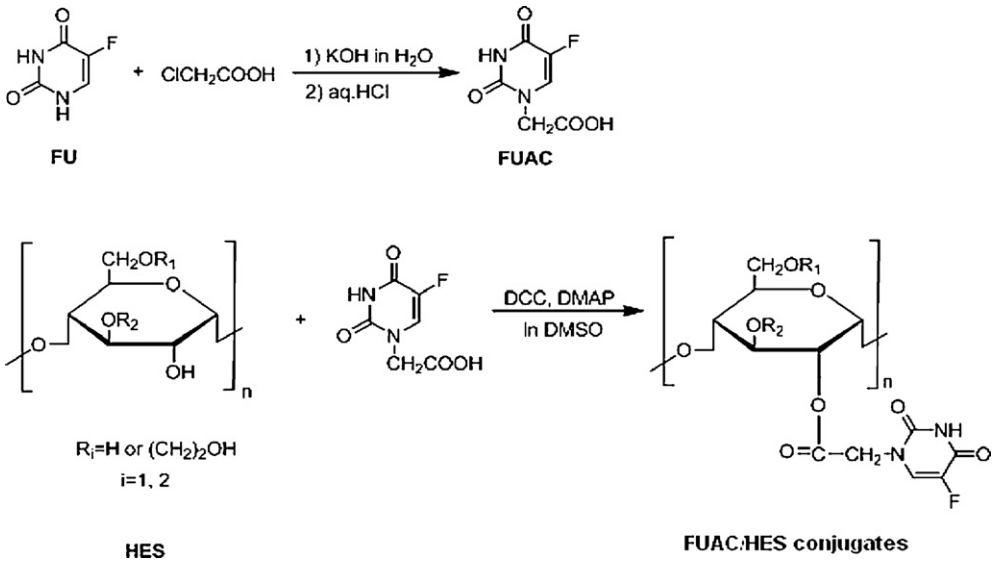
doi:10.1016/j.carbpol.2011.11.039




**Scheme 1.** Synthesis and chemical structure of FUAC/HES conjugates.

linking. In the present study, 5-fluorouracil-1-acetic acid (FUAC) was introduced as a 5-FU derivative which had been shown to be highly effective with a low toxicity in a earlier study (Yang, Wang, Yang, Wang, & Li, 2000). The antitumor activity of FUAC was such that it could inhibit the colony formation of leukemia cells (L1210) and the growth of transplanted tumor sarcoma 180 (S180), hepatic carcinoma (HEPA) and ehrlich ascites (EC) tumor even at a low concentration ($1.0 \times 10^{-5}$ μg/ml). We have described the characteristics of the novel conjugates in vitro, prepared from 5-fluorouracil-1-acetic acid (FUAC) via esterification of the hydroxyl groups from HES. The pharmacokinetic behavior of the formed conjugates following intravenous injection was also investigated.

## 2. Materials and methods

### 2.1. Materials

5-FU was purchased from Nantong Jinghua Pharmaceutical Co. Ltd. (Jiangsu, China). HES (130/0.4) was purchased from Chongqing Daxin Pharmaceutical Co., Ltd. (Chongqing, China). N,N-dicyclhexylcarbodiimide (DCC), 1,1′-carbonyl-di-(1,2,4-triazole) (CDT), and 4-dimethylaminopyridine (DMAP) were purchased from Shanghai Medpep Co., Ltd. (Shanghai, China). Dimethyl sulfoxide (DMSO) was dried with a 4 Å molecular sieve before use. All other solvents and chemicals were obtained from commercial sources and used without further purification.

### 2.2. Synthesis and characteristics of 5-fluorouracil–HES conjugates

#### 2.2.1. Synthesis of 5-fluorouracil-1-acetic acid (FUAC)

Unlike the previously reported method (Tada, 1975), we developed an improved synthetic approach in which 5-fluorouracil (1.54 g) was dissolved in KOH (2.564 g) aqueous solution (8.0 ml) at 50 °C for 30 min. Chloroacetic acid (1.7 g) was then added to the solution slowly over 30 min, and the mixture was stirred at 60 °C. The reaction was monitored by fluorescence thin-layer chromatography. After 5 h, the reaction solution was cooled to room temperature, and then hydrochloric acid was added to adjust pH to 5.5, followed by cooling to 4 °C for 2 h. Unreacted 5-fluorouracil was removed by filtration. More hydrochloric acid was added to adjust the pH to 2, followed by cooling for 12 h. Then, the formed precipitate was filtered, and washed with distilled water three times. After crystallization, needle-like crystals were obtained after drying. The melting point was determined using an X-2 micromelting point apparatus.

#### 2.2.2. Synthesis of 5-fluorouracil-1-acetic acid and hydroxyethyl starch conjugates (FUAC/HES conjugates)

To a solution of HES (1.43 g, in dry DMSO, 20 ml), 5-fluorouracil-1-acetic acid (0.57 g) was added slowly. After dissolution, DCC (0.7 g) and DMAP (0.038 g) were added with stirring, and the reaction continued for 24 h at room temperature with protection from light. The generated dicyclohexylurea (DCU) was removed by filtration. The conjugates were precipitated after addition of a diethyl ether/dehydrated alcohol (1:3, v/v) mixture (200 ml), and washed with 50 ml diethyl ether. Then, the precipitate was re-dissolved in water, and purified by Sephadex G-50 column chromatography. The final conjugates were obtained as a white powder from the eluate after freeze-drying in a VirTis AdVantage Benchtop Freeze Dryer (SP Industries, NY, USA), and stored in a desiccator. The whole process is shown in Scheme 1.

#### 2.2.3. Characterization of the FUAC/HES conjugates and determination of the FUAC content of the conjugates

FTIR spectroscopy and NMR spectroscopy were performed to confirm the ester-bond formation. The obtained conjugates were characterized using $^1$H NMR (ARX-300, Bruker, Switzerland) and IR spectroscopy (IFS-55 Fourier Transform Infrared Spectrophotometer, Bruker Optics, Ettlingen, Germany). Thermal analysis was performed by DSC at a heating rate of 10 °C/min with a closed aluminium pan system over the temperature range of 30–300 °C.

It was found that the conjugates were completely hydrolyzed to release FUAC in strongly alkaline solution. Therefore, the drug content was determined by adding 20 ml NaOH 2 mol/L to 10.0 mg conjugate at a temperature of 60 °C with magnetic stirring. After 1 h, the reaction solution was cooled to room temperature, and 20 ml HCl (2 mol/L) was used to stop the reaction and neutralize the solution. The content of FUAC was calculated by comparison with the FUAC standard curve.

### 2.3. In vitro stability of FUAC/HES conjugates

#### 2.3.1. Hydrolysis in phosphate buffered saline (PBS)

Conjugates were dissolved in a series of various PBS solutions (0.01 mol/L) at pH 1.2, 2.5, 5.8, 7.4, 8.0, 10.0 to give a final

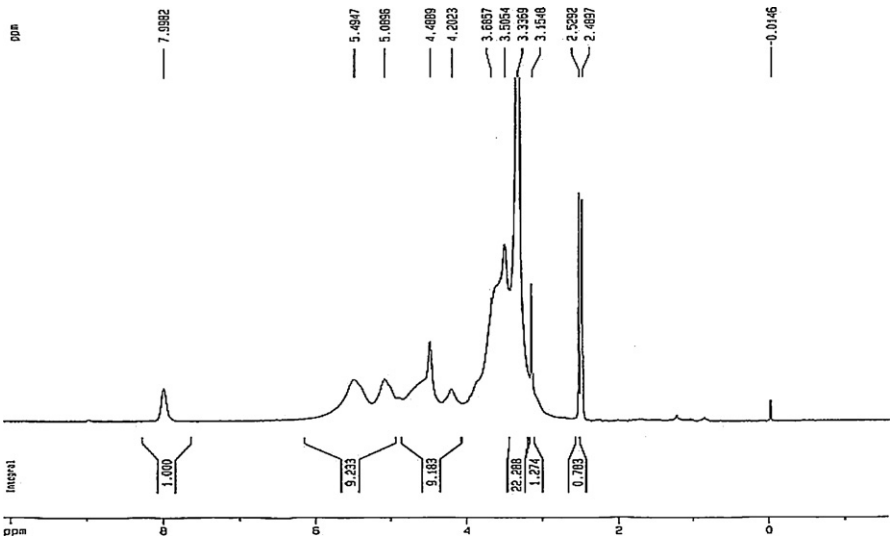



**Fig. 1.** NMR spectrum of FUAC/HES conjugates.

concentration of 0.5 mg/ml. The buffer solutions were incubated at 37 °C for 12 h and 0.1 ml portion of each reaction solution was removed and passed through 0.45 μm membrane filter at 0, 0.25, 0.5, 0.75, 1, 2, 3, 6, and 12 h; 20 μl of each filtrate was analyzed by HPLC. The HPLC system consisted of an LC-20 AD pump (Shimadzu, Japan), an SPD-20A UV detector (Shimadzu, Japan) and an XB-C18 column (250 mm × 4.6 mm, Welch Materials). The mobile phase consisted of a mixture of water and methanol (v/v, 95:5) passed through a 0.45 μm membrane filter before use. The mobile phase was eluted at a flow rate of 1.0 ml/min and monitored by the UV detector at a wavelength of 273 nm. The retention time of the FU, FUAC, and FUAC/HES conjugates was 3.0 min, 3.8 min, and 8.8 min, respectively. The same procedure was also carried out at 60 °C in the series of PBS solutions.

#### 2.3.2. Enzymolysis in plasma and rat liver homogenate

Fresh rat plasma was diluted to 30% with physiological saline. Then, 1.5 mg samples of FUAC/HES conjugates were dissolved in 3 ml diluted rat plasma and kept at 37 °C for 12 h. Then, 100 μl samples were transferred to tubes at appropriate intervals to determine the percentage release. The same operation was also carried out using human plasma and rat liver homogenate (v/v, 30%). The experiments concerning rats plasma and liver were approved by the Committee on Ethical Use of Animals of Shenyang Pharmaceutical University Animal Center.

### 2.4. In vivo release behavior of FUAC/HES conjugates after intravenous administration

SD rats weighing 180–220 g were randomly divided into three equal groups (A, B, and C), each of 5 rats. Group A, with B as a reference group was given the same dose via the femoral vein (24 mg/kg as 5-FU) of 5-FU solution and 5-FUAC solution, respectively. The rats were anesthetized at appropriate intervals (2, 5, 10, 15, 20, 30, 45, 60, 75, 90 min, 2, 4, 6 h) and 0.5 ml blood samples were collected from the retro-orbital sinus and transferred into heparin treated tubes, and then immediately centrifuged at 4000 rpm for 10 min to obtain plasma samples. The plasma samples were stored at −20 °C until required for analysis. Group C was used as a test group and given a solution of FUAC/HES conjugates (12 mg/kg as 5-FU) via the femoral vein. Then, 0.5 ml blood samples were collected at 5, 10, 15, 20, 30 min, 1, 2, 4, 6, 9, 12, 24 h and processed as above. The experimental procedures and the animal use and care protocols were approved by the Committee on Ethical Use of Animals of Shenyang Pharmaceutical University Animal Center.

The pharmacokinetic data were analyzed by drug and statistics (DAS) version software 2.0 (Mathematical Pharmacology Professional Committee of China, Shanghai, China). Differences between groups were considered statically significant when comparisons between groups yielded a value for $P < 0.05$ using statistical analyses performed with the Statistical Package for Social Science (SPSS).

## 3. Results and discussion

### 3.1. Synthesis and characteristic of FUAC/HES conjugates

FUAC was prepared with a melting point at 278 °C measured in a micromelting point apparatus (Ref. 277–279 °C). FUAC/HES conjugates were synthesized using DMAP as an acyl transfer catalyst, and DCC as a condensing agent, and purified by Sephadex column chromatography in high yields (>95%) without any free FUAC. The $^1$H NMR and IR data showed that FUAC was chemically conjugated to HES through an ester bond, $^1$H NMR (DMSO) shown in Fig. 1: $\delta$ (ppm) 3.2–3.75 and 4.45–4.9 (CH–OH, and –$CH_2$O, HES), 3.35 (N–$CH_2$–COO–), 5.14 (CH–OCO), 7.94 ($C_6$–H, FU). The peaks from the IR analysis (KBr) were as follows: 3420 (O–H), 2925 (C–H, stretch, aliphatic and alkene), 1700 (C=O, ester), 1675 (C=O, carboxylic acid), 1609, 1510 (C=C), 1370 (C–N), 1250 (C–F). As seen in Fig. 2, 5-FU has an exothermic peak at 290 °C. The exothermic peak of FUAC shifted to 281 °C showing that the derivative of 5-FU

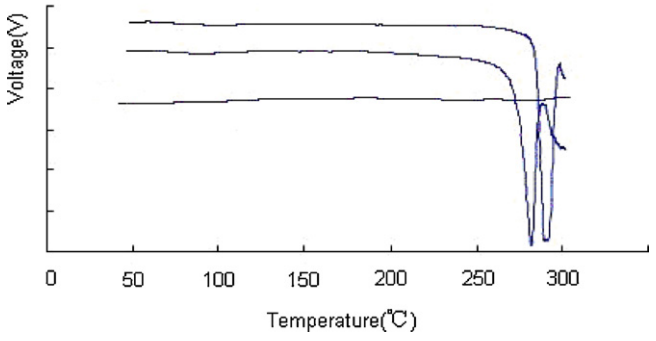



**Fig. 2.** DSC thermogram of samples, from top to bottom: 5-FU, FUAC, and FUAC/HES conjugates.

**Table 1**
Kinetic data for hydrolytic degradation of conjugates in PBS at different pH values at 37 °C and 60 °C.

| pH value | 37 °C | | 60 °C | | $E_{obs}$ (kJ/mol) |
|---|---|---|---|---|---|
| | $k_{obs}$ (l/h) | $t_{1/2}$ (h) | $k_{obs}$ (1/h) | $t_{1/2}$ (h) | |
| 1.2 | 0.0023 | 301.3 | 0.0079 | 87.7 | 46.85 |
| 2.5 | 0.0022 | 315.0 | 0.0101 | 68.6 | 57.86 |
| 5.8 | 0.0012 | 577.5 | 0.0076 | 91.2 | 70.07 |
| 7.4 | 0.0130 | 53.3 | 0.0812 | 8.5 | 69.54 |
| 8 | 0.0178 | 38.9 | 0.1041 | 6.7 | 67.05 |
| 10 | 0.09002 | 7.7 | 0.3408 | 2.0 | 50.54 |

$k_{obs}$, the observed hydrolysis rate constant; $t_{1/2}$, half-life; $E_{obs}$, the observed activation energy.

was generated after carboxymethylation. The DSC thermogram of the final FUAC/HES conjugates was smooth and no characteristic peaks of 5-FU or FUAC appeared which suggested that linking the drug molecule to the amorphous carrier reduces the degree of drug crystallization so that there were no exothermic peaks. All these results confirmed that FUAC was successfully covalently bound to HES.

The content of FUAC loaded in the conjugates depends on the hydroxyl groups in HES and the reaction conditions without water. After repeated experiments, the content was basically stable at 15% meaning that 15 mg FUAC was loaded in 100 mg conjugate.

### 3.2. In vitro stability of FUAC/HES conjugates

As 5-FU was not detected in any buffer, FUAC/HES conjugates degraded according to first-order kinetics releasing FUAC as same as the hydrolysis of FUAC and other macromolecular conjugates (Hao et al., 2006; Jiang et al., 2008; Udo et al., 2010; Wang et al., 2007). Chung (Sang Mok et al., 1991) explained that FUAC might be too stable to degrade to 5-FU in acidic or alkaline solution. Stability studies performed in the various media using 12 h incubation showed that no matter what the temperature of the media was, the conjugate was relatively stable in acidic buffer solution at pH 5.8 in which the FUAC cumulative release was less than 3% at 37 °C and 18.0% at 60 °C. However, it was more sensitive to hydrolysis when the pH changed from 7.0 to 10.0. The observed hydrolysis rate constant ($k_{obs}$) could be calculated for FUAC using the pseudo-first-order kinetics equation. The results shown in Table 1 indicate that a specific base-catalysis was involved in the hydrolysis of the ester conjugate which was usually observed in weak alkaline solution in previous studies. It is known that the hydroxide ion supplied as a strong nucleophile by the media directly attacks the ester carbonyl leading to transfer of the hydrolysis balance of the ester bond irreversibly increasing the hydrolytic rate constant. Larsen (1989) suggested that another reason may be that the intramolecular hydrogen bonding is affected by the adjacent hydroxy groups which prevents the hydrolytic degradation. With an increase in the pH, the hydrogen bond is destroyed and the hydrolysis is accelerated. The degree of hydrolysis was also related to the temperature of the medium. Hydrolysis was more marked at 60 °C under the same conditions. According to the Arrhenius equation, the observed activation energy ($E_{obs}$) could be calculated from the data at 37 °C and 60 °C. The average of the observed activation energy was 60.32 ± 10.08 kJ/mol. Fig. 3 clearly shows that the degree of hydrolysis is strongly dependent on the pH and temperature.

As shown in Fig. 4, the cumulative release of FUAC from the conjugates in rat plasma and human plasma was 62.0% and 52.3%. There were no obvious differences in the release profiles of FUAC in rat and human plasma which suggested that the pharmacokinetic parameters in rats reflected those in humans since there may be similar enzymes present in the two types of plasma used for

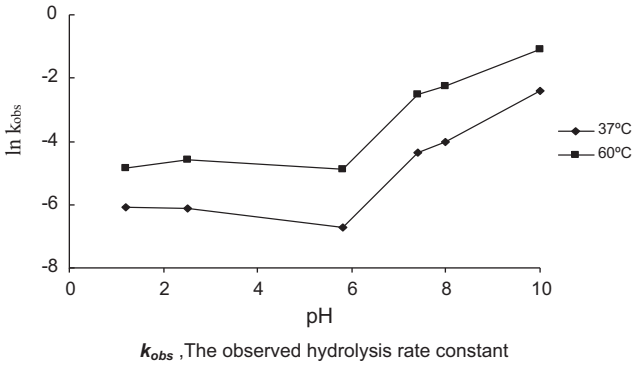



**Fig. 3.** Plots of rate constant versus pH in PBS with different pH values at 37 °C and 60 °C.

the metabolism of the FUAC/HES conjugates. The release profiles fitted first-order kinetics. The half-life calculated for FUAC was 20.4 h for rat plasma and 24.6 h for human plasma. The release of FUAC in the both types of plasma diluted to concentrations of 30% with physiological saline was faster than that in PBS at pH 7.4, showing that the presence of esterases and their isoenzymes helped to catalyze the cleavage of the ester bond (Lockridge & Quinn, 2010). At the same time, the results predicted that the conjugate could act as a prodrug to release FUAC since the circulation time could be significantly prolonged by linking the drug to a macromolecular polymeric chain.

It was noted that both 5-FU and FUAC were detected after a 12 h incubation of the conjugate in rat liver homogenate at 37 °C. The release of FUAC was smooth and there was no apparent rapid increase, being far slower than that in plasma, while there was a rapid increase in the release profile of 5-FU shown in Fig. 4. Rat liver homogenate is rich in esterases, like plasma, which would help free FUAC. The degradation to 5-FU from the conjugate may be carried out chemically or enzymatically in the liver via the different processes shown in Scheme 2. The probable mechanism involves the nucleophilic attack on the carbon connected to the nitrogen or possible hydroxylation of the carbon leading to free 5-FU, although the exact mechanism of the release of 5-FU from FUAC/HES conjugate remains unknown. Chung (Sang Mok et al., 1991) synthesized a 5-flourouracil-1-acetic acid human serum albumin conjugate which was also degraded to 5-FU in liver homogenate, but no 5-FU was found in rat liver homogenate when FUAC was incubated for 24 h. We believe that it is easier to release 5-FU when coupled to a macromolecule compared with FUAC.

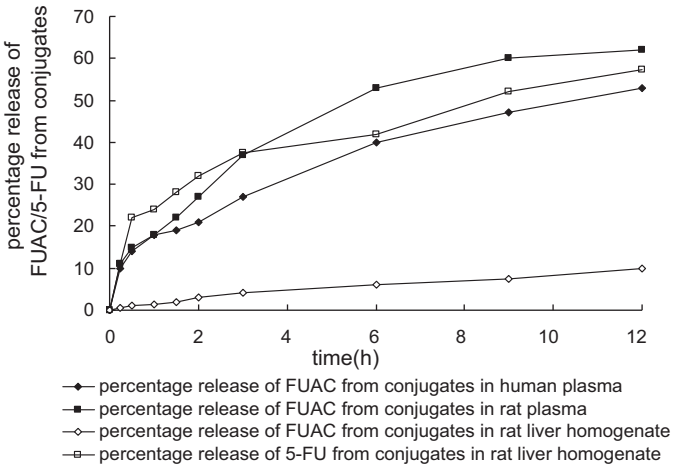



**Fig. 4.** Percentage release of metabolites in plasma and rat liver homogenate at 37 °C.

**Table 2**
Pharmacokinetic parameters of group A, B, and C after intravenous injection to rats ($n = 5$).

| Parameters | Unit | Group A[a] | Group B[b] | Group C[c] |
|---|---|---|---|---|
| $AUC_{(0-t)}$ | mg/L·min | 816.31 ± 209.744 | 3160.879 ± 994.815 | 3850.795 ± 1239.174 |
| $MRT_{(0-t)}$ [d] | min | 18.955 ± 4.458 | 21.119 ± 5.687 | 117.47 ± 39.146 |
| $t_{1/2}$ [d] | min | 17.117 ± 5.394 | 40.071 ± 21.809 | 121.618 ± 49.98 |
| CL[d] | L/min/kg | 0.031 ± 0.012 | 0.014 ± 0.003 | 0.006 ± 0.001 |
| V | L/kg | 0.739 ± 0.272 | 0.803 ± 0.37 | 0.603 ± 0.311 |

The data are mean ± S.D. ($n = 5$). $t_{1/2}$, half-life; AUC, area under the concentration–time curve; MRT, mean residence time; CL, clearance; V, apparent volume of distribution.
[a] Pharmacokinetic parameters of 5-FU after administration of 5-FU solution 24 mg/kg as 5-FU.
[b] Pharmacokinetic parameters of FUAC after administration of FUAC solution 24 mg/kg as 5-FU.
[c] Pharmacokinetic parameters of FUAC after administration of the conjugates solution 12 mg/kg as 5-FU.
[d] Significant difference between group B and C ($P < 0.05$).

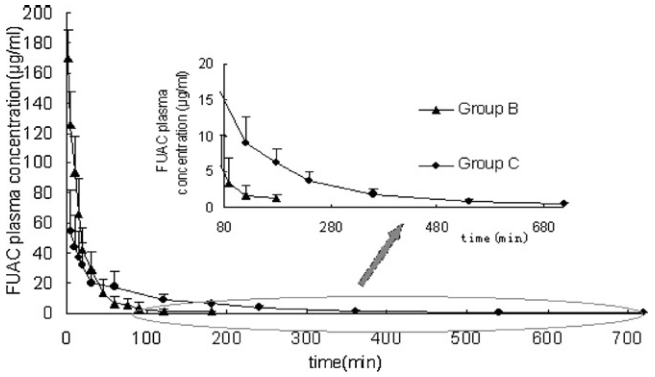


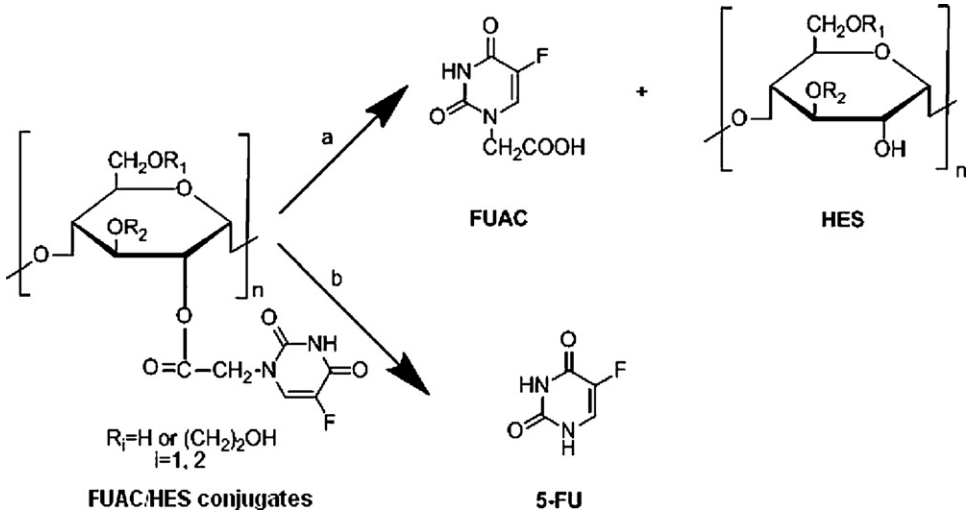

**Fig. 5.** Mean plasma concentration–time profiles of FUAC (group B, 24 mg/kg as 5-FU) and FUAC/HES conjugates (group C, 12 mg/kg as 5-FU) after intravenous administration to rats ($n = 5$). The inset figure is the detailed view of mean FUAC plasma concentration–time profiles from group B and group C time range from 80 min to 720 min.

### 3.3. *In vivo release behaviors of FUAC/HES conjugates after intravenous administration*

The mean plasma concentration–time curves of FUAC from group B and C are shown in Fig. 5. The plasma concentrations of FUAC in group B following a dose of 24 mg/kg as 5-FU reached a mean peak concentration of 169.7 μg/ml after intravenous injection and declined rapidly with a half-life of 40.1 ± 21.8 min. The FUAC plasma concentrations of two of the five rats was below the lower limit of quantification after 180 min while, as soon as the conjugates from group C were given as a dose of 12 mg/kg as 5-FU by injection into blood vessels, the ester bond broke releasing FUAC to give a mean peak concentration of 54.5 μg/ml with a half-life of 121.618 ± 49.98 min. It was worth noting that there was no sign of arise in the FUAC plasma concentration profile at the initial intervals and FUAC could be detected up until 720 min.

All in all, the FUAC plasma concentration in group B fell quickly showing a very fast distribution, while the FUAC in plasma from group C was the result of the release of the FUAC/HES conjugates and distribution within the body followed by a slower elimination. The difference between the profiles of the two groups is due to the following reasons. HES (130/0.4) is largely composed (nearly 98%) of highly branched amylopecin (Westphal et al., 2009). FUAC covalently linked to HES macromolecules which exist under two conditions: one is exposure on the surface of macromolecules; the other is concealment in the starch branches. Immediately after intravenous injection of the conjugates, the exposed ester bond breaks releasing FUAC. However, the rate of degradation of FUAC in plasma is based on the enzymolysis in vitro which does not equal that of distribution so that there is no increase and the plasma concentration of FUAC fell slowly. As for the FUAC hidden in the macromolecules, after HES is degraded into fragments by plasma amylase, the hidden ester bond is exposed and FUAC is released from the fragments. The long time needed for FUAC to escape from HES significantly prolongs the half-life of FUAC in the blood circulation compared with free FUAC.

The prolonged plasma retention of FUAC from the conjugate is undoubtedly associated with the fate of the macromolecular HES in vivo. When conjugates loaded with FUAC were administered, the pharmacokinetic behavior agreed with that of HES. Due to the lack of capillaries in most organs and the size restriction of most tissues, immediately after intravenous injection, macromolecules




**Scheme 2.** The enzymolysis in rat liver homogenate of FUAC/HES conjugates.

are basically locked in the intravascular space. The kidneys play an important role in their fate. Macromolecules smaller than the renal threshold with a molecular weight ranging from 45 to 60 kDa are rapidly excreted in the urine, while larger molecules are degraded by plasma amylase into smaller fragments until they reach the renal threshold for excretion. HES 130/0.4, as the third generation of HES with a mean molecular weight at 130 kDa, are not easily excreted via the kidney and, at the same time, FUAC is released slowly from the conjugates (Fig. 5) in plasma or tissues. The conjugates exhibited more than a 2-fold increase in the AUC of the unit dose when compared with free FUAC for which the AUC of the unit dose was calculated as $131.703 \pm 41.451$ mg/L·min for group B and $320.899 \pm 103.264$ mg/L·min for group C. Therefore, the clearance (CL) following administration of the conjugate could be reduced. The pharmacokinetic parameters of FUAC are shown in Table 2. Some of the conjugate distributed into the liver released 5-FU and FUAC through cleavage of the C–N bond and ester bond according to the study involving rat liver homogenates in vitro. However, the plasma concentration of 5-FU may be too low to be detected.

Since no 5-FU was detected, 5-FU was administered (group A) in order to obtain the pharmacokinetic parameters of 5-FU in rats. The plasma concentration of 5-FU reached a mean peak concentration of 40.9 μg/ml and then fell quickly with a half-life of $17.1 \pm 5.3$ min (Table 2) which agrees with the short half-life of 5-FU in a previous report (Ouchi et al., 1992).

## 4. Conclusion

The low molecular weight anticancer drug FUAC was initially successfully linked to hydroxyethyl starch through an ester bond. FUAC, not 5-FU, was released from the conjugate in vivo. The 5-FU prodrug of HES as a carrier for injection was potential to make up for the deficiency of the tedious injection of 5-FU with high doses to reach effective therapy but always accompanied with toxicity. The pharmacokinetic study indicated that the conjugates exhibit clear sustained-release of FUAC and have potential clinical applications for the treatment of tumors.

## Acknowledgements

The authors thank Cong Liu for valuable discussion; also thank Dr. David for providing language help.

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