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Pharmacokinetics of 5-fluorouracil after intravenous infusion of 5-fluorouracil-acetic acid-human serum albumin conjugates to rabbits

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Summary

The pharmacokinetics of 5-fluorouracil (5-FU) was compared after 30 min intravenous infusion of the same dose (20 mg/kg as 5-FU) of 5-FU (treatment I), 5-FU-acetic acid (5-FU-AA, treatment II) and 5-FU-AA-human serum albumin conjugates (5-FU-AA-HSA, treatment III) to rabbits. After post-infusion, plasma levels of 5-FU declined rapidly with a mean half-life of 8.0 min from treatment I, however, they were not detected until 10–50 min and the mean plasma concentration of 1 μg/ml was maintained from 3 to 24 h for treatment III. It might be possible to maintain constant plasma concentrations of 5-FU for a long period of time by 30 min infusion of 5-FU-AA-HSA conjugates instead of tedious time-consuming infusion of 5-FU. The mean values of 24 h AUC (623 vs 1290 μg min ml^{-1}) were significantly higher from treatment III than that from treatment I. 5-FU was not detected from treatment II nor 5-FU-AA from treatment III in both plasma and urine samples. In treatment II, 5-FU-AA was eliminated rapidly with a mean apparent terminal half-life of 18.7 min based on urinary excretion rate data. 5-FU was not detected in brain after 30 min intravenous infusion of both 5-FU and 5-FU-AA, however, significant amounts of 5-FU were found in brain after administration of 5-FU-AA-HSA conjugates. The in vitro release of 5-FU from 5-FU-AA-HSA conjugates was increased in the presence of protease or liver homogenates, however, 5-FU was not detected for up to 24 h incubation of 5-FU-AA with the various solutions.

Introduction

5-Fluorouracil (5-FU), a uracil analogue, has been widely used in chemotherapy of various types of neoplastic disease (Balis et al., 1983; Calebresi and Parks, 1985). It is usually administered intravenously (i.v.) over a long period of time (e.g., 12 h) in order to maintain adequate concentrations of 5-FU in cancer cells.

The ideal dosage form in cancer chemotherapy is 'the one that provides a specific delivery of anticancer drug to the tumor site in a sufficient amount, for a long period of time with no interaction with the normal tissues' (Yoshioka et al., 1981). For this purpose, anticancer drug-macro-

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molecules (such as human serum albumin, HSA or polypeptides) conjugates were synthesized (Chu and Whiteley, 1977) and their in vitro anticancer activities were reported (Chu and Howell, 1981; Kato et al., 1982; Trouet and Masquelier, 1982; Roos et al., 1984; Halbert et al., 1987). It was reported that albumin which is synthesized in the liver is not confined to intravascular space, and that over 30% of total exchangeable albumin might be in extravascular space, such as in muscle and skin (Jusko and Gretch, 1976). Moreover, it was found that albumin is absorbed into the neoplastic cell (Bushi et al., 1961) and is actively taken up into tumor cells by pinocytosis (Ryser, 1963). Methotrexate (MTX)-rabbit serum albumin (RSA) conjugates seemed to be taken up into tissues and MTX is released slowly from the conjugates when they were infused into rabbits (Yoon et al., 1990). The results led us to study 5-FU-acetic acid (AA)-HSA conjugates as a means of maintaining constant concentrations of 5-FU in tissues or plasma instead of tedious, lengthy infusion of 5-FU.

The introduction of the AA group into the N^1-position of 5-FU has been attempted to improve the antitumor activities of 5-FU (Baker and Cheda, 1965; Tada, 1975). Since there is no functional group to make a conjugate with HSA in the structure of 5-FU, 5-FU-AA which has a carboxylic group has been chosen to make a conjugate with HSA in the present study.

The purpose of this paper is to report the pharmacokinetics and tissue distribution of 5-FU after i.v. infusion of the same dose (20 mg/kg as free 5-FU) of 5-FU, 5-FU-AA, and 5-FU-AA-HSA conjugates to rabbits. The in vitro release of 5-FU from 5-FU-AA and 5-FU-AA-HSA conjugates in the various solutions is also discussed.

Materials and Methods

In vitro release of 5-FU from 5-FU-AA and 5-FU-AA-HSA conjugates

5-FU-AA or 5-FU-AA-HSA conjugates, equivalent to 2 mg as 5-FU, were incubated in a water-bath shaker kept at 37 °C and a rate of 50 oscillations per min with 50 ml of phosphate buffer of pH 7.4, the buffer being in the presence of 200 mg of protease (activity of 31 400 U/g at pH 8.0, kindly supplied by Dong-A Pharm. Co., Seoul, Korea), human plasma and rat liver homogenate (liver homogenized with 5 volumes of the buffer), respectively. The syntheses of 5-FU-AA (Tada, 1975) and 5-FU-AA-HSA conjugates (Lee et al., 1989) have been reported previously. The possible structures of 5-FU-AA-HSA conjugates are shown in Fig. 1.

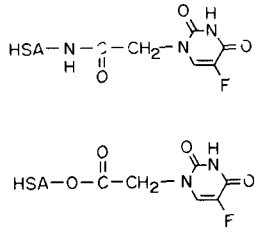

Fig. 1. Possible structures of 5-fluorouracil-acetic acid-human serum albumin (HSA) conjugates.

Animals

Twenty-four male, New Zealand white rabbits (A–X, 1.60–2.70 kg) were anesthetized with 50–100 mg of i.v. ketamine (kindly supplied by Yu-Han Pharm. Co., Seoul, Korea). The carotid artery and jugular vein were catheterized with silastic tubing (Dow Corning, Midland, MI). The cannulas were exteriorized on the dorsal side of the neck where each cannula terminated in a three-way stopcock. The animals were allowed to recover for 4–5 h before the study. Urine samples were collected using a pediatric Foley catheter (Dover, Searle Medical Products, USA Inc., Dallas, TX) which was introduced into the urinary bladder.

Intravenous infusion study

The same dose (containing 20 mg/kg as 5-FU) of 5-FU (250 mg/5 ml, Roche Co. Nutley, NJ), 5-FU-AA, and 5-FU-AA-HSA conjugates, which were freshly diluted with or dissolved in 0.9% NaCl injectable solution to make 25 ml, were infused over 30 min using an infusion pump (Furue Science Co., Japan) via the jugular vein of rabbits A–D (treatment I), E–H (treatment II) and I–L (treatment III), respectively. The blood samples were withdrawn (0.5 ml each) via the carotid artery,

and heparinized normal saline (10 U/ml; 3 ml) was used for flushing the cannula after each blood sampling. The blood sampling times were −30 (to serve as a control), −20, −10, −5, 0 (the end of infusion), 1, 5, 10, 20, 30, 40, 50, 60, 90, 120, 180, and 240 min, and every 2 h for up to 24 h after the infusion. The blood samples were centrifuged immediately to minimize the potential 'blood storage effect' in the plasma concentration determination of 5-FU or 5-FU-AA (Lee et al., 1981, 1984). Two 0.1-ml aliquots of plasma were stored in the freezer prior to the HPLC assay. Urine samples were collected at $-\frac{1}{2}$–0, 0–$\frac{1}{2}$, $\frac{1}{2}$–1, 1–1$\frac{1}{2}$, 1$\frac{1}{2}$–2, 2–3, 3–4, 4–8, 8–12, and 12–24 h after the dose. 10 ml of distilled water or air was used for flushing the bladder just before the end of each urine collection period. The washings were then combined with the urine, and the total volume was measured. Two 0.1-ml aliquots of the combined urine were stored in the freezer prior to the HPLC assay.

Tissue distribution study

The same dose (containing 20 mg/kg as 5-FU) of 5-FU, 5-FU-AA, and 5-FU-AA-HSA conjugates was similarly infused over 30 min using the infusion pump to rabbits M–P (treatment IV), Q–T (treatment V), and U–X (treatment VI), respectively. After 30 min of infusion, blood was collected through the carotid artery as much as possible, and each rabbit was exsanguinated. Approx. 1 g of each organ or tissue was quickly removed, rinsed, minced and homogenized with 4 volumes of Soerensen phosphate buffer of pH 7.4 (Kar et al., 1986) in a tissue homogenizer (Tissuemizer, Tekman Co., Model SDT-1810, Cincinnati, OH). Plasma was also diluted 4 times with the buffer. After centrifugation, two 0.1-ml aliquots of the diluted plasma or the supernatant of tissue homogenates were stored in the freezer prior to the HPLC assay.

HPLC analysis

The concentrations of 5-FU in biological samples were measured by the reported HPLC method (Kar et al., 1986). The concentrations of 5-FU-AA were also measured in this HPLC system. The retention time of 5-FU-AA was approx. 3.0 min

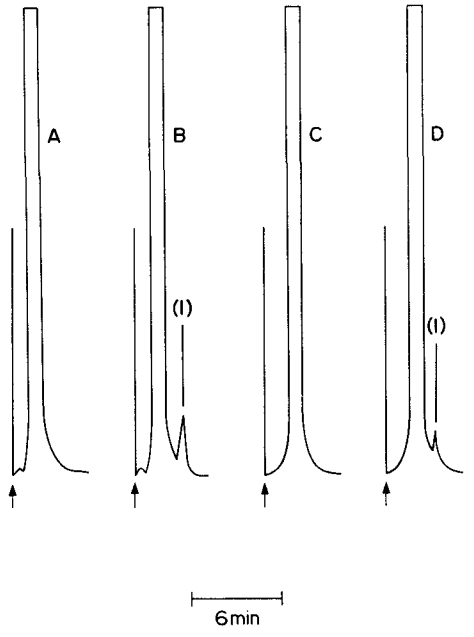

Fig. 2. Chromatograms of: (A) blank rabbit plasma; (B) rabbit plasma spiked with 5 μg/ml of 5-FU-AA; (C) blank rabbit urine; (D) rabbit urine spiked with 30 μg/ml of 5-FU-AA. Peak (1), 5-FU-AA. Detector setting: 0.01 a.u.f.s. Recorder setting: 2 mV for plasma and 20 mV for urine. The arrows mark the points of injections.

(Fig. 2), and detection limits were 2.0 and 10.0 μg/ml for plasma and urine, respectively. The inter-day and intra-day coefficients of variation of 5-FU-AA were less than 4.68 and 6.59% for plasma and urine, respectively. In this study, the concentrations of 5-FU-AA-HSA conjugates were not measured.

Pharmacokinetic analysis

The areas under the plasma concentration-time curves from time zero to time infinity (AUC) were calculated by the trapezoidal rule-extrapolation method (Chen et al., 1982). Standard methods (Riegelman and Collier, 1980; Chen et al., 1982) were used to calculate the following parameters; the time-averaged total body (CL), renal (CL_R), and nonrenal (CL_{NR}) clearances, area under the first-moment of the plasma concentration-time curve (AUMC), mean residence time (MRT), and

apparent volume of distribution at steady state (V_{SS});

$$CL = dose/AUC \quad (1)$$

$$CL_R = X_U/AUC \quad (2)$$

$$CL_{NR} = CL - CL_R \quad (3)$$

$$AUMC = \int_0^\infty t \times C_p \, dt \quad (4)$$

$$MRT = (AUMC/AUC) - T/2 \quad (5)$$

$$V_{SS} = CL \times MRT \quad (6)$$

where X_U is the amount of 5-FU or 5-FU-AA excreted in the urine to time infinity, C_p is the plasma concentration of 5-FU or 5-FU-AA at time t, and T is the infusion time.

The mean values of clearance, apparent volume of distribution at steady state and the half-life were calculated by the harmonic mean method (Chiou, 1979).

Statistical analysis

The data were analyzed for statistical significance ($p < 0.05$) by unpaired t-test.

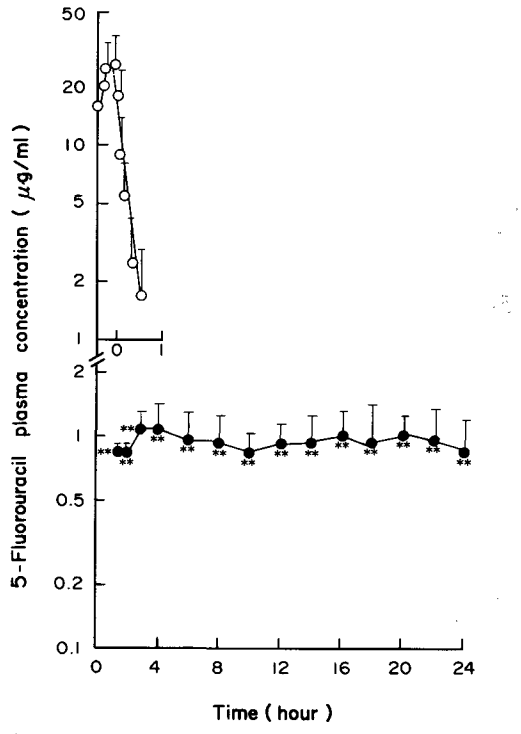

Fig. 3. Mean plasma concentration-time profiles of 5-fluorouracil (5-FU) after 30-min intravenous infusion of 5-FU (○), 20 mg/kg and 5-FU-acetic acid-human serum albumin conjugates (●), 20 mg/kg as 5-FU to rabbits A-D and I-L, respectively. Bars represent standard deviation. ** $p < 0.01$.

Results and Discussion

The mean plasma concentration-time curves of 5-FU from treatments I and III are shown in Fig. 3. The plasma concentrations of 5-FU rose rapidly during infusion with a mean peak concentration (at time 0) of 26.9 μg/ml, and declined quickly post-infusion with a mean apparent terminal half-life of 8.0 min from treatment I. The shorter half-life of 5-FU, 10–20 min, was also reported in human studies (Balis et al., 1983; Calebresi and Parks, 1985). However, the plasma concentrations of 5-FU from treatment III were quite different with those from treatment I; 5-FU was not detected in plasma until 10–50 min post-infusion and approx. 1.0 μg/ml of 5-FU was maintained from 3 to 24 h post-infusion in all four rabbits studied. It was reported that although albumin is a large molecule, it is not confined to the intravascular space, and over 30% of total exchangeable albumin which is synthesized in the liver might be in extravascular space, such as muscle and skin (Jusko and Gretch, 1976). Moreover, albumin is reported to be taken up actively into tumor cells by pinocytosis (Ryser, 1963). MTX-RSA conjugates seemed to be taken up into tissues and MTX was released slowly from the conjugates when the conjgates were infused into rabbits (Yoon et al., 1990). Therefore, it could be expected that some of the injected 5-FU-AA-HSA conjugates were taken up into tissues and that the remainder were present in plasma. The constant plasma concentration of 5-FU from 3 to 24 h post-infusion from treatment III might be due to the slow release of 5-FU from 5-FU-AA-HSA conjugates which are taken up into the tissues or present in plasma, and was expected based on in vitro release

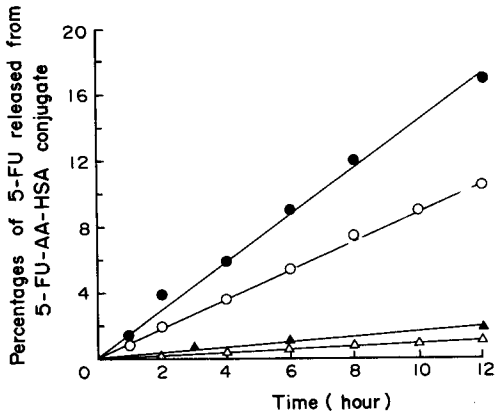

Fig. 4. Percentages of 5-fluorouracil (5-FU) released from 5-FU-acetic acid-human serum albumin (5-FU-AA-HSA) conjugates as a function of time after incubation of the conjugates with phosphate buffer of pH 7.4 (▲), the buffer in the presence of protease (●), human plasma (△), and rat liver homogenate (○), respectively, at 37 °C and 50 oscillations per min.

studies of the conjugate. 5-FU was released from the 5-FU-AA-HSA conjugates via 'apparent' zero-order kinetics; approx. 1.9, 17.0, 1.2, and 10.3% of 5-FU was released from the conjugates for up to 12 h of incubation with phosphate buffer of pH 7.4, the buffer in the presence of protease, human plasma and rat liver homogenates, respectively (Fig. 4). Blood sample was also collected at 36 h post-infusion in rabbit K, and the plasma concentration of 5-FU at that time was also approx. 1.0 μg/ml. The constant plasma concentration of 5-FU from treatment III implies that it might be possible to maintain a constant plasma concentration of 5-FU for longer than 24 h by 30 min infusion of 5-FU-AA-HSA conjugates instead of tedious, time-consuming infusion of 5-FU. In the preliminary study, 5-FU-AA-HSA conjugates were also similarly infused to four other rabbits and blood samples were collected for up to 8 h post-infusion; the 5-FU plasma concentrations of approx. 1 μg/ml were also maintained from 3 h to 8 h post-infusion in all four rabbits studied (Chung, 1989).

Some pharmacokinetic parameters of 5-FU from treatments I and III are listed in Table 1. Since the 'apparent' terminal phase of 5-FU in plasma was not reached for up to 24 h post-infusion from treatment III (Fig. 3), only AUC and renal clearance were estimated based on 24 h data from treatment III. 5-FU was detected in neither plasma nor urine samples after 30 min and 8 h post-infusion, respectively, from treatment I. Therefore, the pharmacokinetic parameters from treatment I which are listed in Table 1 could be very close to the values based on 24 h data. The 5-FU-AA-HSA conjugates themselves are not excreted via kidney nor are they metabolized, and 5-FU is released slowly from the conjugates (Fig. 4) in tissues and plasma, the 24 h AUC was approx. 2-times higher from treatment III than that from treatment I. Therefore, the total body clearance per kg body weight could be significantly reduced from treatment III, although the exact value could not be estimated in this study. However, the renal clearance per kg body weight was not significantly different between treatments I and III (Table 1). 5-FU was extensively eliminated via nonrenal clearance from treatment I; the fraction of nonrenal clearance to total body clearance was 0.919, and a similar result was also reported in human studies (Balis et al., 1983). Therefore, the percentages of dose excreted in 24 h urine as 5-FU was low; the mean value was 7.47% from treatment I. The lower value of MRT from treatment I indicated that 5-FU was eliminated rapidly from rabbits.

Since 5-FU-AA was detected in neither plasma nor urine samples from treatment III (possibly due to our HPLC sensitivity), 5-FU-AA was infused (treatment II) in order to obtain some

TABLE 1

Pharmacokinetic parameters of 5-fluorouracil after 30 min infusion of 5-fluorouracil (20 mg/kg) and 5-fluorouracil-acetic acid-human serum albumin conjugate (20 mg/kg as 5-fluorouracil) to rabbits A–D (treatment I) and I–L (treatment III), respectively

	Treatment I	Treatment III
AUC (μg min ml^{-1}) [b]	623 ± 135 [a]	1290 ± 422
$t_{1/2}$ (min)	8.01 ± 3.24	
V_{SS} (ml kg^{-1})	775 ± 71.0	
CL (ml min^{-1} kg^{-1})	32.1 ± 6.64	
CL_R (ml min^{-1} kg^{-1})	2.40 ± 1.01	1.32 ± 0.69
CL_{NR} (ml min^{-1} kg^{-1})	29.5 ± 5.51	
MRT (min)	9.68 ± 2.64	

[a] Mean ± SD.
[b] $P < 0.05$.

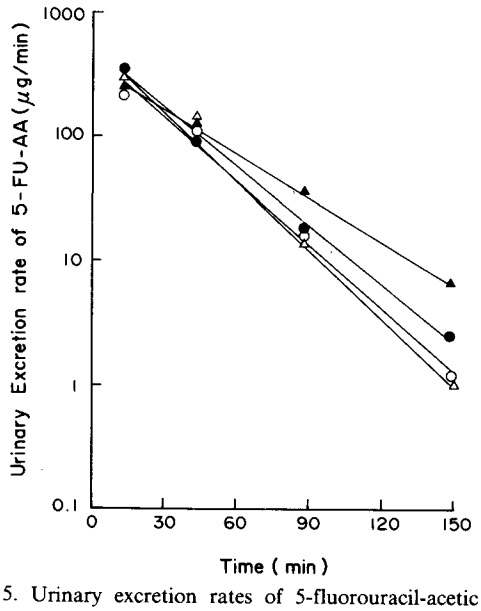

Fig. 5. Urinary excretion rates of 5-fluorouracil-acetic acid (5-FU-AA) as a function of time after 30 min intravenous infusion of 5-FU-AA, 20 mg/kg as 5-FU to rabbit E (●), F (○), G (▲), and H (△), respectively.

pharmacokinetic parameters of 5-FU-AA in rabbits. The plasma concentrations of 5-FU-AA increased during infusion with a mean peak concentration of 11.3 μg/ml (ranging from 7.92 to 15.7 μg/ml) and declined rapidly post-infusion with a mean apparent terminal half-life of 18.7 ± 2.30 min (ranging from 16.6 to 22.0 min) based on urinary excretion rate data (Fig. 5). The elimination of 5-FU-AA was also very fast; the mean total body clearance per kg body weight (AUC from last plasma sampling time to time infinity was estimated by dividing the last plasma concentration of 5-FU-AA by elimination rate constant obtained from urinary excretion rate data) was 37.8 ± 13.4 ml/min per kg (ranging from 25.1 to 55.7 ml/min per kg), and approx. 60.9% (ranging from 45.1 to 76.3%) of the dose was excreted in 3 h urine post-infusion. Since the formation of conjugate such as glucuronidation of 5-FU-AA is quite possible, the rests of the dose could be, at least partly, eliminated by the formation of conjugate. It is of interest to note that 5-FU was detected in neither plasma nor urine samples from treatment II, which was expected based on in vitro release studies of 5-FU-AA; 5-FU was not detected after 24 h incubation of 5-FU-AA with phosphate buffer of pH 7.4, the buffer containing protease, human plasma and rat liver homogenate, respectively. Therefore, it could be concluded that 5-FU which is detected in both plasma and urine samples from treatment III is 'mainly' released directly from 5-FU-AA-HSA conjugates without breaking the bond between 5-FU-AA and HSA. 5-FU could be released chemically or enzymatically from 5-FU-AA-HSA conjugates in rabbits by the following mechanisms; nucleophilic attack on the carbon attached to 5-FU, or hydroxylation on the carbon and spontaneous breaking of the $-CH_2-N<$ bond (Fig. 1). However, the exact mechanism of release of 5-FU from 5-FU-AA-HSA conjugates in rabbits remains unknown.

The amounts of 5-FU remaining per g tissue after 30 min of infusion from treatments IV and VI are listed in Table 2. In treatment IV, tissue to plasma (T/P) ratios of 5-FU were lower than unity in all tissues studied, indicating that the affinity of 5-FU to those tissues studied was lower than that of plasma. 5-FU was not detected in

TABLE 2

Amounts (μg/g tissue) of 5-fluorouracil remaining in tissue after 30 min infusion of 5-fluorouracil (20 mg/kg) and 5-fluorouracil-acetic acid-human serum albumin conjugate (20 mg/kg as 5-fluorouracil) to rabbits M–P (treatment IV) and U–X (treatment VI), respectively

Tissue	Treatment IV		Treatment VI
	Amount (μg/g tissue)	T/P	Amount (μg/g tissue)
Plasma [c]	11.73 ± 3.40 [a]	1.00	N.D. [b]
Brain [c]	N.D.	–	8.65 ± 2.30
Fat [c]	N.D.	–	10.11 ± 4.82
Lung [c]	8.54 ± 1.56	0.728	4.93 ± 1.18
Liver	4.86 ± 2.78	0.414	5.94 ± 2.30
Stomach	7.87 ± 5.99	0.671	9.05 ± 3.85
Kidney	8.27 ± 4.75	0.705	6.27 ± 2.52
Mesentery	5.95 ± 1.19	0.507	17.53 ± 15.4
Small intestine	10.37 ± 1.50	0.884	8.66 ± 3.43
Large intestine	11.11 ± 1.50	0.947	7.51 ± 1.01
Muscle	4.20 ± 1.50	0.358	5.09 ± 0.13
Heart	N.D.	–	N.D.

[a] Mean ± SD.
[b] Not detectable.
[c] $P < 0.01$.

brain, fat and heart from treatment IV. In treatment VI, 5-FU was detected in most of the tissue studied, however, it was not found in plasma and heart. Therefore, the T/P ratios could not be calculated in treatment VI. The amounts of 5-FU remaining per g brain and fat were significantly increased, whereas the values in lung were considerably reduced, in treatment VI as compared with treatment IV. Therefore, it appeared that the lipophilicity of 5-FU-AA-HSA conjugates increased compared with that of 5-FU. 5-FU-AA was detected in none of the tissues studied after administration of 5-FU-AA-HSA conjugates (treatment VI), and is consistent with the result that 5-FU-AA was found in neither plasma nor urine samples from treatment III. It should be noted that 5-FU-AA was detected only in liver and kidney, however, 5-FU was found in none of the tissues studied after administration of 5-FU-AA (treatment V), being also consistent with the results showing that 5-FU was not detected in either plasma or urine samples from treatment II, and that 5-FU was not found for up to 24 h incubation of 5-FU-AA with various solutions as stated in in vitro release studies.

5-FU was not detected in brain from treatments VI and V, however, significant amounts of 5-FU were detected in brain from treatment VI. This indicated that 5-FU-AA-HSA conjugates passed the blood-brain barrier although the exact mechanism remains to be fully explored.

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