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Short communication

Determination of pharmacokinetics of 8-chloroadenosine and its two major metabolites in dogs by high-performance liquid chromatography

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Abstract

High-performance liquid chromatography was used to measure concentration of 8-chloroadenosine (8-Cl-A) and its two major metabolites 8-chloroadenine (8-Cl-Ad) and 8-chloroinosine (8-Cl-I), and their pharmacokinetics in dogs. 8-Cl-A and its metabolites in serum were treated by deproteinization with acetonitrile, then organic impurities were extracted with dichloromethane, followed by centrifuged and direct injection of the supernatant into the liquid chromatograph. After intravenous injection of 8-Cl-A (30 mg/kg), the parent drug and 8-Cl-I were not detected, but the other metabolite, 8-Cl-Ad, was found at a high concentration for 240 min in dog serum. The main pharmacokinetic parameters of 8-Cl-Ad, $t_{1/2\beta}$ and AUC, were 69.30 min and 580 μg min/ml. Our finding indicates that in dogs 8-Cl-A is rapidly metabolized and forms its major metabolites, 8-Cl-Ad and 8-Cl-I. 8-Cl-Ad appeared in many tissues, but 8-Cl-A and 8-Cl-I did not. The concentration of 8-Cl-Ad in dog tissues was highest in the liver and spleen, intermediate in the kidney, intestine, and lowest in the bone marrow, heart, and lungs. However, it was not detected in some liposoluble tissues such as the testes, brain, or uterus. Our study provides useful information for clinical experiment.

Keywords: 8-Chloroadenosine; 8-Chloroadenine; 8-Chloroinosine

1. Introduction

8-Chloroadenosine (8-Cl-A), is a new anti-cancer compound, synthesized by the National Research Laboratories of Natural and Biomimetic Drugs, Beijing Medical University. The chemical name of 8-Cl-A is 8-chloro-β-D-ribofuronosyladenine ($C_{10}H_{12}N_5O_4Cl$). Its chemical structure is shown in Fig. 1, and its molecular weight is 301.5. In early 1995, Fang et al. [1] reported that 8-Cl-A has an obvious inhibitory effect on parenchymatous tumors in mice, in human leukemia HL60, K562 cell. Toxicological study showed that in mice after intravenous injection of 8-Cl-A, the LD_{50} (median lethal dose) was 1025±52.4 mg/kg, and the chronic toxicity of 8-Cl-A was relatively low [2]. Cummings et al. [3] reported a method of determination of 8-Cl-A by high-performance liquid chromatography(HPLC). We established a method of measurement of 8-Cl-A and its matabolites 8-chloroadenine (8-Cl-Ad) and 8 chloroinosine (8-Cl-I) simultaneously in order to study the Pharmacokinetics of this compound in dogs [4]. Pharmacokinetics study of 8-Cl-A in rats showed that 8-Cl-A is rapidly trans-

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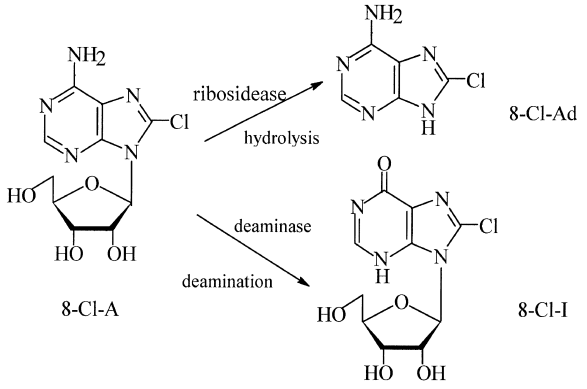

Fig. 1. Chemical structures of 8-chloro-adenosine(8-Cl-A) and its two metabolites, 8-chloroadenine (8-Cl-Ad) and 8-chloroinosine (8-Cl-I).

formed into two main metabolites, 8-Cl-Ad and 8-Cl-I [5] (Fig. 1). In order to further understand the pharmacokinetics of 8-Cl-A in animals, we studied the pharmacokinetics of 8-Cl-A and its main metabolites in dogs.

2. Experimental

2.1. Chemicals and reagents

8-Cl-A, 8-Cl-Ad, and 8-Cl-I were provided by the Scientific Research Group, College of Pharmacy, Beijing Medical University, Beijing, China. Methanol, glacial acetic acid, tetrabutylammonium bromide and dichloromethane were of analytical grade (Beijing Chemical Plant, Beijing, China). Acetonitrile was of chromatographic grade.(Shanghai Institute of Brain, China Academy of Science, Shanghai, China).

2.2. Animals

Beagle dogs (weighing 8.4–9.6 kg, either sex) were provided by the Animal Office of the Fifth Institute, Academy of Military Medical Institute, Beijing, China.

2.3. HPLC conditions

The HPLC system was performed on a Varian system consisting of Varian 9010 pump, Varian 9050 variable-wavelength UV detector, Varian 9020 chromatographic working station, Varian 4270 integrator and rheodyne 7125 injecting valve, all from Varian Assoc. (Walnut Creek, CA, USA). The analytical column was a stainless steel (25 cm×4.6 mm I.D.) packed with 5 μm YWG C_{18} (Beijing Analytical Instrument Plant, Beijing, China) fitted with a guard column packed with C_{18} (Waters Assoc. Milford, MA, USA). The mobile phase consisted of acetonitrile–methanol–1% aqueous acetic acid.(including 0.1% tetrabutylammonium bromide) (3:11:86, v/v). The flow-rate was 1.0 ml/min. The column effluent was monitored at 263 nm. An all-glass filter apparatus with 0.45 μm filters was used to filter the mobile phase before use. All chromatography was done at ambient temperature.

2.4. Sample preparation

The tissue homogenates and unmodified serum samples were treated the same way. Each sample of 0.2 ml was put into a 1.5 ml plastic centrifuge tube with cap and 0.4 ml acetonitrile was added to precipitate protein. The mixture was mingled equally, then vibrated for 5 min, ultrasonicated for 10 min and centrifuged at 7500 *g* for 5 min. Then, the supernatant was put into another tube and 0.4 ml dichloromethane was added to extract organic impurities. The extract was treated in the same way as above. Subsequently, 20 μl aqueous phase of the supernatant was injected into the chromatographic system for analysis.

2.5. Methodological study for the determination of 8-Cl-A and its two metabolites in dog serum

2.5.1. Standard curves

Series of standard solution of 8-Cl-A, 8-Cl-Ad, and 8-Cl-I were added to blank dog serum, which enabled the concentrations of each standard series to be 0.1, 0.5, 1.0, 5.0 and 10.0 μg/ml. Standard curve was evaluated by linear regression analysis and was obtained by plotting peak area against the amount of the standard.

2.5.2. Reproducibility and recovery

The solutions, composed of standards of 8-Cl-A, 8-Cl-Ad, and 8-Cl-I in serum, were made up accurately to enable the concentration of each standard to

be 1.0, 5.0 and 10.0 μg/ml. All samples were dealt with sample's treatment method (2.4). The precision of the assay was determined by replicate analysis samples (n=5). The absolute recovery (extraction efficacy) was determined by comparing the peak area of extracted standard with that of unextracted standard.

2.6. Determination of pharmacokinetics of 8-Cl-A after intravenous infusion in dogs

Two male and two female Beagle dogs were fasted but permitted to drink water for 12 h before administration. The next morning they were given intravenous infusion of 8-Cl-A at dose of 30 mg/kg for 30 min, and blood samples were taken from their forelegs at 0, 15, 30, 45, 60, 90, 120, 150 and 240 min, respectively. The blood samples were centrifuged at 7500 *g* for 5 min, and then the serum was separated and preserved at −20°C for analysis.

2.7. Determination of tissue distribution of 8-Cl-A

The four Beagle dogs were given 8-Cl-A (75 mg/kg) intravenously every day for 35 days. They were killed 36 h after the final drug infusion. The following organs' tissues were removed, symmetrically and identically: the heart, liver, brain, lungs, spleen, kidneys, intestine, fat, bone marrow, testes and uterus. The tissues taken were washed by physiological saline. Then, physiological saline was added to these tissues at the ratio of 1:3 to make up homogenates in the homogenizer, which were preserved at −20°C for analysis.

3. Results

3.1. Determination of 8-Cl-A and its two metabolites in dog serum

The HPLC method established for the determination of 8-Cl-A and its two main metabolites, 8-Cl-Ad and 8-Cl-I, in dog serum was suitable for studying the pharmacokinetics of this compound in dogs. 8-Cl-A, 8-Cl-Ad and 8-Cl-I were well separated. The retention times of 8-Cl-A, 8-Cl-Ad and 8-Cl-I were 20.5, 6.3, and 13.1 min, respectively. The chromatograms are shown in Fig. 2. Standard curves of peak area versus concentration were obtained by analyzing serum standards containing of 8-Cl-A, 8-Cl-Ad, and 8-Cl-I in concentrations ranging from 0.1 to 10.0 μg/ml. The regressive equation and relevant coefficients of standard curves were following: 8-Cl-A: A=12 825+35 890C (r=0.9980); 8-Cl-Ad: A=7551+41 505C (r=0.9966); 8-Cl-I: A=6282+12 911C (r=0.9964). The within-

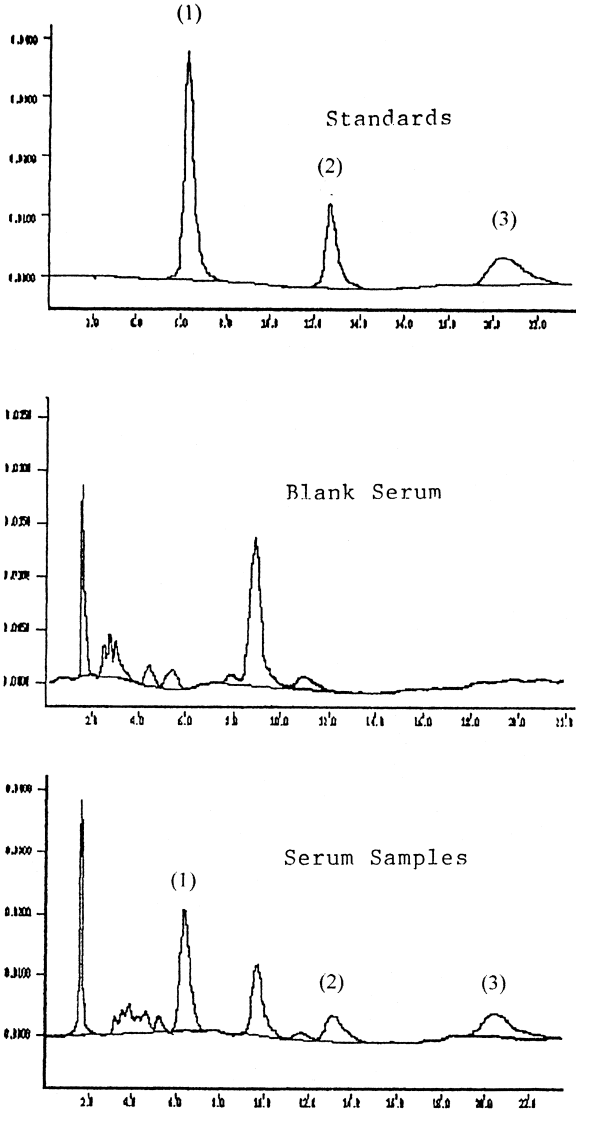

Fig. 2. Chromatograms of (1) 6-Cl-Ad (6.3′), (2) 8-Cl-I (13.1′) and (3) 8-Cl-A (20.5′).

day and day-to-day variations at each concentration were ranged from 0.3% to 21.6%. The mean recoveries were ranged from 54.5% to 76.7% (Table 1). The limits of detection ($S/N=2$) were 4.0, 4.0 and 1.4 ng for 8-Cl-A, 8-Cl-I and 8-Cl-Ad, respectively, while the limits of quantitation ($S/N=10$) were 0.5, 0.5 and 0.1 μg/ml for 8-Cl-A, 8-Cl-I and 8-Cl-Ad, respectively.

3.2. Pharmacokinetics of 8-Cl-A

8-Cl-A and its metabolite 8-Cl-I were not detected in serum samples, but the other metabolite, 8-Cl-Ad, was found at each time (Table 2 and Fig. 3). The main pharmacokinetic parameters of 8-Cl-Ad, $t_{1/2\beta}$ and AUC, were 69.30 min and 580 μg min/ml. The parameters were calculated by the following formulas: $C=A\ e^{-\alpha t}+B\ e^{-\beta t}$, $t_{1/2\beta}=0.693/\beta$, $AUC=A/\alpha+B/\beta$.

3.3. Tissues distribution of 8-Cl-A

The parent compound, 8-Cl-A, and the metabolite, 8-Cl-I, were undetected in all tissues, whereas the metabolite, 8-Cl-Ad, was present in most tissues. The concentration was highest in the liver and spleen, intermediate in the kidney and intestine and lowest in the bone marrow, heart, lung, and fat (Table 3 and Fig. 4). 8-Cl-Ad was not found in highly liposoluble tissues (testes, brain, and uterus). This finding may reflect the fact that 8-Cl-Ad is strongly hydrophilic and does not easily penetrate the blood–testis and blood–brain barriers.

Table 2
Serum Concentration of 8-Cl-Ad in dogs ($n=4$) after i.v. infusion of 8-Cl-A (30 mg/kg)

Time (min)	Conc. (μg/ml)	SD	RSD (%)
0	8.99	1.35	15
15	6.93	1.39	20
30	4.86	0.37	7.6
45	3.72	0.41	11
60	2.39	0.48	20
90	1.84	0.07	3.8
120	1.53	0.28	18
150	0.88	0.22	25
240	0.42	0.19	45

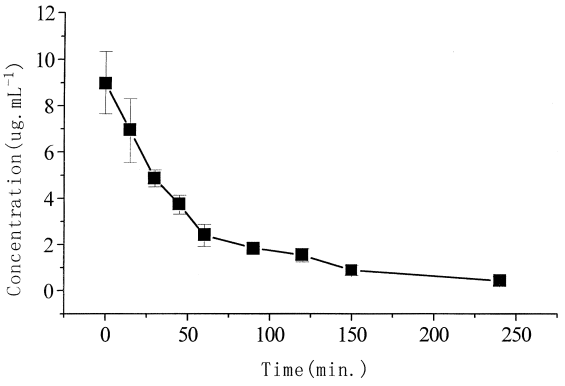

Fig. 3. Mean drug concentration versus time curve of 8-Cl-Ad in serum after i.v. infusion of 8-Cl-A at dose of 30 mg/kg in four dogs.

Table 1
Precisions and Recoveries of 8-Cl-A, 8-Cl-Ad and 8-Cl-I in dog serum ($n=5$)

Compound	Standard Conc. (μg/ml)	Precision (%)		Recovery (%) (mean±RSD)
		Within-day ($n=5$)	Day-to-day ($n=5$)	
8-Cl-Ad	1.00	8.0	21.6	64.4±7.97
	5.00	8.2	16.4	66.4±8.22
	10.00	1.1	13.2	66.2±1.06
8-Cl-I	1.00	0.3	15.7	76.7±9.14
	5.00	8.3	13.8	57.0±8.34
	10.00	0.4	17.0	75.2±0.42
8-Cl-A	1.00	13.2	11.7	68.5±13.2
	5.00	8.1	7.8	70.2±8.08
	10.00	1.5	8.2	54.5±1.51

Table 3
Distribution of 8-Cl-Ad in dog tissues after i.v. infusion of 8-Cl-A (75 mg/kg/day)

Sample	Concentration (μg/g tissue)	SD	RSD (%)
Liver	23.4	1.1	4.7
Spleen	17.7	1.1	6.2
Kidney	10.4	0.90	8.7
Intestine	8.24	0.59	7.2
Marrow	2.53	0.13	5.1
Heart	1.37	0.14	10
Lungs	1.02	0.14	14
Fat	0.62	0.05	8.1

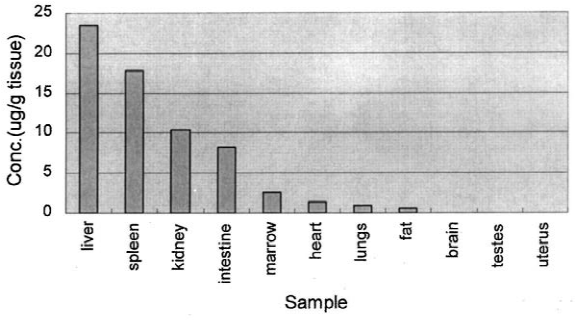

Fig. 4. Distribution of 8-Cl-Ad in dog tissues after i.v. infusion of 8-Cl-A (75 mg/kg/day) for 35 days.

4. Conclusion

A simple, accurate, and reliable HPLC method was established for the determination of 8-Cl-A and its main metabolites, 8-Cl-Ad and 8-Cl-I, in dogs. The results are satisfactory for pharmacokinetics study of 8-Cl-A in dogs. Our finding indicates that 8-Cl-A is mainly biotransformed into 8-Cl-Ad rapidly in dogs. It differs from that of Dou, et al. [6], who found both 8-Cl-Ad and 8-Cl-I in rats.

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