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Short communication

Pharmacokinetics study of calf thymus DNA in rats and beagle dogs with ^{3}H-labeling and tracing method

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ABSTRACT

This study developed a radioisotope detection and tracing method to investigate the pharmacokinetic properties of calf thymus DNA (ctDNA) in rats and beagle dogs. The radioactivity labeling result was detected through gel electrophoresis analysis, and pharmacokinetic analytical methods for ^{3}H-ctDNA in rat and beagle dog plasma were developed, respectively. Full method validation indicated that the established radioisotope method was sensitive, specific, rapid and reliable, and the results were all in accordance with the analysis requirement in biological samples. After intravenous administration of the planned doses of ^{3}H-ctDNA to the rats and beagle dogs, plasma concentrations from the various dose groups declined rapidly. In addition, the radioactive concentration of ^{3}H-ctDNA in the plasma from single and multiple dosings decreased in a similar trend. Through comparative analysis of the pharmacokinetic parameters, we inferred that the elimination of ctDNA accorded with the linear pharmacokinetic characteristic. The results demonstrated that ctDNA was rapidly eliminated in rat and beagle dog plasma and would not accumulate, indicating the safe use of ctDNA as an immunoadsorptive material without bringing out potential risk.

1. Introduction

Nucleic acid drugs have been increasingly developed and used in clinical practice along with biotechnology and biomedicine advancements [1,2]. Biological macromolecule compounds, nucleic acids, and nucleotides have high molecular weight, complicated structure, and can be easily degraded; thus characterizing the pharmacokinetic profile and quantitating these compounds in vivo through conventional approaches in preclinical and clinical pharmacokinetic research are quite difficult [3,4]. At present, commonly used methods include capillary gel electrophoresis (CE), liquid chromatography–tandem mass spectrometry (LC–MS–MS), enzyme immunoassay (EIA), and so on [5–8]. However, all these methods have definite flaws and shortcomings in determining nucleic acid compounds [9–12]. Radioisotope labeling and tracing method is a highly specific and sensitive new analytical technique, which is independent of the molecular structure of analytes [13]. Given its prominent advantages compared with other approaches in excretion and mass balance analysis [14], this technique has been successfully applied for the pharmacokinetic study of nucleic acid drugs [15–17].

Calf thymus DNA (ctDNA), one of the absorptive materials used in leprosy hemodialysis in vitro, effectively clears leprosy bacillus through physical adsorption, in which blood is purified and endogenous diseases are alleviated remarkably [18,19]. However, some ctDNA may fall off from the immunoadsorption column during the application process, which may enter the circulatory system along with the returning purified blood, consequently, bringing potential risk and damage to patients' health. Thus, pharmacokinetic and elimination study of ctDNA must be carried out before its therapeutic use. In our previous study, we evaluated the pharmacokinetic property of ctDNA solely in rats after intravenous administration by applying ^{3}H-labeling, including plasma pharmacokinetics, tissue distribution, mass balance and excretion [20]. However, according to the guiding principles of nonclinical pharmacokinetic research technique, at least two kinds of animals should be used and three dose groups should be set up in an in vivo pharmacokinetic study [21]. Therefore, the research needed to be further deepened to acquire complete pharmacokinetic and disposition information for ctDNA.

The present article aims to investigate the pharmacokinetics of ctDNA according to the guiding principles of nonclinical pharmacokinetic research technique using radioisotope detection, to further evaluate the use of ctDNA as an immunoadsorptive carrier material for in vitro therapy.

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2. Materials and methods

2.1. Chemicals and reagents

The ctDNA was provided by Jianfan Biotechnology Company (Zhuhai, China). Isopropanol was purchased from Sinopharm Chemical Reagent Company. Purified water was prepared through a MilliQ® ultrapure water purification system in XenoBiotic Laboratories, Inc. – China (Nanjing, China). Ultima Gold TM scintillation fluid was purchased from PerkinElmer Life Sciences (Boston, MA). All other reagents were of analytical grade.

2.2. Radiolabeling and verification of ctDNA

Radiolabeling of ctDNA was conducted by Shanghai Institute of Applied Physics, Chinese Academy of Sciences (Shanghai, China). Isotope exchange method was used to synthesize the radiolabeled ctDNA as reported previously. In brief, a certain amount of ctDNA was dissolved in phosphate buffer, and then 5% PdO/$BaSO_4$ was added as catalyst. The exchange reaction was conducted in 50 °C water bath, with continuous stirring. Tritiated product was obtained after removing the unstable tritium. The specific activity of the final preparation was 1.2 mCi/mg. The ^{3}H-labeled ctDNA was stored at 4 °C until use.

Five microliters of ^{3}H-labeled ctDNA was taken and added onto the gel well containing 1% agarose. The gel was cut longitudinally into 10 small pieces after electrophoresis for 40 min. The entire radioactivity in the gel strap, the buffer solution in the electrophoresis system, and the blank gel well were detected with a radioactive analyzer. The radioactive concentration of the initial solution was 8×10^5 DPM/μL (DPM: disintegrations per minute); thus, the gross radioactivity in 5 μL of ^{3}H-ctDNA sample was 4×10^6 DPM (1.8 μCi).

2.3. Preparation of dosing form

Radiolabeled ctDNA was mixed with the "cold" sample for animal experiments. Sufficient ^{3}H-ctDNA diluted with alcohol as solvent was mixed with the unlabeled sample, and then added to 5% glucose solution, followed by heating at 50 °C and continuous stirring until the solution was mixed uniformly. During the administration process, the ^{3}H-ctDNA solution was continuously vortexed at a concentration of 10 μCi/mL.

2.4. Animals

Male and female Sprague-Dawley rats weighing between 200 and 220 g were purchased from the Center of Experimental Animals of the Academy of Military Medical Sciences (Beijing, China). Beagles (10.0 ± 0.5 kg) of both genders were supplied by Zhongshan Xike Experimental Animal Company, Ltd. (Suzhou, China). The animals were kept under controlled temperature and relative humidity, and acclimatized to the housing environment for 1 week before the study. The rats were housed in polypropylene cages (one rat per cage) and allowed access to food and water ad libitum. The beagles were fasted, but had free access to water overnight before the administration. All studies were in compliance with the Guidelines for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee in XBL-China.

2.5. Method validation

2.5.1. Sample processing

The samples included all the calibration curve solutions, quality control (QC) samples, stock solutions, and testing samples. All the samples were adequately vortexed before determination; then 50 μL solutions were placed into scintillation vials and mixed with scintillation fluid. A liquid scintillation counter was used for radioactivity determination.

Table 1
Plasma pharmacokinetic experimental design and sample collection.

Animal	Number and gender	Dose level (mg/kg)	Time points and intervals
Rats	6 Male 6 Female	1.67	0.033, 0.25, 1, 2, 4, 6, 8, 12 and 24 h after administering
	6 Male 6 Female	5[a]	
	6 Male 6 Female	15[a]	
	2 Male 6 Female	0.167	
Beagles	2 Male 1 Female	0.5[a]	
	2 Male 1 Female	1.5	

[a] Animals in the group were administered with single-dose and multiple-dose, respectively.

2.5.2. Linearity and lower limit of quantification (LLOQ)

The ^{3}H-ctDNA solutions containing different radioactivities were mixed with blank rat or beagle plasma. The calibration curve samples at six radioactivity levels were assayed in triplicate, using the measured DPM values used as ordinates (Y) and the calculated ones as abscissa (X). The standard curves of ^{3}H-ctDNA in rat and beagle plasma were obtained by linear regression analysis to demonstrate the linearity of this method.

LLOQ, defined as the lowest radioactivity (DPM) that could be measured accurately, was evaluated by analysis in the five replicates.

2.5.3. Accuracy and precision

Precision and accuracy were assessed by determining the replicate QC samples on the same day (intra-day precision) and three consecutive days (inter-day precision). Accuracy was described by relative error and precision was evaluated by intra- and inter-day relative standard deviation (RSD).

2.5.4. Recovery

Recovery of analytes was evaluated by QC samples, and data were determined by comparing the mean radioactivity obtained from the plasma sample spiked with ^{3}H-ctDNA solution with that of the neat standard samples. Three different radioactivity levels of analytes were evaluated by analyzing the three samples at each level.

2.5.5. Stability

The stability of ^{3}H-ctDNA in rat and beagle plasma was evaluated by analyzing the samples stored at 20 °C for 24 h, 4 °C for 24 h, three successive freeze–thaw (−20 °C) cycles, and −20 °C for 30 days [22,23]. The samples were considered stable when the deviation from nominal radioactivity value was within ±15.0%.

2.6. Application to pharmacokinetic study

The developed method was employed for the pharmacokinetic investigation of ctDNA in rat and beagle plasma. The rats and beagle dogs were randomly divided into three dose groups, as presented in detail in Table 1. ^{3}H-ctDNA was then administered by intravenous injection via the lateral tail vein for rats or the anterior limb vein for beagle dogs. Both pharmacokinetic experiments were conducted once a day or consecutively for seven days in the given dose group for single and multiple dosing. Before dosing, each rat had a