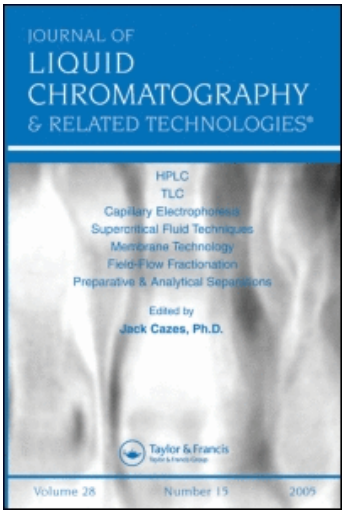

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Determination of Lidamycin: Application in Mice

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Determination of Lidamycin: Application in Mice

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Abstract: A rapid and simple method, radioactivity assay after separation with high performance liquid chromatography (HPLC–RA method), was validated and developed for determination of lidamycin (C-1027) in mouse serum samples. ^{125}I-C-1027 was prepared by the Iodogen method, and separated and purified by size exclusive high performance liquid chromatography (HPLC). The pharmacokinetic parameters after intravenous injection of ^{125}I-C-1027 to mice were as follows: apparent volume of distribution (V_d) were 0.35 ± 0.12, 0.35 ± 0.09, and 0.35 ± 0.05 L/kg; biological half-lives ($T_{1/2\beta}$) were 2.93 ± 1.21, 2.78 ± 1.33, and 2.68 ± 1.02 h; Elimination rate constant (K_e) were 2.27, 2.49, and 2.53 hr^{-1} AUC were 12.55, 57.52, and 130.92 ng hr/mL; Clearance (Cl) were 0.80, 0.87, and 0.76 L/hr/kg at doses of 10, 50, and 100 μg/kg, respectively. The results showed that the radiolabeling method was a specific, accurate, and sensitive way to assay ^{125}I-C-1027 in mouse serum.

Keywords: Lidamycin, pharmacokinetics, radiolabeling, high performance liquid chromatography

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INTRODUCTION

A new macromolecular antitumor antibiotic, lidamycin (C-1027), produced by *Streptomyces globisporus* in soil, consisted of a noncovalently bound apoprotein and a labile chromophore, which was responsible for most of the biological activities, and showed highly potent cytotoxicity to cultured cancer cells and marked DNA cleaving ability.[1–3] The protein moiety of C-1027 has a single polypeptide chain, cross-linked by two disulfide bonds with a molecular weight of 10.5 Da.[4,5] Like other enediyne agents, antibiotic C-1027 was believed to exert its biological activity through the induction of cellular DNA damage.[6,7] After pre-clinical evaluation studies, lidamycin had been carried out as a phase I clinical trial. In pre-clinical studies, we investigated the pharmacokinetics. This article is a study on the pharmacokinetics by an radio active assay after separation with high performance liquid chromatography (HPLC–RA) method in mice.

EXPERIMENTAL

Chemicals and Animals

Lidamycin (Lot: 20020519), purity 95.0%, was manufactured by the Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical University (Beijin, China). ^{125}I-labeled C-1027, which was radio iodinated by the Iodogen method,[8] had a specific radioactivity of 275.65 MBq (7.45 mCi) per 1 mg of protein and the radiochemical purity was more than 95%. Iodogen was from Academy of Military Medical Sciences (Beijing, China). Sodium dihydrogen phosphate (analytical grade) was provided by Chemical Company (Beijing, China); 0.9% sodium chloride was purchased from Dazhong Pharmaceutical Company (Tianjin, China); Distilled water, prepared from demineralized water, was used throughout the study.

Kunming mice, male and female, with body weights ranging from 17 to 24 g, were purchased from the Center of Experimental Animals of Tianjin Institute of Pharmaceutical Research (Certificate No: 20020804, Tianjin, China).

Instrumentation

The Gamma counter (FJ630G/12 model) was produced by Beijing Nuclear Company (Beijing, China). This chromatographic system (LC-6A, Shimadzu, Japan) consisted of a pump (LC-6AT), temperature box, and variable wavelength UV detector (Spectra 100, Shimadzu, Japan). A Biosep SEC-S 2000 gel column (300 mm × 7.8 mm I.D.) was purchased from Phenomenex Company (UK).

Preparation of ^{125}I-labeled C-1027

Iodogen (50 ng) in 100 μL of chloroform was placed in a sample tube and evaporated to dryness with nitrogen gas. C-1027 (500 ng), and 0.37 MBq of ^{125}I sodium iodide were pipetted, mixed, and allowed to react at 15°C for 30 min.[8] The mixture was chromatographed on a Sephadex G-20 column (200 mm × 10 mm, I.D). The mobile phase consisted of 0.05 mol/L phosphate buffer solution (pH 7.0) at a flow rate of 0.8 mL/min. The eluted fractions, detected by gamma counter as the same chromatographic behavior as standard C-1027 and Na^{125}I, were components of ^{125}I-C-1027 and Na^{125}I, respectively. The fraction, collected from 10 to 12.5 min, was a single radioactive peak of ^{125}I-C-1027, and was concentrated to a specific activity and applied to a pharmacokinetic study.

Radiochemical Purity and Biological Activity

The radiochemical purity of ^{125}I-C-1027 was calculated from the ratio of radioactivity of ^{125}I-C-1027 to the collected total radioactivity. The biological activity of ^{125}I-C-1027 was assayed in mice as described,[9] and the biological activities were compared with C-1027. The biological activity of ^{125}I-C-1027 hardly changed. ^{125}I-C-1027 and C-1027 had the same chromatographic behavior. This indicated that the ^{125}I-labeled method had little effect on the biological activity of C-1027.

Solution Preparation and Quality Control Samples

The stock solution (2 μg/mL) (275.65 kBq/mL) of the ^{125}I-C-1027 was prepared in water, and stored at −20°C. The solution was prepared in the serial concentrations of standard solution of 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, and 100.0 ng/mL with 0.9% sodium chloride solution. The standard solution was used to prepare standard curves. The serial concentrations of calibration curves were prepared with blank serum of mice instead of 0.9% sodium chloride solution, as mentioned earlier. Quality control (QC) samples were prepared in concentrations of 0.5, 5.0, and 50 ng/mL in serum.

Sample Preparation

Serum samples (100 mL) were separated by high performance liquid chromatography (HPLC). The elution, from 10.0 to 12.5 min, was collected and was determined by gamma counter.

Specificity, Precision, Accuracy, and Stability

Specificity was determined in blank serum from six mice. The chromatograms of the blank and the serum sample were compared.

QC samples of low, middle, and high concentration levels (0.5, 5.0, and 50 ng/mL, respectively) were prepared for the determination of the precision and accuracy of intra-day (on the same day) and inter-day (on the different days, $n = 6$) in double replicates for three consecutive runs. Precision, which was evaluated by a one-way analysis (ANOVA), was defined as the relative standard deviation (RSD). Accuracy was defined as the relative errors (RE) between the measured and the nominal value on each of the three concentration levels.

Sampling Procedure

Each sampling time was randomly distributed among the six mice. The serum samples (0.4 mL) were collected at 0.033, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h after a single intravenous administration at doses of 10, 50, and 100 μg/kg (275.65 kBq/mL). Serum samples were obtained by centrifuging at 2000*g* for 10 min, and stored at −20°C until analysis.

Pharmacokinetic Analysis

The concentration-time data were computed using a 3p87 Practice Pharmacokinetic Calculation Program edited by the Mathematic Pharmacological Committee, Chinese Pharmacological Society (China). The calculated pharmacokinetic parameters were as follows: half-life ($t_{1/2}$), area under curve (AUC), volume of distribution (V_d), clearance (CL_s), elimination rate constant (K_e), and other parameters.

RESULTS

Validation of Methodology

Under the optimized chromatographic condition, the ^{125}I-C-1027 was separated and determined. The eluent, separated by HPLC, was collected and assayed by the gamma counter. The chromatogram showed that the decomposed products and ^{125}I flowed out of column after the unchanged ^{125}I-C-1027. The standard curve equation and the calibration curve equation of serum showed that the concentrations and the corresponding radioactivity

had good linear correlation. The typical curve equations and correlation coefficients were as follows:

$Y = 52.1 + 1266.9X$ ($n = 8$, $r = 0.9997$) for the standard ^{125}I-C-1027.

$Y = 127.6 + 969.5X$ ($n = 7$, $r = 0.9999$) for serum at the concentration range from 0.5 to 100.0 ng/mL.

Precisions and accuracy of the assay were evaluated by assaying QC samples (0.5, 5.0, 50.0 ng/mL), in six replicates on three different days. The RSD was $<11.6\%$ for intra-day and $<6.9\%$ for inter-day assay at range of 0.5–100.0 ng/mL. The accuracy was within $\pm 4.0\%$ (Table 1). The limit of quantitation is the lowest concentration on the calibration curve if the following conditions are met: (1) There is no interference present in blanks at the retention time of the analyte, or the determination response is at least 10 times greater than any interference in blank sample at the retention time; and (2) the analyte peak should be identifiable, discrete, and reproducible, with a precision of $\leq 15\%$ and accuracy of $\leq 15\%$. The limit of quantitation of ^{125}I-C-1027 for the HPLC-RA method was 0.5 ng/mL.

Stability

Bench top stability was experimented at room temperature over 12 hr. The QC samples (0.5, 5.0, and 50 ng/mL) in six replicates were analyzed at room temperature on the same day. The results, determined at time intervals of 0, 1, 2, 4, 8, and 12 hr, showed that the RSD was within 3.8%. The samples were stable at room temperature over 12 hr. The freeze-thaw stability was also done on QC samples (0.5, 5.0, and 50 ng/mL) for at least three freeze-thaw cycles. The results displayed that there were no significant differences (RSD was $<7.0\%$).

Table 1. Precision and accuracy for the analysis of C-1027 by HPLC–RA method in mouse serum (in pre-study validation, $\bar{x} \pm$ SD, $n = 5$)

	Intra-day			Inter-day		
Added (ng/mL^{-1})	Found	RSD (%)	Accuracy (%)	Found	RSD (%)	Accuracy (%)
5.0	4.86 ± 0.05	11.6	−2.8	4.80 ± 0.04	6.9	−4.0
50.0	49.05 ± 0.05	1.9	−1.9	49.10 ± 0.25	6.2	−1.8
200.0	197.40 ± 0.14	1.8	−1.3	196.80 ± 1.00	3.7	−1.6

Specificity

Analysis of blank serum from six different mice showed no endogenous interference with the quantification of ^{125}I-C-1027. Representative chromatograms of blank serum, and serum sample with ^{125}I-C-1027 are shown in Figures 1 and 2.

The validation of two methods satisfied the need of the bioanalysis.[10–12]

Pharmacokinetic Parameters

The concentration-time curves after intravenous administration of ^{125}I-C-1027 are shown in Figure 3.

The pharmacokinetic parameters are presented in Table 2. The pharmacokinetics of ^{125}I-C-1027 was fitted to a two-compartment open model. It was eliminated with terminal half-lives ($T_{1/2\beta}$) of 2.93 ± 1.21, 2.78 ± 1.33, and 2.68 ± 1.02 hr, respectively. No statistically significant differences ($p > 0.05$) were found in the pharmacokinetic parameters $T_{1/2\alpha}$, $T_{1/2\beta}$, CL_s, V_d, and K_e among three doses. The AUC values depended on the administration doses.

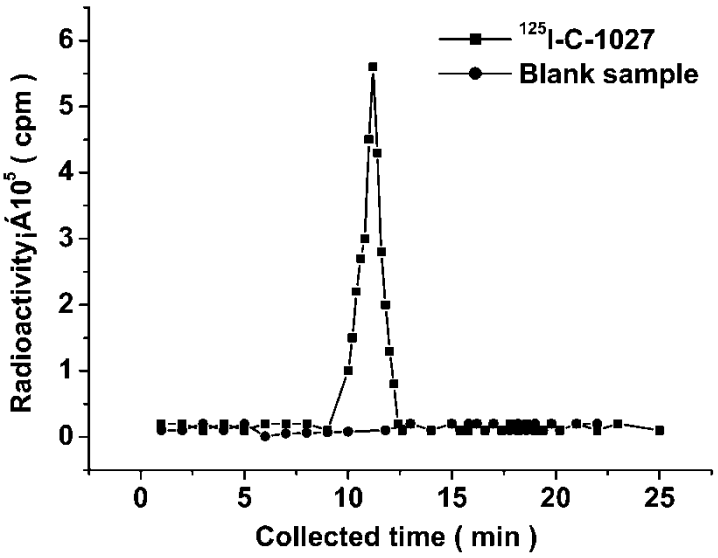

Figure 1. Chromatograms of radioactivity assay of ^{125}I-C-1027 and mouse blank serum sample.

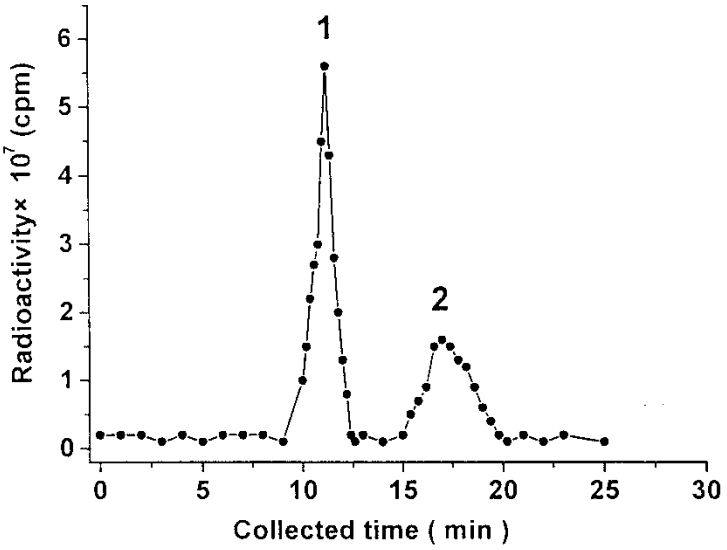

Figure 2. Chromatogram of radioactivity assay of the serum sample of ^{125}I-C-1027: 1, ^{125}I-C-1027; 2, the decomposed products of ^{125}I-C-1027.

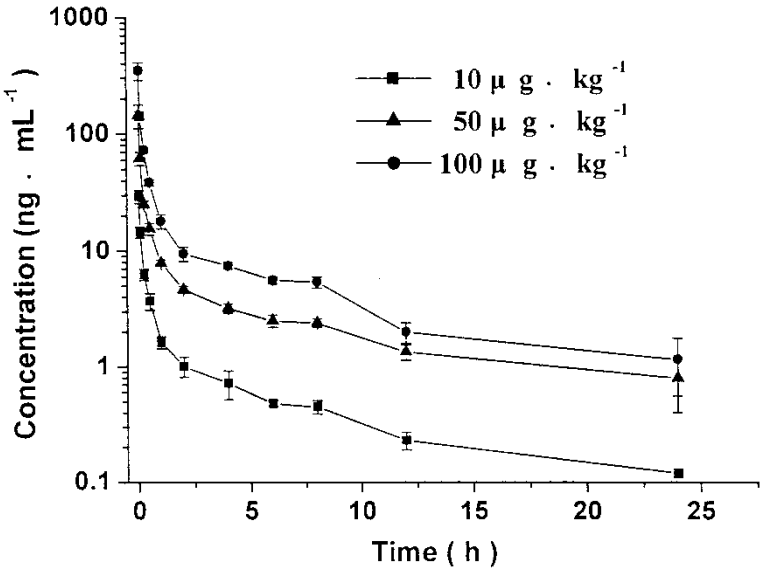

Figure 3. The mean serum concentration–time curves of ^{125}I-C-1027 at three doses in mice.

Table 2. Pharmacokinetic parameters of ^{125}I-C-1027 measured by HPLC–RA method at three doses in mice ($n = 6$)

Parameters	Unit	10 μg/kg	50 μg/kg	100 μg/kg
A	ng/mL	26.33 ± 2.31	132.36 ± 33.12	307.10 ± 51.42
α	hr^{-1}	7.47 ± 1.23	9.80 ± 1.55	8.57 ± 1.08
B	ng/mL	2.14 ± 0.59	10.95 ± 1.64	24.54 ± 3.05
β	hr^{-1}	0.24 ± 0.11	0.25 ± 0.10	0.26 ± 0.12
V_d	L/kg	0.35 ± 0.12	0.35 ± 0.09	0.35 ± 0.05
$T_{1/2\alpha}$	hr	0.09 ± 0.01	0.07 ± 0.02	0.08 ± 0.01
$T_{1/2\beta}$	hr	2.93 ± 1.21	2.78 ± 1.33	2.68 ± 1.02
K_{21}	hr^{-1}	0.78 ± 0.32	0.98 ± 0.41	0.87 ± 0.50
K_{10}	hr^{-1}	2.27 ± 1.43	2.49 ± 1.63	2.53 ± 0.75
K_{12}	hr^{-1}	4.66 ± 2.07	6.57 ± 1.76	5.42 ± 1.08
AUC	ng hr/mL	12.55 ± 1.75	57.52 ± 8.36	130.92 ± 21.24
$Cl_{(tot)}$	L/hr/kg	0.80 ± 0.07	0.87 ± 0.81	0.76 ± 0.69

DISCUSSION

The analytical methods of biotechnological pharmaceuticals were the critical step. At present, bioassays and immunoassays were the methods that were used most often, but were often nonspecific, inaccurate, and imprecise in the study on peptides and proteins. The disadvantage of the radiolabeling method was that the labeled substances were easily decomposed, and the bioactivity could be damaged for the research of peptides and proteins in animals and humans. With the development of science and technology, there were many methods, such as the enzyme-linked immunosorbent assay and solid-phase radioimmunoassay, and so on which could be used to investigate the pharmacokinetics of biotechnology pharmaceuticals. The isotope labeling method, which was used to analyze pharmacokinetic properties of biotechnological products, could eliminate the interference of the endogenous substances and improve the specificity, accuracy, limit of quantitation, and the speed of analysis.

In this article, the serum pharmacokinetic profiles of ^{125}I-C-1027 were determined by HPLC–RA after intravenous injection in mice. ^{125}I-radio labeling had little effect on the bioactivity of C-1027. The behavior of radioactivity, measured by HPLC–RA in serum, could reflect the behavior of the unchanged ^{125}I-C-1027. The validation results exhibited that this method was suitable for the study on the pharmacokinetics of ^{125}I-C-1027.

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