Pharmacokinetics of C-1027 in mice as determined by TCA-RA method

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Supported by: the National “863” Project of China, No. 2003AA22347D
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Abstract

AIM: To validate a radioactivity assay, the TCA-RA method, for the measurement of C-1027 in serum and to evaluate its application in determination of pharmacokinetics of C-1027 in mice.

METHODS: [125]I-C-1027 was prepared by the Iodogen method and separated by HPLC. The radioactivity assay was established and used to determine [125]I-C-1027 in mice at doses of 10, 50 and 100 μg/kg after precipitation with 20% trichloroacetic acid (TCA-RA method). Several pharmacokinetic parameters were determined after intravenous injection of [125]I-C-1027 in mice.

RESULTS: After intravenous injection of [125]I-C-1027 to mice, at doses of 10, 50 and 100 μg/kg, the apparent distribution volumes (V) were 0.26, 0.31 and 0.33 L/kg; the biological half-lives (T1/2b) were 3.10, 3.40 and 3.90 h; the areas under curve (AUC) were 18.41, 103.69 and 202.74 ng/h/mL; the elimination rate constants (K) were 1.04, 1.26 and 0.58/h; and the total body clearance (Cl) were 0.54, 0.48 and 0.49 L/kg/h, respectively.

CONCLUSION: TCA-RA is a sensitive, reliable and suitable method for the determination of [125]I-C-1027 in mouse serum.

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Key words: C-1027; TCA-RA method; Pharmacokinetics

Liu YP, Li QS, Huang YR, Zhou MJ, Liu CX. Pharmacokinetics of C-1027 in mice as determined by TCA-RA method. World J Gastroenterol 2005; 11(5): 717-720
http://www.wjgnet.com/1007-9327/11/717.asp

INTRODUCTION

Lidamycin (C-1027), produced by Streptomyces globisporus in soil, consists of a non-covalently bound apoprotein, and a labile chromophore that is responsible for most of the biological activities[1-7]. The structure of C-1027 has been studied by several methods[8-10]. C-1027 shows a remarkable inhibition on the growth of human liver cancer, colon cancer and epithelial tumor cells[11-18], and exhibits highly potent cytotoxicity to cultured cancer cells and marked DNA cleaving ability[19-25]. The protein moiety of C-1027 has a single polypeptide chain cross-linked by two disulfide bonds with a molecular weight of 10 500 Da[26,29]. The protein protects the stability of the chromophore. Like other enediyne agents, antibiotic C-1027 is believed to exert its biological activity through the induction of cellular DNA and RNA damage[30-35]. The pre-clinical studies on the pharmacodynamics, pharmacokinetics and toxicology have demonstrated that C-1027 appears to be a very promising anticancer candidate, and has been used in clinical trial in China. The aim of this study was to validate a radioactivity assay after precipitation with 20% trichloroacetic acid (the TCA-RA method) for the measurement of serum C-1027 and to evaluate its application in determination of pharmacokinetics of C-1027 in mice.

MATERIALS AND METHODS

Chemicals and instruments

C-1027 (Lot: 20020525, purity 95.0%) was produced by the Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical University (Beijing, China). [125]I-C-1027, which was radioiodinated by the Iodogen method[36], had a specific radioactivity of 7.45 mCi (275.65 MBq)/mg. The radiochemical purity was more than 95%. Iodogen was from Academy of Military Medical Sciences (Beijing, China). Trichloroacetic acid (TCA, analytical grade) was provided by the Chemical Company (Beijing, China), and 0.9% sodium chloride was purchased from Dazhong Pharmaceutical Company (Tianjin, China). Distilled water, prepared from demineralized water, was used throughout the study. Gamma counter (FJ630G/12 model) was produced by Beijing Nuclear Company (Beijing, China). The chromatographic system (LC-6A, Shimadzu, Japan) consisted of a pump (LC-6AT), temperature box and variable wavelength UV detector (Spectra 100, Shimadzu, Japan). Sephadex G-50 column (300 mm × 7.8 mm I.D) was purchased from Pharmacia Company (USA).

Animals

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

Preparation of [125]I-C-1027

Iodogen (100 μg) in 100 μL of chloroform was placed in a sample tube, and evaporated to dryness with nitrogen gas. C-1027 (50 μg), and 50 μL of Na[125]I (74 MBq) were pipetted, mixed, and allowed to react at 15 °C for 30 min[39]. The mixture was chromatographed on Sephadex G-50 column. The mobile phase consisted of 0.05 mol/L sodium dodecyl sulfate phosphate buffer solution (pH 7.0) at a flow rate of 0.8 mL/min. The eluted
fractons, detected by gamma counter as the same chromatographic behavior as standard C-1027 and Na\(^{21}\)I, were components of \(^{125}\)I-C-1027 and Na\(^{21}\)I, respectively (Figure 1). The fraction, collected from 10 min to 12.5 min, was a single radioactive peak of \(^{125}\)I-C-1027, and was concentrated to a specific activity and applied for pharmacokinetic study.

Application for mouse pharmacokinetics
Each sampling time was randomly distributed in 6 mice. Blood samples (0.4 mL) were collected at 0 min (pre-dose) and 2, 5, 15, 30 min and 1, 2, 4, 6, 8, 12, and 24 h after intravenous administration of \(^{125}\)I-C-1027 at doses of 10, 50, and 100 \(\mu\)g/kg. Serum samples were obtained by centrifuging at 2 000 g for 10 min, and stored at -20 °C until analysis.

Pharmacokinetic datum analysis
The concentration-time data were computed using a 3p97 Pharmacokinetic Calculation Program developed by the Mathematic Pharmacological Committee, Chinese Pharmacological Society (Beijing, China). The following pharmacokinetic parameters were calculated: biological half-life \((T_{1/2})\), area under concentration-time curve (\(AUC\)), apparent distribution volume \((V_d)\), the total body clearance \((CL)\), elimination rate constant \((K_e)\), and other parameters.

RESULTS
Validation of the bioanalytical method
The standard and calibration curve equations of \(^{125}\)I-C-1027 showed that the concentrations and their own radioactivity had a good linear correlation. The typical curve equations and correlation coefficients were as follows: \(y = 49.5 + 1235.9x (n = 8, r = 0.9994)\) for the standard solution of \(^{125}\)I-C-1027 at the concentration from 0.5 to 100.0 ng/mL, and \(y = 127.6 + 969.5x (n = 7, r = 0.9999)\) for serum samples from 0.5 to 100.0 ng/mL.

Precision and accuracy of the assay were evaluated by analyzing QC samples (0.5, 5.0 and 50.0 ng/mL) in 6 replicates on 3 different days. The relative standard deviation (RSD) was less than 5.0% for intra-day assay and less than 10.1% for inter-day assay at 0.5-100.0 ng/mL. The accuracy was between 96.0% and 99.0% (Table 1). The limit of quantitation was the lowest concentration on the calibration curve if the following conditions were met. (1) There was no interference present in blanks at the retention time of the analyte, or the determination response was at least 10 times greater than any interference in blank sample at the retention time; (2) Analyte peak should be identifiable, discrete and reproducible with a precision of less or equal to 15% and accuracy within ±15%. The limit of quantitation of \(^{125}\)I-C-1027 for the TCA-RA method was 0.5 ng/mL.

Table 1 Precision and accuracy for determination of \(^{125}\)I-C-1027 by TCA-RA method in mouse serum (mean±SD, \(n = 5\))

| Added (ng/mL) | Within-day | Between-day |
|--------------|------------|-------------|
| Found (%)    | RSD (%)    | Accuracy (%)|
|             | Found (%)  | RSD (%)     | Accuracy (%)|
| 1.0          | 0.98±0.05  | 5.0         | 98.0         | 0.96±0.04    | 4.6         | 96.0        |
| 2.5          | 2.47±0.05  | 1.9         | 98.6         | 2.45±0.25    | 10.1        | 98.2        |
| 20.0         | 9.81±0.14  | 0.7         | 99.0         | 19.65±1.00   | 5.1         | 98.4        |

Stability
The bench top stability of \(^{125}\)I-C-1027 in mouse serum was determined over 12 h at room temperature. The results showed that \(^{125}\)I-C-1027 was stable at the concentrations (0.5, 5.0 and 50.0 ng/mL) at room temperature (more than 92.0%, 91.0% and 90.0%, respectively). Similarly when \(^{125}\)I-C-1027 underwent 3 freeze-thaw cycles, the percentage differences were 8.9%, 7.3% and 8.2% at 3 different concentrations, respectively.

Specificity
The comparison of blank serum samples and serum samples spiked with \(^{125}\)I-C-1027 showed no endogenous interference.

Specificity, precision, accuracy and stability
Specificity of the assay was demonstrated by comparison between the radioactivity of the mouse serum spiked with \(^{125}\)I-C-1027 and the mouse blank serum. QC samples of low, middle and high concentration levels (0.5, 5.0, and 50 ng/mL) were prepared for the determination of the precision and accuracy of intra- and inter-day. Precision, which was evaluated by 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.

Bench top stability was experimented at room temperature over 12 h. The QC samples in 6 replicates were analyzed at room temperature on the same day. Three freeze-thaw cycles were done on the QC samples.

Radiochemical purity
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 previously described[1], and the biological activity was compared with C-1027. Only the \(^{125}\)I-C-1027, whose biological activity remained unchanged, was used to study the pharmacokinetics of \(^{125}\)I-C-1027.

Solution preparation and quality control samples
Stock solution (10.0 µg/mL) of the \(^{125}\)I-C-1027 (275.65 KBq/mL) was prepared in water, and stored at -20 °C. The stock solution was prepared into 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 in 0.9% sodium chloride solution. The standard solution was used to prepare standard curves. The serial concentrations of calibration curves were prepared with mouse blank serum instead of 0.9% sodium chloride solution as mentioned above. Quality control (QC) samples were prepared into low, middle and high concentrations (0.5, 5.0 and 50 ng/mL) in mouse serum.

Sample preparation
To 100 µL of mouse serum samples, 100 µL of 20% TCA was added. The mixture was vortexed for 2 min, and the supernatant was removed. Then, radioactivity of the precipitate was determined by the gamma counter.

Sample preparation

![Figure 1](https://example.com/figure1.png)

Figure 1 Chromatograms of the separation of \(^{125}\)I-C-1027 assayed by UV and radioactivity. A: \(^{125}\)I-C-1027; B: Decomposed I-C-1027; C: Blank serum. The radiochemical purity of \(^{125}\)I-C-1027 was determined by the gamma counter as the same chromatographic behavior as standard C-1027 and Na\(^{21}\)I, respectively (Figure 1). The fraction, collected from 10 min to 12.5 min, was a single radioactive peak of \(^{125}\)I-C-1027, and was concentrated to a specific activity and applied for pharmacokinetic study.
with the measurement of $^{125}$I-C-1027.

The validation of the TCA-RA method satisfied the requirements for bioanalysis[38-42].

**Pharmacokinetics**

After intravenous injection of 10, 50 and 100 µg/kg $^{125}$I-C-1027 to mice, the serum concentrations of $^{125}$I-C-1027 were determined by the TCA-RA method. Figure 2 shows the serum concentration-time curve of $^{125}$I-C-1027 after intravenous administration ($n=6$). The results of this experiment showed that the pharmacokinetic parameters: $T_{1/2}$, $Cl$, $V_t$ and $K$ did not exhibit statistically significant differences ($P>0.05$) among the three doses, and the AUC values depended on the administration dose (Table 2).

![Figure 2 Mean serum concentration-time curves of C-1027 after IV administration to mice at three different doses by TCA-RA method.](image)

**Table 2 Mean pharmacokinetic parameters of $^{125}$I-C-1027 measured by TCA-RA method at three doses in mice ($n=6$)**

| Parameters | Unit | Doses (µg/kg) | $P$ |
|------------|------|---------------|-----|
| A          | ng/mL| 10            | 125 |
| $\alpha$   | /h   | 50            | 42  |
| B          | ng/mL| 100           | 42  |
| $\beta$    | /h   |               |     |
| $V_{ss}$   | L/kg |               |     |
| $T_{1/2}$  | h    |               |     |
| $V_{ss}$   | L/kg |               |     |
| $K_{el}$   | /h   |               |     |
| $K_{2}$    | /h   |               |     |
| $K_{12}$   | /h   |               |     |
| AUC        | ng/h/mL|           |     |
| $Cl_{(0)}$ | L/(kg·h)|             |     |

$V_{ss}$: Apparent distribution volume; $T_{1/2}$: Half-life; $K$: Elimination rate constant; AUC: Area under curve; $Cl_{(0)}$: Clearance.

**DISCUSSION**

Biotechnological pharmaceuticals can be analyzed by many methods, such as bioassays, immunoassays, enzyme-linked immunosorbent assay and solid-phase radioimmunoassay. However, these methods are limited due to the interference of endogenous substances. On the other hand, isotopic labeling methods used to analyze pharmacokinetic properties of biotechnological products, can eliminate the interference of endogenous substances, and improve the specificity, accuracy, limit of quantitation, and the speed of analysis.

The method of $^{125}$I labeling C-1027 is simple, quick and acceptable. The highly purified $^{125}$I-C-1027 (with purity of more than 95.0%) is obtained by the Sephadex G-50 gel filtration, indicating that Sephadex G-50 gel filtration is an effective procedure to yield the high quality $^{125}$I-labeled C-1027. Only the $^{125}$I-C-1027 with purity and biological activity in accordance with the regulation can be used for the pharmacokinetic experiments in mice.

The TCA-RA method has been considered to be an accepted method for assay of the precipitated $^{125}$I-C-1027 in [38]. In this study, the mouse serum determination and pharmacokinetic profiles of $^{125}$I-C-1027 were measured by the TCA-RA method after intravenous injection of 10, 50, and 100 µg/kg $^{125}$I-C-1027 to mice. The pharmacokinetic results show that the areas under curves of three doses (10, 50, and 100 µg/kg) depend on the doses. The biological half-lives ($T_{1/2}$) do not change with the doses. The limit of quantitation indicates that this method is a sensitive method for analysis of C-1027.

**ACKNOWLEDGEMENTS**

The authors are indebted to the Institute of Medicinal Biotechnology (Beijing, China) for their financial support during the course of the study.

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Edited by Xia HHX and Wang XL. Proofread by Chen WW