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Short communication

Liquid chromatography with multichannel electrochemical detection for the determination of *trans*-resveratrol in rat blood utilizing an automated blood sampling device

Yongxin Zhu, Tiehua Huang, Meloney Cregor, Hong Long, Candice B. Kissinger, Peter T. Kissinger*

Bioanalytical Systems, Inc. 2701 Kent Avenue, West Lafayette, IN 47906, USA

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Abstract

A sensitive and selective multichannel liquid chromatography–electrochemistry method was developed for the determination of the natural product *trans*-resveratrol (resveratrol) in rat blood. After administration of resveratrol, blood samples were periodically collected by an automated blood sampling device. Resveratrol was extracted from 150 μl of diluted blood (blood and saline at a ratio of 1:1) with acetonitrile containing 1% of trichloroacetic acid. Chromatographic separation was achieved within 12 min using a C_{18} (100×2.0 mm) 3 μm column with a mobile phase containing 20 m*M* sodium acetate, 0.5 m*M* EDTA, pH 4.5 and 21% acetonitrile at a flow-rate of 0.4 ml/min. A multichannel detector with glassy carbon electrodes was used, which can control up to four working electrodes simultaneously with applied potentials of +800, 700, 600, 500 mV vs. Ag/AgCl. The limit of detection was 2 ng/ml at a signal-to-noise ratio of 3:1 and the limit of quantitation was 4 ng/ml. The linearity of the calibration curve was obtained over the analytical range of 5–1000 ng/ml. The intra- and interassay precision was in the range of 2.5–4.4% and 1.2–4.3%, respectively. Using this method it was possible to quantify blood concentration following a single dose of resveratrol to rats with good accuracy and precision. Thus the pharmacokinetic properties of resveratrol in rats can be examined for intraperitoneal, oral and intravenous dosing.

Keywords: Resveratrol

1. Introduction

Resveratrol (3,5,4′-trihydroxistilbene) exists in two isomeric forms. Fig. 1 shows the structure of *trans*- and *cis*-resveratrol. The *trans*-isomer is found in many plants, such as mulberries, particularly the berry skins of most grape cultivars and some Asian folk medicines [1–3]. Both *trans*- and *cis*-resveratrol are found in red wine [4]. It has been reported that resveratrol may be effective in inhibiting platelet aggregation, altering eicosanoid synthesis and modulating lipoprotein metabolism [5–8]. Recent findings suggest that resveratrol may also be anticarcinogenic [9] and a potential chemopreventive agent for breast cancer [10]. So far, resveratrol has proven remarkably potent at preventing experimental skin tumors in mice and at inhibiting the replication in vitro of human leukemia cells [9].

*Corresponding author.

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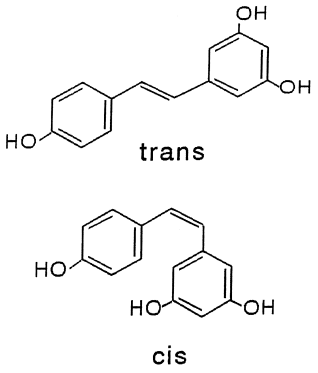

Fig. 1. The structures of *trans*- and *cis*-resveratrol.

LC methods have been developed for the analysis of this stilbene, the bulk of which deal with the measurement of resveratrol content in red wines or in plant sources [11–14]. Recently a few reports have described the determination of resveratrol in plasma and tissue [15–17], but no complete data have been published concerning absorption, bioavailability and pharmacokinetics.

This paper describes a LC method to determine *trans*-resveratrol in rat blood. The samples were periodically collected by an automated blood sampling device. Multichannel electrochemical detection provided a selective and sensitive approach for the determination of resveratrol in blood. By monitoring four potentials simultaneously one can easily determine the optimum potential during the method development and also verify peak purity by ratioing response at different energies. This method has been applied to evaluate the pharmacokinetics of resveratrol.

2. Experimental

2.1. Apparatus

The LC with electrochemical detection system was comprised of a chromatographic pump (PM-80, BAS, West Lafayette, IN, USA), an in-line filter (0.5 μm, Rheodyne) before an analytical column, an ODS 3 μm column (PEEK 100×2.0 mm, BAS), a manual injector (Rheodyne), an experimental multichannel amperometric detector (epsilon™, BAS) coupled to a four glassy carbon working electrode and referenced to a Ag/AgCl electrode. The mobile phase was 20 m*M* sodium acetate, 0.5 m*M* EDTA, pH 4.5 containing 21% of acetonitrile (v/v). The pump flow-rate was 0.4 ml/min. Injection loop volume was 20 μl. Applied potentials of +800, 700, 600 and 500 mV vs. Ag/AgCl were utilized. Data was acquired and integrated using BAS ChromGraph version 9.13 chromatography software.

The rat blood collecting system consisted of a freely moving rat containment device (Raturn™, BAS) [18], and an experimental automated blood sampler (Culex™, BAS). The blood samples were collected into a fraction collector (HoneyComb™, BAS). The temperature of the 300 μl vials in the fraction collector was maintained at 4°C.

2.2. Chemicals and reagents

trans-Resveratrol was purchased from Sigma (St. Louis, MO, USA), *cis*-resveratrol was prepared from *trans*-resveratrol as reported by Goldberg et al. [12]. Acetonitrile was of HPLC grade (Burdick and Jackson). Sodium acetate and ethylenedinitrilo-tetraacetic acid disodium salt (EDTA) were of analytical grade (Mallinckrodt).

2.3. Standard curve and quality control (QC) samples

Resveratrol was dissolved in methanol at a concentration of 1 mg/ml and stored in the dark at 4°C until used. Resveratrol was added to pooled rat blood to yield final concentrations of 5, 10, 50, 100, 500, 1000 ng/ml. These spiked samples were used to construct the standard curve. QC samples were prepared in pooled rat blood to contain concentrations of resveratrol within the standard curve range.

2.4. Sample preparation

A total of 150 μl of blood solution, which contained 75 μl rat blood and 75 μl of physiological saline were transferred to a 1.7 ml centrifuge tube. A total of 150 μl of acetonitrile containing 1% of

trichloroacetic acid were added, vortex-mixed, and centrifuged for 3 min at 11 200 *g*. Following centrifugation, a 75 μl aliquot of the clear supernatant was diluted with 75 μl of water and mixed. A volume of 20 μl of the solution was injected into the LC system.

2.5. Assay validation

2.5.1. Calibration

A calibration curve was constructed by plotting peak height (nA) of the analyte vs. the analyte's concentration (ng/ml). The weighted ($1/x$) linear regression was fitted over the concentration range 5–1000 ng/ml.

2.5.2. Accuracy and precision

The inter- and intraassay validation was performed by assaying QC samples (50, 100, and 500 ng/ml) with three replicates on three different days. The accuracy and precision were reported as the Bias (%) the RSD (%) respectively.

2.6. Preliminary animal study

Traditional pharmacokinetic studies involve manual intermittent blood sampling and subsequent determination of blood or plasma drug concentrations. Automated blood sampling as a continuous blood withdrawal approach can provide a means of accurately measuring an ''integrated concentration'' without having to manually draw intermittent samples [19–21]. Sprague-Dawley rats weighting 280–330 g were used. For the automated intermittent blood sampling experiments, the rats were implanted with a jugular vein cannula (0.3 I.D.×0.6 O.D.×81.3 mm L, polyurethane, BAS). After surgery, the rats were installed in the Raturn™, then allowed to recover for 1 day with free access to food and water. The rats were connected to sterile tubing on the Culex™ and were dosed with resveratrol intraperitoneally, orally or intravenously. The blood was automatically withdrawn from the jugular vein and followed by a heparin/saline flush. A total 150 μl of blood and saline (1:1) was collected by the fraction collector.

3. Results and discussion

3.1. Method development

Liquid chromatography–electrochemistry with multielectrode detection has proven useful in the identification and determination of phenolic compounds [22–24]. In this study, four-electrode detector experiments were performed with applied potentials: +800, 700, 600 and 500. Simultaneously monitoring four potentials by a multichannel detector gave a better voltammetric characterization of resveratrol in blood. Peak purity can be assured by comparing ratios at different energies for both standards and samples [22]. It was found that +700 mV was the optimum potential for the determination of resveratrol in blood. The separation of *trans*- and *cis*-resveratrol was investigated. The two isomers were well separated under the condition described in Section 2.1. Fig. 2 shows a typical chromatogram of

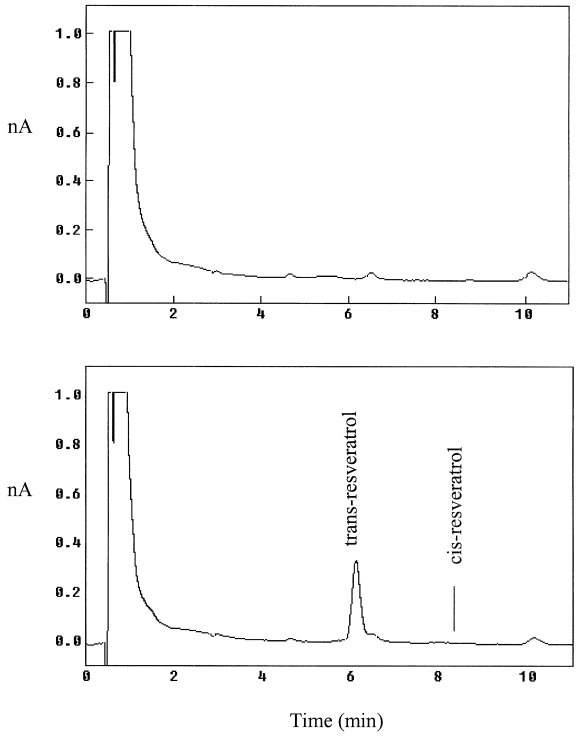

Fig. 2. Chromatogram of extract from blank rat blood (top) and blood spiked with 100 ng/ml of resveratrol (bottom). Applied potential: +700 mV vs. Ag/AgCl.

blank rat blood (top) and rat blood spiked with 100 ng/ml resveratrol (bottom). The retention times for the *trans*- and *cis*-resveratrol were 6.1 and 8.4 min respectively.

3.2. Validation of assay

3.2.1. Selectivity

Chromatograms were obtained and compared between the blank blood and blood containing resveratrol (Fig. 2) at +700 mV vs. Ag/AgCl applied voltage. No interfering peaks were detected at the retention times of the *trans*- and *cis*-resveratrol. Samples could be injected every 12 min.

3.2.2. Linearity

A weighed linear regression of the peak height vs. standard concentrations was performed for *trans*-resveratrol using a weight of 1/concentration. The observed peak heights were linear over the concentration range of 5–1000 ng/ml in rat blood. The mean values (±SD) (n=3) for the slope, intercept and r^2 were 214±4.6, 1100±1240 and 0.998±0.001 respectively.

3.2.3. Limit of detection and quantitation

The detection limit of resveratrol in rat blood was determined at 2 ng/ml with a signal-to-noise ratio of 3. The limit of quantitation was 4 ng/ml.

3.2.4. Accuracy and precision

The intra- and interday accuracy and precision values for QC samples are provided in Table 1. The precision values (%RSD) at the three concentrations in the intraassay study varied between 2.5 and 4.4% and in the interassay study varied between 1.2 and 4.3%. The accuracy (Bias%) values for all three concentrations deviated less than 6.2% from the corresponding nominal concentrations.

Table 1
Accuracy and precision for resveratrol assay in rat blood

Concentration added (ng/ml)	Concentration measured (mean±SD) (ng/ml)	RSD (%)	Bias (%)
Intraday (n=3)			
50	53.1±2.3	4.4	6.2
100	98.1±3.7	3.8	−1.9
500	496±13	2.5	−0.7
Interday (n=3)			
50	51.2±1.0	1.9	2.4
100	97.6±4.2	4.3	−2.4
500	501±6	1.2	0.3

3.2.5. Extraction recovery and stability

A comparison of neat standard vs. blood-extracted standard indicated that the extraction recovery of the analyte from rat blood was 63.5%. Resveratrol was stable in rat blood in the dark and at 4°C for up to 6 h. As far as the stability of processed samples after purification was concerned, no significant loss of resveratrol was observed at 4°C for 6 h.

3.3. Pharmacokinetic results

The proposed method was used for the determination of resveratrol in rat blood. Fig. 3 illustrates data for a single 2 mg/kg intraperitoneal dose administration of resveratrol to rats (n=3). The compound was rapidly absorbed. The blood concentration declined in a two-exponential fashion. The elimination rate constant for the first phase (k_{e1}), the half-life ($t_{1/2}$) and area under curve (AUC) of resveratrol were 0.185 min^{-1}, 3.74 min, 9917 min ng/ml respectively. This method is being used to study resveratrol kinetics using various routes of administration to better understand the potential role

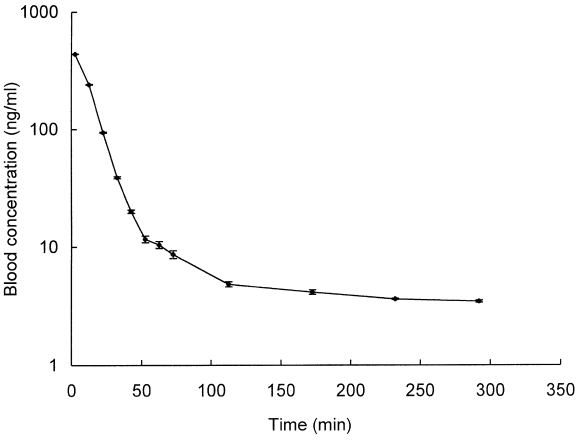

Fig. 3. Mean (±SD) blood concentration vs. time profile of resveratrol in rats (n=3) following a single 2 mg/kg intraperitoneal dose administration.

of this compound in human consumption of red wine.

4. Conclusion

A LC procedure with multichannel electrochemical detector was developed and validated for the determination of resveratrol in rat blood. The automated blood sampling device and the reported method offer several advantages, such as easy and accurate withdrawal of blood, a rapid and clean extraction scheme, and a short chromatographic run time with a superior lower limit of quantitation.

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