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A sensitive assay for the aminoimidazole-containing drug GP531 in plasma using liquid chromatography with amperometric electrochemical detection: a new class of electroactive compounds

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Abstract

Aminoimidazole-containing compounds have been found to be electroactive and can be detected by amperometric electrochemical detection (ECD) with a high degree of sensitivity. A liquid chromatography (LC) method using ECD was developed for measuring plasma concentrations of the aminoimidazole-containing drug GP531, a potent adenosine-regulating agent. Plasma samples were extracted with 2-propanol and analyzed by LC under isocratic conditions using a mobile phase of methanol–sodium phosphate (pH 6.3; 3.3 mM) (32:68, v/v). The potential of the glassy carbon working electrode was set at +800 mV. The limit of quantitation was 12.5 ng ml^{-1} of GP531 using 100 μl of plasma. The method was used to define the pharmacokinetics of GP531 in monkey following i.v. administration.

Keywords: Aminoimidazole; Amperometric electrochemical detection; Adenosine regulating agent; Reversed-phase liquid chromatography; Assay validation; Pharmacokinetic studies

1. Introduction

GP531 (5-amino-1-β-D-(5′-benzylamino-5′-deoxyribofuranosyl)imidazole-4-carboxamide, Fig. 1) is a novel riboside with similar structural and anti-ischemic properties to the adenosine-regulating agent (ARA) acadesine (Protara™; AICA-riboside) [1–4]. Since acadesine was found to be an electroactive compound [5], an assay for GP531 which, like acadesine, contains a 5-aminoimidazole group was developed using liquid chromatography (LC) and amperometric electrochemical detection (ECD) to determine plasma concentrations of GP531 after i.v. dosing of the drug in monkeys. The limit of quantitation (LOQ) was determined to be 12.5 ng ml^{-1}, which is tenfold lower than the LOQ in an assay using detection by ultraviolet absorbance. The 5-aminoimidazole species represents a new class of electroactive compounds that can be detected by ECD [6,7].

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2. Materials and methods

All chemicals used were either HPLC or reagent grade. Methanol, 2-propanol, water, and sodium hydroxide were obtained from Fisher (Fairlawn, NJ, USA). Phosphoric acid (85%, w/w) was purchased from Curtin Matheson Scientific (Houston, TX, USA). GP531 and GP343 (Fig. 2) were obtained from Gensia, Inc. (San Diego, CA).

The LC system consisted of a pump and autosampler (Hewlett Packard HP1090, Wallbronn, Germany) and an amperometric electrochemical detector (HP1049A, Wallbronn, Germany). The working electrode potential was set at +800 mV based on good selectivity and optimized signal-to-noise ratios at the LOQ. The Ag/AgCl reference electrode was filled with 3 M KCl. Samples were chromatographed on a Beckman Ultrasphere C-18 column (4.6 mm × 150 mm; 5 μm; Beckman, Fullerton, CA, USA) with a Brownlee precolumn (3.2 mm × 15 mm; 7 μm; Applied Biosystems, Foster City, CA, USA) eluted with a mobile phase consisting of methanol–sodium phosphate (pH 6.3; 3.3 mM) (32:68, v/v). The sodium phosphate buffer was prepared by adjusting 0.01 N phosphoric acid with a 10% (w/v) solution of sodium hydroxide to pH 6.3. The mobile phase was filtered by vacuum through an HV-filter (Millipore, Bedford, MA, USA) and was continuously degassed with helium during sample analyses.

The standard solutions were prepared by successive 1:2 dilutions of 6400 ng ml^{-1} of GP531 in plasma down to a concentration of 12.5 ng ml^{-1}. High, medium and low in vitro quality control plasma samples were prepared at 4480 ng ml^{-1}, 448 ng ml^{-1} and 22.4 ng ml^{-1}, respectively. These quality controls were stored in aliquots at

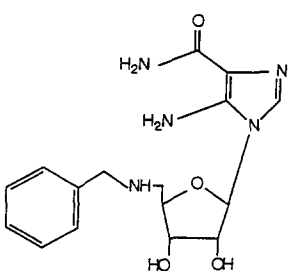

Fig. 1. Structure of GP531.

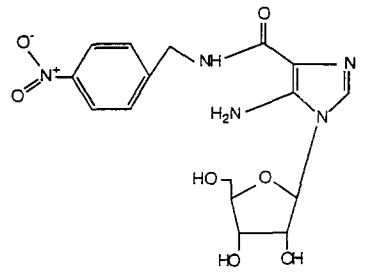

Fig. 2. Structure of GP343.

−20°C and used in the daily method validation [8]. Plasma samples (100 μl) were added to 25 μl of the internal standard GP343 (12.5 μg ml^{-1}) and 250 μl of 2-propanol. After vortexing for 10 s and centrifuging for 5 min at 12000 rev min^{-1} in a microcentrifuge at room temperature (MARATHON 13K/M, Fischer Scientific, Fairlawn, NJ, USA), 330 μl of the supernatant were transferred into a new microvial and evaporated on a Speed Vac (Savant SC110A, Farmingdale, NY, USA). The residue was reconstituted with 200 μl of mobile phase, vortexed, and centrifuged

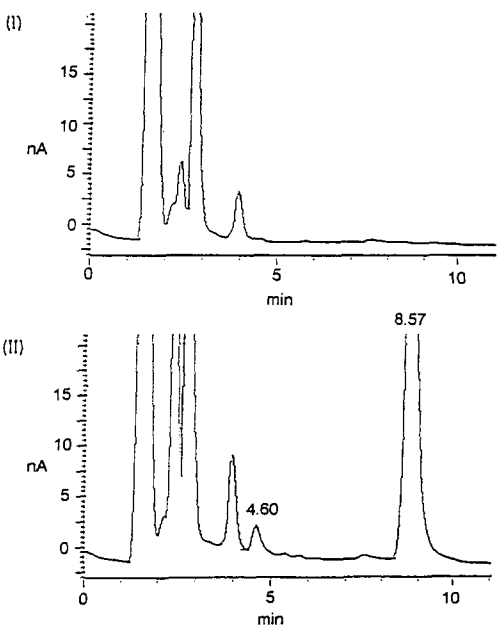

Fig. 3. Chromatograms of (I) blank monkey plasma and (II) a 12 h plasma sample from a monkey i.v. bolus administered 10 mg kg^{-1} of GP531 (GP531 elutes at 4.60 min; internal standard GP343 elutes at 8.57 min).

Table 1
Mean intra-day precision ($n = 5$) of GP531 in rat plasma

Amount GP531 added (ng ml^{-1})	Amount GP531 found (ng ml^{-1})	RSD (%)	Relative error (%)
6400	7150	2.71	+ 11.72
3200	3470	2.30	+ 8.44
1600	1670	2.94	+ 4.38
800	796	3.23	− 0.50
400	366	3.20	− 8.50
200	189	14.74	− 5.50
100	88	3.63	− 12.00
50	48	5.68	− 4.00
25	27	2.06	+ 8.00
12.5	13.4	3.10	+ 7.20

Table 3
Standard curve statistics ($n = 5$, mean ± S.D.) for GP531 in rat plasma

Date	Slope	Y-intercept	Correlation coefficient
21/04/94	0.726	− 0.004	0.9999
22/04/94	0.759	− 0.003	0.9998
23/04/94	0.813	− 0.001	0.9996
24/04/94	0.783	− 0.001	0.9997
25/04/94	0.885	− 0.003	0.9996
Mean	0.793	− 0.002	0.9997
SD	0.060	0.001	0.0001
RSD (%)	7.62	− 55.90	0.01

as before. The supernatant (70 μl) was injected into a liquid chromatograph for analysis. The data were collected and analyzed using ChemStation-based software (Hewlett Packard, Kennet Square, PA, USA). Variance stabilized transformation regression analysis was used to fit the standard curve [9].

3. Results

Although the assay was validated with rat plasma and cross-validated with monkey plasma, for simplicity only the rat validation data will be presented here. The LOQ and precision were the same for both species. Chromatograms of control blank plasma from either rat or monkey were also similar. Concentrations of GP531 in plasma remained constant at room temperature for at least 2 h. The extraction recovery of GP531 from plasma was 81 ± 9% (mean ± SD) with GP531 and the internal standard eluting at 4.60 min and 8.57 min, respectively, using the LC system (Fig. 3). The mean intra-day ($n = 5$) and inter-day ($n = 5$) precision were less than 15% (Tables 1 and 2). The standard curves ($n = 5$) were linear throughout the range tested ($r > 0.999$) (Table 3). The relative standard deviation of the slope during the 5-day analysis was 7.62% (Table 3). The accuracy using in vitro quality control samples in high, medium, and low concentrations was between 95 and 105% (Table 4). The precision of these quality control

Table 2
Mean inter-day precission ($n = 5$) of GP531 in rat plasma

Amount GP531 added (ng ml^{-1})	Amount GP531 found (ng ml^{-1})	RSD (%)	Relative error (%)
6400	6330	3.10	− 1.71
3200	3290	2.56	+ 2.81
1600	1650	3.74	+ 3.13
800	836	4.97	+ 4.50
400	403	4.46	+ 0.75
200	209	14.02	+ 4.50
100	91	2.38	− 9.00
50	46	6.74	− 8.00
25	24	3.95	− 4.00
12.5	12.9	2.97	+ 3.20

Table 4
Mean accuracy (%) ($n = 10$) of GP531 *in vitro* quality control sample in rat plasma

Amount GP531 added (ng ml^{-1})	Amount GP531 found (ng ml^{-1})	RSD (%)	Accuracy (%)
4480	4720	6.54	105.38
448	463	3.04	103.24
22.4	21.3	6.67	95.04

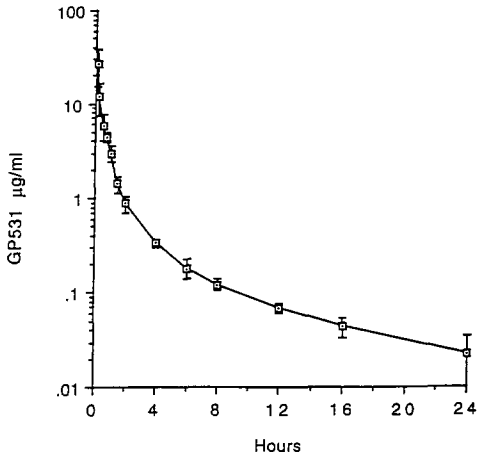

Fig. 4. Plasma concentration–time profile ($n = 4$, mean $\pm$ SD) of GP531 in the monkey after an i.v. bolus administration of 10 mg kg^{-1} of GP531.

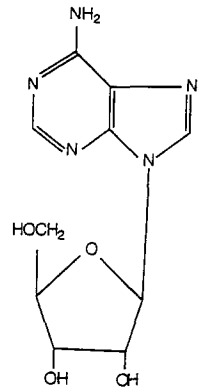

Fig. 5. Structure of adenosine.

samples (Table 4) indicated that GP531 is stable in plasma at -20°C for at least 2 months and in extraction medium at room temperature for at least 2 h.

The assay was used to define the pharmacokinetics of GP531 in the monkey ($n = 4$) following an i.v. bolus of 10 mg kg^{-1} of the drug. The individual plasma concentration data (Fig. 4) were well fitted to a biexponential equation using PCNONLIN. Mean (plus or minus the standard deviation) total plasma clearance, volume of distribution and terminal elimination half-life were 0.71 ± 0.14 l h^{-1} kg^{-1}, 1.15 ± 0.42 l kg^{-1}, and 5.3 ± 1.7 h, respectively.

4. Conclusions

We have developed a highly sensitive and specific LC-amperometric electrochemical detection assay for GP531 in plasma. Whereas aminopurine-containing compounds such as adenosine (Fig. 5) are not electroactive [10], the aminoimidazole-containing compounds acadesine, GP531, and GP343 can be oxidized easily. The aminoimidazole group is a new class of oxidative species that can be measured by amperometric electrochemical detection [6,7]. Because of the sensitivity of this LC assay, volumes of plasma as low as 100 μl can be analyzed. The assay has the advantage of being simple and superior in sensitivity over previously employed UV absorbance-based LC methods. The utility of the assay for pharmacokinetic studies in certain animals has been demonstrated.

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