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Note

Reversed-phase high-performance liquid chromatographic method to determine vidarabine and hypoxanthine arabinoside in biological fluids

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Vidarabine is an arabinosyl nucleoside used to treat various herpes virus infections including herpes simplex keratitis, herpes simplex encephalitis, and herpes zoster in immunosuppressed patients.

Humans rapidly deaminate vidarabine to arabinosyl hypoxanthine (ara-Hx) [1]. Ara-Hx exhibits antiviral activity although to a lesser extent than vidarabine. Methods for measuring vidarabine in biological fluids for clinical or pharmacokinetic studies must be able to measure ara-Hx as well as vidarabine, because of the rapid appearance of the deaminated metabolite [2].

Previously described methods to determine vidarabine and ara-Hx concentrations have relied on measurement of total antiviral activity in tissue culture [3] or on ion-exchange high-performance liquid chromatography (HPLC) methods [4]. The biological assays are cumbersome, suffer from considerable random variation inherent with the assay system, and do not distinguish between parent drug and active metabolites. The ion-exchange HPLC method of Schneider and Glazko [4] requires a minimal chromatography time of 30 min and is subject to interference by endogenous and exogenous arabinoside analogues.

We describe here a reversed-phase HPLC method to quantitate vidarabine and ara-Hx. This method requires a small sample volume, provides the required sensitivity and precision, and substantially reduces chromatographic time. Other arabinosides, as well as a number of other drugs, do not interfere with the assay.

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MATERIALS AND METHODS

Reagents

Pentostatin (adenosine deaminase inhibitor) was supplied by Warner-Lambert (Ann Arbor, MI, U.S.A.). Vidarabine and ara-Hx analytical standards and isoamyl alcohol were obtained from Sigma (St. Louis, MO, U.S.A.). HPLC grade sodium pentanesulfonate, chloroform, acetonitrile and acetone were obtained from Fisher Scientific (Fairlawn, NJ, U.S.A.).

Vidarabine and ara-Hx stock standards containing 0.5 mg/ml and 1.0 mg/ml, respectively, were prepared monthly in deionized water alkalinized with two drops of concentrated ammonium hydroxide and stored at 5°C. The mobile phase consisted of 20 ml of acetonitrile in 480 ml of a 5 m*M* sodium pentanesulfonate buffer, pH 7.2 at room temperature.

Chromatography

The chromatography was performed with a Perkin-Elmer Model 601 high-performance liquid chromatograph equipped with an LC 55 UV/VIS variable-wavelength detector interfaced to a Sigma 10 data system (Perkin-Elmer, Norwalk, CT, U.S.A.). The data system provided the integrated areas under the peaks and the retention times for vidarabine and ara-Hx. A 25 × 0.46 cm LiChrosorb RP-8, 5-μm particle size, reversed-phase column (Supelco, Bellefonte, PA, U.S.A.) was used for the chromatography. The oven temperature was maintained at 40°C, the flow-rate was 1.0 ml/min, and the effluent was monitored at 250 nm.

Procedure

Pentostatin was added to all blood samples at the time of venipuncture to inhibit any in vitro deamination of vidarabine to ara-Hx in patient and control sera. Two hundred μl of sample (serum, cerobrospinal fluid, or urine diluted 1:10 with distilled water) were placed into a 1.5-ml Eppendorf tube. Twenty μl of isoamyl alcohol (to prevent emulsion formation) and 50 μl of chloroform were added. The tube was vortexed for 30 sec and centrifuged for 10 min at 20,931 *g* in an Eppendorf 3200 centrifuge. The aqueous layer was transferred to another Eppendorf tube to which was added 1.0 ml cold acetone (0°C). This mixture was vortexed for 10 sec and centrifuged for 5 min. The supernatant was transferred to a clean 10 × 75 mm culture tube and evaporated to dryness at 40°C under nitrogen. The dried sample was reconstituted with 50 μl of deionized water just prior to injection into the chromatograph.

RESULTS

Typical chromatograms of vidarabine and ara-Hx are shown in Fig. 1. Retention times for ara-Hx, pentostatin, and vidarabine were 4.30, 5.82, and 6.65 min, respectively. Fig. 1A shows a chromatogram of extracted blank serum. Fig. 1B illustrates a chromatogram of drug-free serum spiked with 5.0 μg/ml ara-Hx and 2.5 μg/ml vidarabine. Fig. 1C is a chromatogram obtained from a patient serum sample in which the determined concentration of ara-Hx was 3.4 μg/ml. Similar chromatograms were obtained from urine and cerebrospinal fluid.

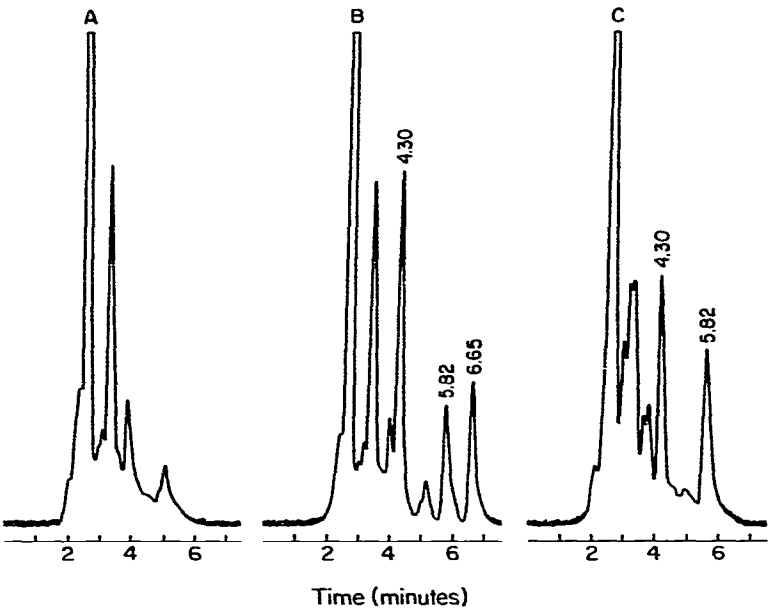

Fig. 1. Chromatograms obtained from: (A) blank serum; (B) serum prepared with 5.0 μg/ml ara-Hx and 2.5 μg/ml vidarabine; (C) patients' serum determined as 3.4 μg/ml ara-Hx. Vidarabine was not detected. The retention times for ara-Hx, pentostatin and vidarabine were 4.30, 5.82, and 6.65 min, respectively.

A standard curve was constructed by adding ara-Hx and vidarabine to five sets of drug-free serum or urine samples to yield concentrations in the range of 1.0—40.0 μg/ml and 0.5—20.0 μg/ml, respectively. Concentrations and peak areas were linearly related over the respective ranges in both serum and urine. The least squares regression for vidarabine was $y = 0.943x + 0.48$ and for ara-Hx was $y = 1.05x - 0.558$. The coefficient of correlation for both lines was 0.998.

Within-run precision was evaluated by assaying five aliquots of each of the following prepared serum standard concentrations: 0.5, 5.0, and 20.0 μg/ml of vidarabine; 1.0, 10.0, and 40.0 μg/ml of ara-Hx (Table I). Between-run

TABLE I

WITHIN-RUN PRECISION IN SERUM

In all cases $n = 5$.

	Mean (μg/ml)	S.D. (μg/ml)	C.V. (%)
Vidarabine	0.6	0.1	16.7
	5.0	0.3	6.0
	21.3	0.9	4.0
Ara-Hx	1.3	0.1	7.6
	10.0	0.6	6.0
	28.0	0.9	3.2

precision was determined by freezing aliquots of a pooled, drug-free serum to which vidarabine and ara-Hx were added and assaying the aliquots over a period of one month (Table II). There was no significant loss of vidarabine or ara-Hx during storage at −20°C for one month.

Five samples at each of the three different concentrations were assayed to determine the analytical recovery of known amounts of vidarabine and ara-Hx. Recovery of vidarabine from serum concentrations of 1.25, 5.0, and 20 μg/ml was 78, 80, and 84%, respectively. Recovery of ara-Hx from serum concentrations of 2.5, 10.0, and 40.0 μg/ml was 76, 80, and 78%, respectively.

The detection limit of this assay for both vidarabine and ara-Hx is 0.5 μg/ml in serum and cerebrospinal fluid and 2.5 μg/ml in urine.

Penicillin G, hydroxyzine, chlorothiazide, phenobarbitol, theophylline, prednisone, cytosine arabinoside, uracil arabinoside, guanine arabinoside, nystatin, kanamycin, sulfamethoxazole, trimethoprim and metaproterenol did not produce interfering peaks. Serum and urine samples obtained from patients receiving cancer chemotherapeutic agents were also free of interfering substances.

TABLE II

BETWEEN-RUN PRECISION

		Vidarabine	Ara-Hx
Serum	Mean (μg/ml)	8.5	7.0
	S.D. (μg/ml)	0.6	0.7
	C.V. (%)	7.0	10.0
	n	8	8
Urine	Mean (μg/ml)	7.6	7.3
	S.D. (μg/ml)	1.1	0.6
	C.V. (%)	14.5	8.2
	n	9	9

DISCUSSION

Previously described methods for measuring vidarabine and ara-Hx in biological fluids have relied on bioassays in tissue culture or ion-exchange HPLC methods. Biological assay systems measure total antiviral activity and do not distinguish between parent drug and active metabolites. In addition, tissue culture assays require prolonged incubation time resulting in excessively long turn-around time for the assay. The ion-exchange HPLC method described by Schneider and Glazko [4] also requires long turn-around time because of the chromatography time of 30 min. The latter method is also susceptible to interference from endogenous and exogenous analogues of vidarabine.

The method described here reduces the chromatography time to approximately 8 min, requires a sample size of only 200 μl and is not subject to interference from a number of concomitantly administered drugs or endogenous nucleosides. The short chromatographic time and small sample size make this

method particularly useful in monitoring vidarabine and ara-Hx levels during therapy in infants and small children.

We have observed serum and urine concentrations of vidarabine and ara-Hx in infants and children during therapy which are comparable to those reported in adult patients. Vidarabine is rarely detected because it is rapidly deaminated in vivo and is present in concentrations less than the minimum level of detection of our method (< 0.5 μg/ml in serum and cerebrospinal fluid and < 2.5 μg/ml in urine). Ara-Hx concentrations typically range from 2.0 to 12.0 μg/ml in serum depending on the dose and the time after the dose at which the sample is obtained.

The reproducibility, precision, and simplicity of the method described herein make it practical for use in any clinical laboratory with HPLC capability.

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