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Isoniazid/acetylisoniazid urine concentrations: markers of adherence to isoniazid preventive therapy in children

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SUMMARY

The Arkansas colorimetric method monitors adherence to isoniazid (INH) by the detection of INH metabolites in urine. Urine samples 4 h after INH administration in 31 human immunodeficiency virus infected children receiving daily or thrice weekly INH preventive therapy were Arkansas test-positive for 29/31 (94%), while acetylisoniazid (AcINH) was detected in 30/31 (97%) using mass spectrometry. At 24, 48 and 72 h, only 78%, 23% and 0 samples, respectively, were Arkansas-positive, while INH or AcINH was detected in respectively 94%, 69% and 33%. The Arkansas test reliably predicted INH ingestion at a clinic visit 4 h after morning doses, but did not perform well at 24 h.

Keywords

HIV-infected; Arkansas test; mass spectrometry

Isoniazid Preventive Therapy (IPT) is recommended for children at risk of developing tuberculosis (TB) disease.¹ Adherence to therapy is important for efficacy.² The Arkansas method is a qualitative urine assay used to monitor isoniazid (INH) ingestion³⁻⁷ by detecting urinary metabolites reacting with cyanogen chloride and barbituric acid, to produce a blue-purple dye indicating a positive reaction.⁸ A limitation of the test is that its sensitivity decreases with increasing time after INH ingestion. Moreover, the Arkansas method has not been evaluated against objective measurements of INH and acetylisoniazid (AcINH) in urine.

The present study aimed to measure urine concentrations of INH and AcINH at different time points after INH administration in children to evaluate the sensitivity of the Arkansas test against mass spectrometry.

STUDY POPULATION AND METHODS

A cohort of human immunodeficiency virus (HIV) infected children randomised to daily or intermittent IPT (8 – 12 mg/kg/dose) or placebo, as part of a larger IPT randomised controlled trial (NCT00330304) at the Red Cross War Memorial Children's Hospital, Cape Town, were recruited into the study. Children aged 1–15 years and established on antiretroviral therapy were requested to provide urine samples for the determination of INH and AcINH concentration levels and Arkansas testing.

A parent or legal guardian gave consent for their child's participation. The study was approved by the Human Research Ethics Committee of the University of Cape Town, Cape Town, South Africa (HREC ref no. 299/2005).

Children were recruited sequentially from a list generated by the study pharmacist, at a rate of three children on INH to one on placebo. The remainder of the study team were blinded to treatment allocation until after the urine assay. Parents were asked to withhold giving INH or placebo on the day of urine sampling. For the intermittent arm, urine samples were collected on a Monday, approximately 72 h after the last dose. The children then ingested an observed INH dose in the clinic, and urine samples were taken 4 and 48 h later. Urine samples were collected approximately 24 h after a dose for children on daily INH, followed immediately by an observed dose of INH and urine sampling 4 h later. Adherence to INH doses before the clinic visit was maximised by phone calls to the child's care giver. A 1 ml aliquot of urine was stored at –80°C.

The Arkansas test was carried out on urine samples as previously described.³ Digital colour photographs of the samples documented the colour changes after allowing the tubes to stand for approximately 10 min. Colours were read by comparing the digital images with a colour chart (obtained from www.art-paints.com). Two observers initially read the colours in a subset of 48 samples independently; however, due to excellent concordance between the observers, one observer read the subsequent samples. The range of colours observed is reported in the Figure. Based on reports from previous studies,^{3–7} and supported by high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) findings, yellow was defined as negative, while the other colours in the Figure were recorded as positive.

INH and AcINH concentrations were determined using HPLC-MS/MS. The urine samples were centrifuged and then diluted 10-fold with water before injection. Gradient chromatography was performed on a Phenomenex Luna 5 µm PFP(2) 100 Å, 50 mm × 2 mm analytical column (Phenomenex, Macclesfield, UK), using acetonitrile and 5 mM ammonium acetate as a mobile phase. The calibration range for both compounds was between 0.125 mg/l and 20 mg/l. Samples with concentrations below 0.125 mg/l were assigned a value of zero.

RESULTS

A total of 41 children with a median age of 7.7 years (interquartile range 6.6 – 9.5) participated in the study; 18 children received daily doses and 13 received intermittent doses of INH, while 10 children were assigned a placebo. For each time point, all the urine

samples were collected, except for one missing 72-h sample. All of the samples from children taking the placebo gave a yellow colour with the test. The Arkansas test results for children assigned to INH are shown in the Figure. The 4-h urine concentrations of INH and AcINH for the daily and intermittent arms were not statistically significantly different ($P = 0.517$; Mann-Whitney U -test).

The proportions of 4-h urine samples with INH/AcINH detectable using HPLC-MS/MS and a positive Arkansas test were similar (Table). A number of outlying observations were noted. The 4 and 24-h samples from a child assigned to daily INH did not have detectable INH or AcINH and were Arkansas-negative, while the 4-h samples from two children assigned to INH had low INH and AcINH concentrations (<2.1 mg/l), and were respectively pale green and yellow on the Arkansas test. These are in line with the results for 24 and 72-h samples. Conversely, a 48-h sample had high INH (137 mg/l) and AcINH (26.87 mg/l) concentrations, in line with recent ingestion of INH. When excluding the outlying observations, likely due to protocol nonadherence, both tests detected INH/degradation products in 100% of the samples. At 24, 48 and 72 h, respectively 94%, 69% and 33% of the samples had AcINH detectable using HPLC-MS/MS, while only 78%, 23% and 0% of the samples, respectively, were Arkansas test-positive.

DISCUSSION

This is the first study to evaluate the Arkansas test for measuring adherence to INH treatment against the objective measurement of INH and AcINH urine concentrations. We found the Arkansas test to be reliable 4 h post dose, indicating its utility for monitoring ingestion of morning INH doses in children attending the clinic on the same day. It is also significantly cheaper than HPLC-MS/MS. However, with the reduction in INH metabolite concentrations, the Arkansas test became less sensitive for monitoring INH ingestion with increasing time after the dose, and was not useful 24 h after the dose. These results are in line with the report of Hanifa et al.,⁵ and are useful for the interpretation of the Arkansas test results. However, this is an exploratory study and should be followed by larger studies to confirm the results with better characterisation of urinary INH excretion between 4 and 12 h, during which clinic visits might occur.

In conclusion, the Arkansas test can reliably detect recent INH ingestion up to 4 h after the dose, while HPLC-MS/MS is useful for detecting a dose for up to 24 h after ingestion.

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Figure.

Bar chart showing colours observed from the Arkansas test and their occurrence in percentage at different sampling times in the 31 children assigned to daily or intermittent isoniazid. The 4 h result is a combination of the daily and intermittent arms, which had 18 and 13 samples, respectively.

Table
Proportion of samples with a positive Arkansas test, and INH and AcINH concentrations using mass spectrometry

	Daily		Intermittent	
	4h	24 h	4h	72 h
	(n = 18)	(n = 18)	(n = 13)	(n = 12)
	n (%)	n (%)	n (%)	n (%)
Arkansas-positive	17 (94)	14 (78)	12 (92)	0 (0)
Samples with detectable INH (HPLC-MS/MS)	17 (94)	17 (94)	12 (92)	3 (25)
INH concentration, mg/l, median [IQR]	139 [18.0–273]	0.53 [0.34–4.90]	122 [52.0–255]	0.00 [0.00–0.07]
Samples with detectable AcINH (HPLC-MS/MS)	17 (94)	17 (94)	13 (100)	4 (33)
AcINH concentration, mg/l, median [IQR]	103 [36.0–227]	3.91 [1.39–9.95]	217 [69.0–421]	0.00 [0.00–0.18]

INH = isoniazid; AcINH = acetylisoniazid; HPLC-MS/MS = high-performance liquid chromatography-mass spectrometry; IQR = interquartile range.