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RESEARCH ARTICLE

HPLC/MS/MS methodology for sensitive quantitation of monic acid A, the metabolic product of the antibiotic mupirocin

Elizabeth A.C. Haidar[1], Terence D. Lee[2], Jonathan D. Barton[3], Anthony R.M. Coates[1], and Peter G. Mantle[4]

[1]Medical Microbiology, Centre for Infection, Division of Clinical Sciences, St George's - University of London, Cranmer Terrace, London SW17 0RE, UK, [2]Division of Cardiac and Vascular Sciences, St George's– University of London SW17 0RE, UK, [3]Chemistry Department, Imperial College London, London SW7 2AZ, UK, and [4]Centre for Environmental Policy, Imperial College London, London SW7 2AZ, UK

Abstract

Patients who are treated by self-medication with intranasal mupiricin (Bactroban™) for controlling meticillin-resistant *Staphylococcus aureus* may, or may not, adhere to their regimen. Herein, we describe a potential methodology for assessing adherence by measuring the gastric degradation product, monic acid A (MA), as a biomarker in urine. MA was isolated (~80% recovery) through a Waters Oasis HLB cartridge and detected (e.g. 25 pg on the column) by HPLC/MS/MS (API4000). Within a calculated 10^6-fold margin, this analytical sensitivity should facilitate urinary MA quantitation if, for example, 1% of intranasal mupirocin is swallowed and degraded characteristically to MA by gastric acidity.

Keywords: Monic acid A, hospital-acquired infections, patient adherence, intranasal medication, pseudomonic acid A, Bactroban™

Introduction

Mupirocin is the generic name for pseudomonic acid A, an antibiotic produced by *Pseudomonas fluorescens*. The discovery was made at Imperial College (Fuller et al. 1971) and the structure was described by Chain and Mellows (1977). Study of its commercial potential proceeded in the Beecham Research Laboratories. Meanwhile, at Imperial College, research continued on its biosynthesis (Feline et al. 1977) and mode of antibiotic action (Hughes et al. 1980), and it was used as a teaching and research model in fermentation technology and microbial biochemistry until 1999 (Mantle & Macgeorge 1990; Mantle et al. 2001).

Commercial development revealed a unique mode of antibacterial action, but wide exploitation was constrained by its instability in monogastric animals as an oral therapeutant, whereby acidic cleavage of the molecule to give monic acid A (MA) precluded other than topical application. Nevertheless, mupirocin is in a niche market which has expanded recently through use for intranasal administration to control hospital-acquired infectious bacteria and associated disease. Mupirocin's unique mode of action is of particular advantage concerning meticillin-resistant *Staphylococcus aureus*.

Pharmaceutical industry focus on metabolism of mupirocin has been relatively muted because of its topical application, poor skin absorption, low mammalian toxicity and simple *in vivo* degradation to biologically inactive MA. However, chemistry of MA has been well defined (Clayton et al. 1979). Consequently, there has been no published methodology for recognition or quantitation of MA as an excretory biomarker of mupirocin exposure. The most informative Web site is http://www.

Address for Correspondence: Prof. Peter G. Mantle, Centre for Environmental Policy, Imperial College London, London, SW7 2AZ, UK. Tel.: +44 20 7594 5245; Fax: +44 20 7594 9334. E-mail: p.mantle@imperial.ac.uk

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rxlist.com/bactroban_nasal-drug.htm, which only states that MA was below the LOD in a prescribed treatment regimen with mupirocin in its formulation as Bactroban. Analytical sensitivity of HLPC/MS/MS instrumentation should, however, enable detection of patient adherence of prescribed treatment, which is a general matter of concern in medication, and particularly so with respect to antibiotic resistance (Thomas et al. 2010). We have developed a sensitive assay for quantitative detection of human urinary MA as a basis for subsequent study of patient adherence.

Method development

Mass spectrometric characteristics of MA

Electrospray ionization (ESI) mass spectrometry (Waters LCT Premier) confirmed the purity of the crystalline MA, sourced in 1985 courtesy of Dr. J Clayton, Beecham Pharmaceuticals, and provided fragmentation characteristics in positive and negative modes (Figure 1) of relevance to developing HPLC/MS/MS methodology for analysis. Spectra were obtained under the following conditions: capillary voltage 2 KV, cone voltage 30 V, source temperature 120°C, desolvation temperature 350°C, N_2 flow rates (cone) 10 L/h and (desolvation) 400 L/h.

Important ions in positive mode were *m/z* 367 (100%) $C_{17}H_{28}O_7Na$ found 367.1722 (–3.0 ppm); *m/z* 362 (5%) $C_{17}H_{32}NO_7$ found 362.2174 (1.4 ppm); 345 (7) $C_{17}H_{29}O_7$ found 345.1912 (–0.1 ppm); *m/z* 327 (75%) $C_{17}H_{27}O_6$ found 327.1799 (2.8 ppm). *m/z* 367 was the sodium adduct of MA; *m/z* 362 was the ammonium adduct; *m/z* 345 was $M+H^+$; *m/z* 327 was $(M+H-H_2O)^+$.

In negative mode, the molecular ion (M-H) of MA was prominent (85%) $C_{17}H_{27}O_7$ found 343.1766 (2.6 ppm); *m/z* 389 (100%) was the formic adduct $C_{18}H_{29}O_9$ found 389.1827 (3.9 ppm); *m/z* 411, the formic + sodium adduct (55%), $C_{18}H_{28}O_9Na$ found 411.1638 (1.7 ppm).

HPLC/MS/MS: optimum sensitivity for analytical dose-response relationship

An API4000 mass spectrometer (MS) coupled to an Agilent 1100 series HPLC system was used for this assay. The mobile phase was a mixture of acetonitrile (12.5%) and deionized water (87.5%) containing formic acid (0.1%) and ammonium acetate (2 mMol/L), pumped isocratically at 400 µL/min. The HPLC column used was a 150 cm × 2.1 mm Supelco Discovery® BIO wide pore C18 (part number 568202-U).

The MS detector was operated in positive ionization multiple reaction monitoring mode, set to detect the ammonium adduct at *m/z* 362.3 ± 0.5 and the product ion at *m/z* 327.1 ± 0.5. Ammonium ions in the HPLC mobile phase enhanced the natural low intensity of the *m/z* 362 ion in the ESI positive mode (Figure 1) to provide a useful mass spectrometry characteristic.

A linear relationship was obtained between the concentration of MA injected, in the range of 5–100 ng/mL, and the recorded MS ion current signal intensity (Figure 2). This translated into a confident detection of as little as 25 pg of MA on column.

Assay validation

With the analytical instrumentation above, an assay was performed to evaluate the sensitivity of the test, the recovery of MA from urine and the reproducibility of the extraction procedure.

A set of calibrators covering the range 0.1–2 µg/mL were prepared in mobile phase. Two urine samples spiked with MA were prepared at 0.1 and at 1 µg/mL and six aliquots of each were extracted. In order to protect the

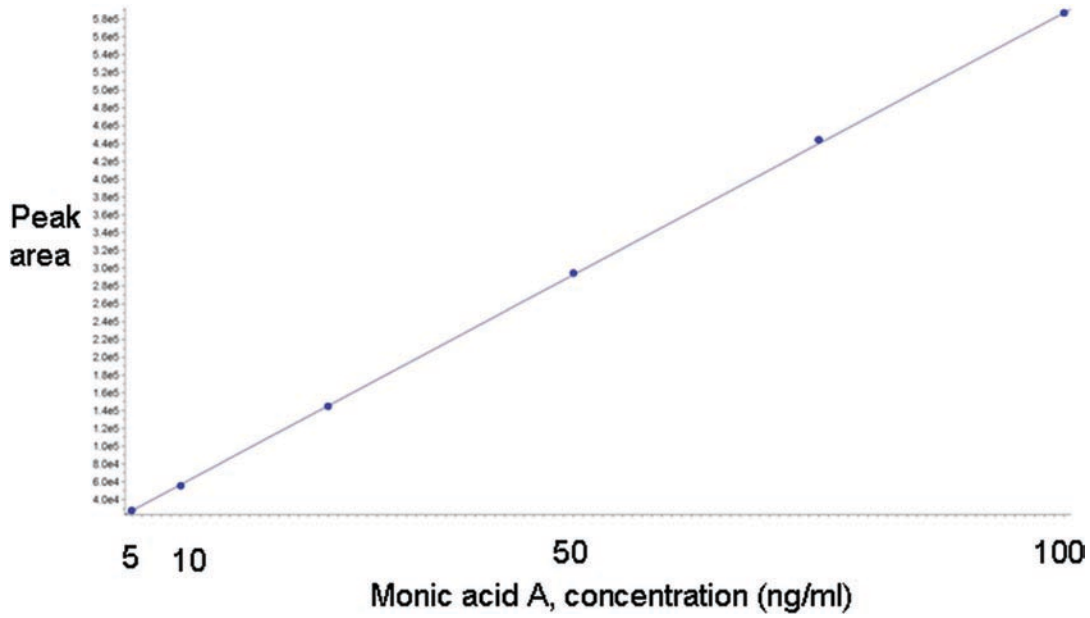

Figure 1. Positive (below) and negative (above) electrospray ionization spectra of MA.

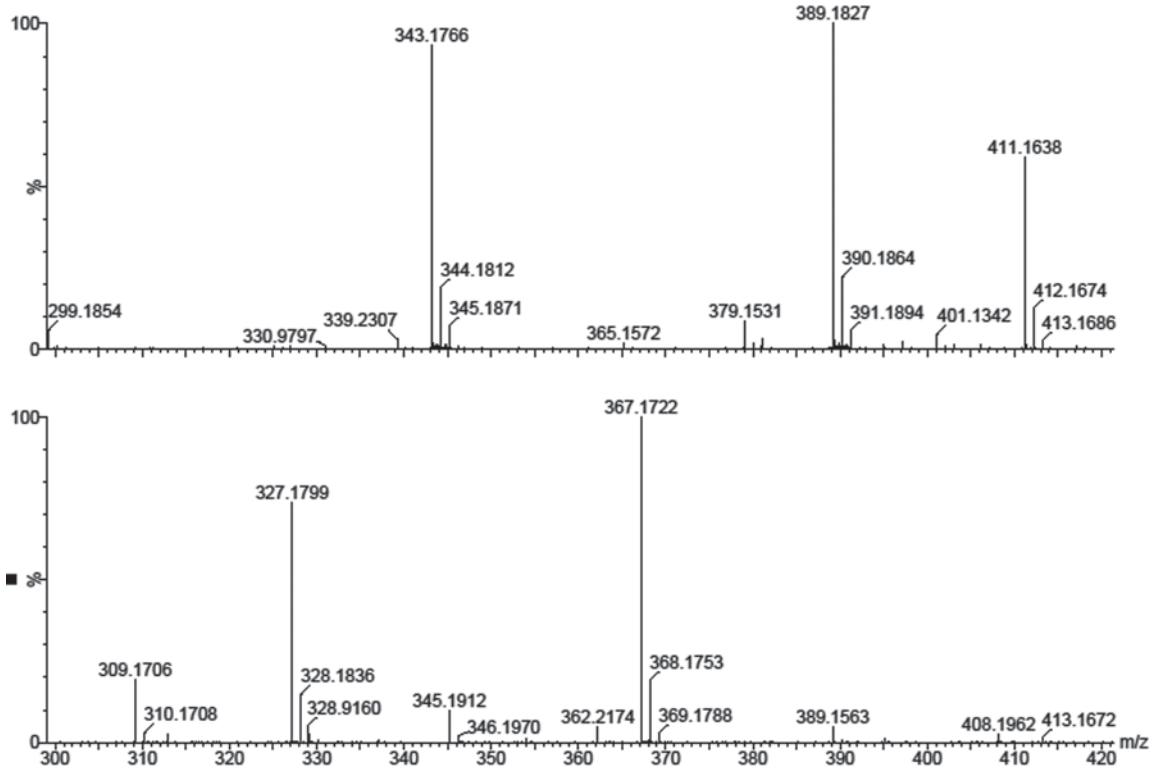

Figure 2. The linear relationship between changes in concentration of monic acid A in the 10 µL injection volume for HPLC and the MS/MS specifically defined detection response. The Y-axis values relating to the six test injections translate into the detectable range 25–500 pg on the column.

HPLC column from the complex solutes in urine, to keep the assay as simple as possible and to avoid unnecessary solvents and evaporation stages, a solid-phase extraction assay was developed using Waters Oasis® HLB, 1 cc (30 mg), extraction columns (part number WAT094225). The columns were preconditioned with 1 mL methanol followed by 1 mL deionized water. One milliliter of urine was mixed with 100 µL of 10% acetic acid and applied to the preconditioned extraction columns. The columns were then washed twice with 1 mL deionized water. MA was eluted with 250 µL methanol and mixed with 750 µL deionized water before injecting 10 µL into the HLPC/MS/MS. At 1 µg/mL, the mean recovery was 79.3%; at a concentration of 0.1 µg/mL, the mean was 81.9%. Extraction reproducibility was 5.3 and 7.1%, respectively.

Assay sensitivity

For this test, 1 mL of human urine, spiked with MA at 1 or 0.1 µg/mL, was used. In each example, MA was extracted via the Waters cartridge and eluted into the same (1 mL) final volume of 25% methanol and 75% water. Only 10 µL of the final extract was analyzed by HPLC/MS/MS. The chromatogram in Figure 3 shows clear recognition of MA (~1 ng) in the more dilute extract (0.1 µg/mL).

Discussion

The clinical intranasal use of mupirocin to decolonize the nose of *S. aureus* is usually with three times per day application to the anterior nares for 5 consecutive days. Each dose is about 100 µL of a glycol-based cream containing 2% of the antibiotic to each nostril. Inevitably, a small amount can pass to the pharynx and be swallowed. This presents no problem, but it does offer opportunity to detect the application indirectly through recognition of MA excreted in urine. The pharmacokinetics of the proportion of a single application that may enter the acidic stomach environment during the following few hours is unclear, but degradation to MA is likely to be rapid and there is no evidence of subsequent protracted half-life in blood. However, the present finding of specific detection of 25 pg of MA isolated from urine, coupled with the potential bioavailability of ~25 µg if only 1% of an intranasal dose is swallowed, offer a 10^6-fold window for clinical chemistry development.

The demonstrated efficiency of Waters Oasis HLB solid-phase extraction for selective adsorption of MA from urine, and the opportunity for concentrating the extracted MA via the volatile methanolic eluate, confirms the potential for indirect detection of microgram amounts of ingested mupirocin. Urine sample collection in the evening after the three-dose daily regimen may further optimize detection of MA, and larger Waters cartridges offer potential for analysis of greater urine volumes.

The new sensitive method will facilitate model kinetic study of the fate of very small amounts of ingested mupirocin, detected as MA, as a foundation for forensic detection of adherence to the treatment regimen in the clinical setting.

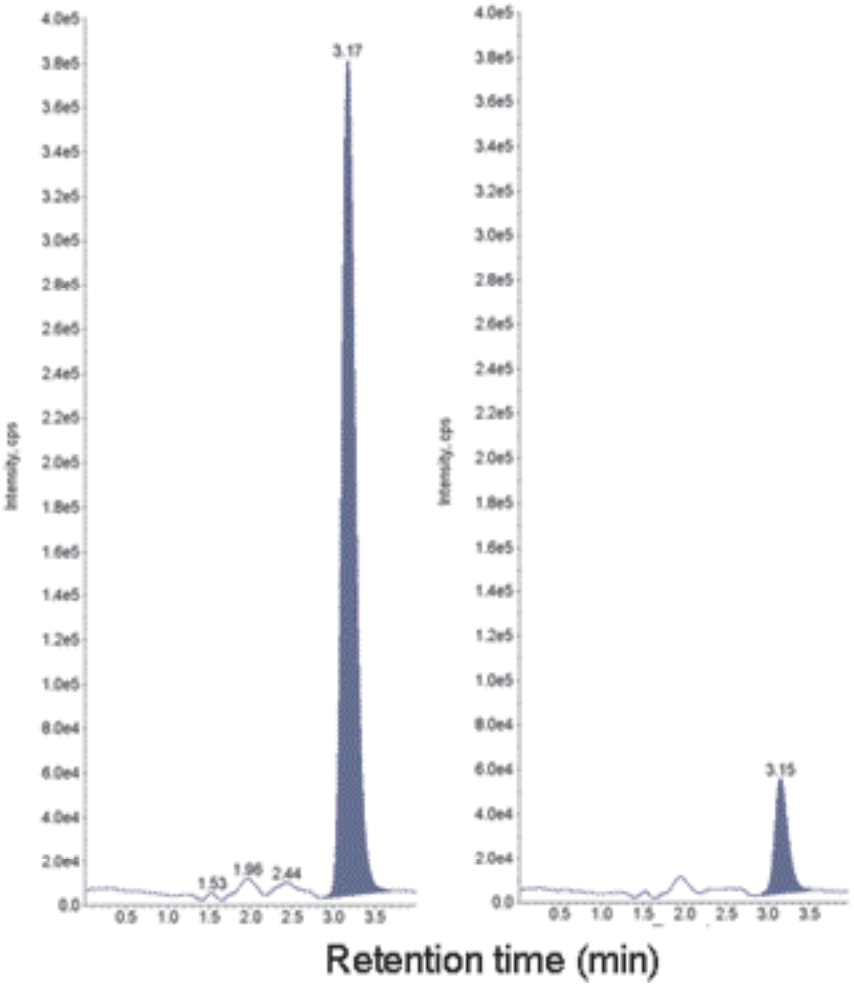

Figure 3. Comparison of HPLC/MS/MS detection of MA extracted from urine spiked at 1 μg/mL (left) and at 100 ng/mL (right), the latter representing detection of ~1 ng of MA.

Declaration of interest

The authors report no conflicts of interest

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