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Short communication

Analysis of minocycline by high-performance liquid chromatography in tissue and serum

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Abstract

A sensitive and rapid reversed-phase high-performance liquid chromatography assay can be used to accurately determine serum and tissue minocycline concentrations. Minocycline is a broad spectrum tetracycline derivative with many applications. Tissue and serum samples were obtained from guinea pigs that had received either topical or intravenous minocycline. Samples were extracted using a Sep-Pak C_{18} cartridge and were injected into a μBondapak C_{18} column with an isocratic methanol mobile phase. Samples were analyzed using UV detection and produced sharp peaks with a retention time of 2.5 min. The lower limit of detection was 100 ng and drug recovery was 61%. This method greatly facilitated the analysis of minocycline while allowing for sensitivity.

Keywords: Minocycline

1. Introduction

The quantification of antibiotic serum concentrations is an essential analytical technique in the clinical laboratory. Antibiotics have been shown to have enhanced efficacy based on treatment, the modality and dosing schedules [1–3]. We evaluated the broad spectrum antibiotic minocycline in an animal model of surgical wound infection, comparing local and systemic drug delivery. In order to determine antibiotic levels, we developed a high-performance liquid chromatography (HPLC) technique that was capable of evaluating minocycline concentrations in both tissue and serum.

Minocycline is a tetracycline derivative with a bacteriostatic effect through the inhibition of protein synthesis. It is an effective broad spectrum antibiotic against both Gram-negative and Gram-positive organisms. Minocycline is the treatment of choice for chlamydial infection, mycoplasmal infection and brucellosis. It can, however, produce toxic side effects, such as vestibular dysfunction and damage.

Sensitive techniques are necessary for the determination of drug concentrations to permit optimal dosing with minimal toxicity. The techniques that are currently available are directed towards the analysis of tetracycline in general and not the specific drug minocycline [4–7]. This method is reliable, can be replicated and is suitable for tissue samples as well as serum samples. This paper details the extraction

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from tissue and serum and the HPLC analysis of minocycline.

2. Materials and methods

Minocycline was obtained from Lederle Labs (Pearl River, NY, USA) and HPLC-grade reagents were from Fisher Scientific (Pittsburgh, PA, USA). A clinically relevant model of surgical wound infection was used in male guinea pigs. Wounds (1 cm) were created on the dorsum of animals and were contaminated with 1×10^8 *Escherichia coli* and 1×10^8 *Staphylococcus aureus*. Animals were subsequently treated either intravenously with 1.1 mg of minocycline or locally with 4 mg of polyglycolic acid microspheres containing 1.1 mg of minocycline. The wounds were excised in toto with a 1.5-cm radius down to the level of retroperitoneal fat (including muscle).

2.1. Equipment

A HPLC system (Waters, Milford, MA, USA) was utilized for analysis of minocycline in both serum and tissue samples. The mobile phase was delivered by a Waters M600E system. Chromatographic patterns were recorded with a Waters M996 photodiode array detector. A Waters C_{18} μBondapak (220×30 mm) column was used for sample separation. Data were analyzed using a Waters Millennium Chromatographic Manager package, v2.0, in a NEC/486 computer.

2.2. Tissue extraction

Wound tissue samples (7.9±2.6 g) were suspended in 2 ml of HPLC-grade water and homogenized for 1 min in a Thomas homogenizer (Thomas Scientific, Swedesboro, NJ, USA). All of the concentrations were standardized to tissue weight. The samples were placed in an ice bath during homogenization. The homogenate was centrifuged at 2000 *g* for 30 min at 4°C. Sep-Pak C_{18} cartridges (Waters) were primed with 2 ml of HPLC-grade water, then washed with 2 ml of methanol and finally with 2 ml of water. For each sample, 500 μl of the supernatant were applied to the cartridge. The cartridge was then rinsed with 2×500 μl of HPLC-grade water. The resulting solution (1.5 ml) was frozen at −70°C until HPLC analysis.

2.3. Serum extraction

Whole blood was allowed to coagulate and was subsequently centrifuged at 2500 *g* for 5 min at 4°C. The serum was collected, thus removing fibrinogen and various proteins. Serum (500 μl) was passed through a Sep-Pak C_{18} cartridge as in Section 2.2 and the cartridge was rinsed with 2×500 μl of HPLC-grade water. The resulting solution (1.5 ml) was frozen at −70°C until HPLC analysis.

2.4. HPLC analysis

Analysis was conducted using a μBondapak C_{18} column (250×30 mm). The mobile phase was isocratic methanol at a flow-rate of 1 ml/min. As minocycline is water soluble, the sample was ready for injection without any additional preparation since HPLC-grade water was used as the eluent. Precisely 30 μl of the eluent from the cartridge were injected into the column via the autosampler (Waters M717).

3. Results

A 1 μg/μl solution of minocycline in water was used to determine the standard curve. The injection

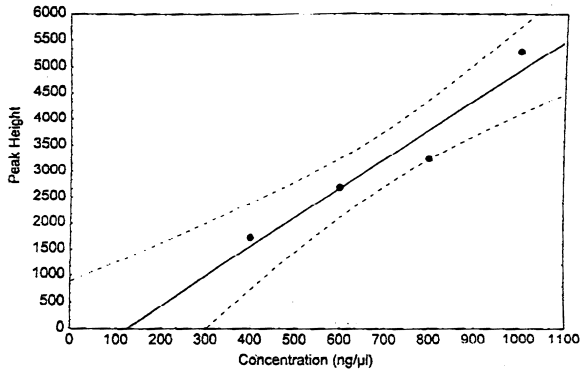

Fig. 1. Standard curve for minocycline, produced using stock solutions at concentrations of 400, 600, 800 and 1000 ng/μl. Correlation of the regression line was $r^2=0.931$. Symbols: - - -, 95% confidence intervals; —, linear regression.

volume was varied to produce a standard curve (100, 300, 600, 800 and 1000 ng of minocycline; Fig. 1). The curve was linear with a minimum detection of 100 ng. A small peak representing the water was seen at 2.2 min followed by minocycline at 2.5 min (Fig. 2). Drug recovery was 61% for the tissue samples and 54% for serum samples. Samples that were collected had concentrations that were directly correlated to the time of tissue harvest or blood withdrawal. The time at which the peak eluted (Fig. 2) remained consistent (2.52±0.13 min), indicating the stability of minocycline up to day seven of the study.

4. Discussion

The extraction of minocycline presented a challenge. The compound is both light-sensitive and degrades rapidly, having a reconstituted shelf-life of 24 h. The Sep-Pak cartridge enables a rapid extraction that has been used with a variety of HPLC

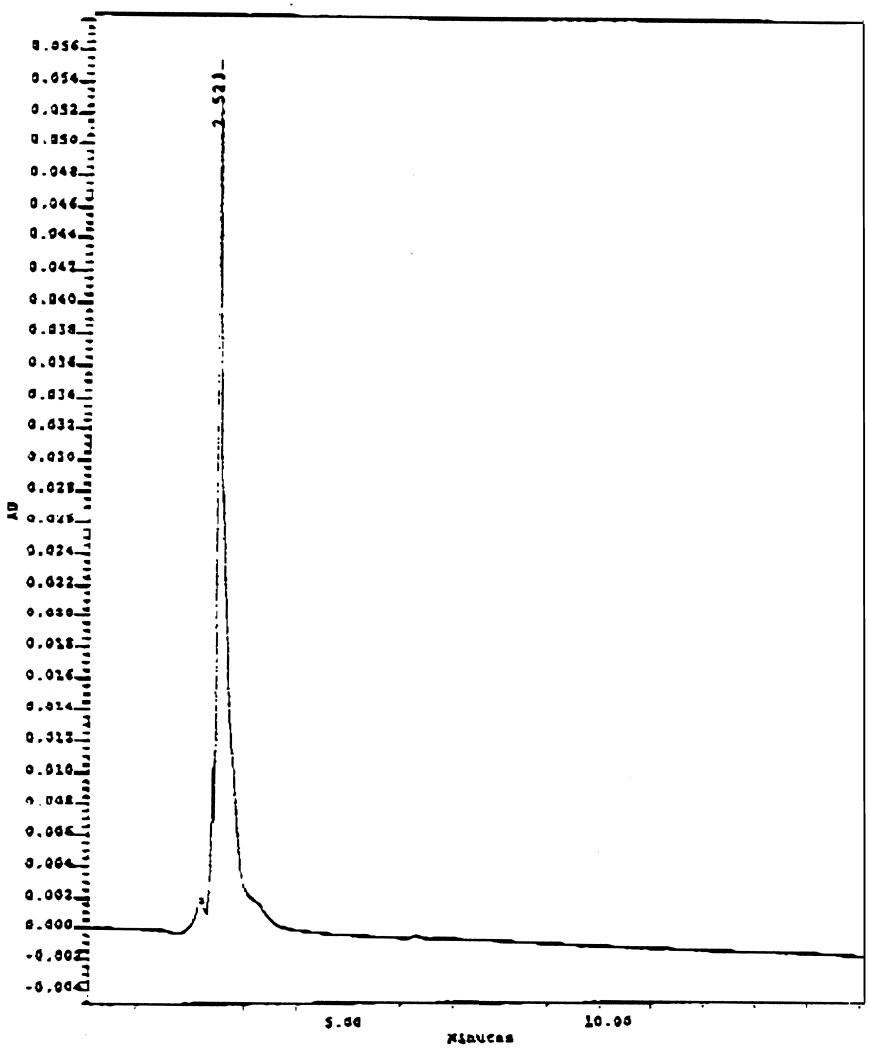

Fig. 2. Minocycline samples from an animal at day one following minocycline administration. The concentration in the sample was determined to be 27 μg/g of tissue. AU=absorbance.

methods [4,6]. We chose to use the cartridge because of this characteristic and its ability to eliminate contaminants. We believe that the carrier, as well as protein contaminants, were eliminated with this method. There was no significant degradation of the compound following cartridge extraction when compared to minocycline that had been freshly applied to the column. We found that there was a 17% loss secondary to degradation in samples that had been left at room temperature for 24 h compared to fresh samples. When samples were maintained at cool temperatures (−70°C), this loss was not seen.

Minocycline's cyclic structure allows for UV detection (350 nm). In addition, its solubility in water and methanol make selection of the mobile phase a simple determination of the ratio of methanol to water. We evaluated mobile phases with water to methanol ratios of 100:0, 75:25, 50:50, 25:75 and 0:100. The higher water ratios showed multiple and asymmetric peaks, indicating possible minocycline degradation. The most efficient mobile phase, which did not cause distortion, was 100% methanol and this was used for the study. The r^2 value (0.931) of the standard curve was within acceptable limits to allow for consistent and accurate analysis of minocycline. The r^2 value increased to 0.987 if the 800 ng sample injection was eliminated (Fig. 1). The HPLC method described above provides a simple and reproducible analytical method for the detection of minocycline in tissue and serum.

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References

[1] S. Galandiuk, H.C. Polk Jr., D.G. Jagelman, V.W. Fazio, Surg. Gynecol. Obstet. 169 (1989) 219–222.
[2] H.C. Polk Jr., L. Trachtenberg, C.D. George, Am. J. Surg. 152 (1986) 517–521.
[3] W.G. Cheadle, Am. J. Surg. 164 (1992) 44S–47S.
[4] J. Walsh, L. Walker, J. Webber, J. Chromatogr. 596 (1992) 211–216.
[5] G.D. Mack, R.B. Ashworth, J. Chromatogr. Sci. 16 (1978) 93–101.
[6] J. Bauchet, E. Pussard, J.J. Garaud, J. Chromatogr. 414 (1987) 472–476.
[7] T. Hasan, B.S. Cooperman, J. Chromatogr. 321 (1985) 462–466.