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Doxycycline assay hair samples for testing long-term compliance treatment

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Summary *Objectives:* Many patients undergoing long-term doxycycline treatment do not regularly take their treatment because of photosensitivity. Our objective was to create an assay for determining doxycycline levels and to use hair samples for monitoring the compliance over a longer period of time.
Methods: We tested sera and hair samples from patients treated with doxycycline by a suitable ultra-high performance liquid chromatography (UHPLC) based assay.
Results: We estimated that the speed of hair growth is roughly 1.25 cm per month and we were able to determine doxycycline levels over a 6-month period. We tested 14 patients treated with doxycycline and we found similar levels of doxycycline in the serum and the hair samples representing the last 4 months. Linear regression analysis revealed that the level of doxycycline in the serum remained stable over time ($p = 0.7$) but the level of doxycycline in the hair decreased significantly over time ($p = 0.03$) indicating a degradation of this molecule in the hair. We detected two patients who did not have antibiotic in the hair, indicating a lack of compliance that was also confirmed by interview.
Conclusion: Hair samples can be used to test long-term compliance in patients to explain failures or relapses.

Introduction

Long-term hydroxychloroquine and doxycycline is the reference treatment for Q fever endocarditis caused by *Coxiella burnetti*[1] and for Whipple's disease (WD) caused by *Tropheryma whipplei*.[2] Q fever endocarditis is associated with surgery for 15%—73% of patients, causes death for 5%—65% of patients, and induces a large number of relapses when the endocarditis is inadequately treated.[3,4] The most serious risk factor for endocarditis is a substantial underlying valvulopathy, but progression to endocarditis is also found in patients with clinically silent,

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previously undiagnosed, valvulopathies.[5] *T. whipplei* can be involved in chronic, localized infections without systemic involvement and asymptomatic carriage.[2] *T. whipplei* causes endocarditis which differs from classic Whipple disease, which primarily affects the gastrointestinal system.[2] The incidence rate of *T. whipplei* endocarditis among blood culture-negative endocarditis cases at Germany was estimated to be 6.3% and at our center (Assistance Publique Hôpitaux de Marseille, Marseille, France) was estimated 2.6%.[2] A combination of doxycycline and hydroxychloroquine is proposed for WD,[2,6] for the long-term treatment of Q fever endocarditis: 18 months for native valves and 24 months for prosthetic valves[3,4] and 18 months for Q fever osteoarticular infection.[7] Tetracyclines are known to have side effects such as gastrointestinal symptoms, candidiasis, photosensitivity, pigmentation changes, and central nervous system effects.[8] Photosensitivity, including sunburn, photosensitive eruptions, blistering, rash, pruritus, and photoonycholysis has proposed side effects that significantly affect the quality of life of patients treated with long-term doxycycline and hydroxychloroquine.[8–10] As a result, patients with long-term doxycycline treatment may fail to take their treatment regularly and the monitoring of doxycycline and hydroxychloroquine levels in the blood is critical for the management of Q fever endocarditis and WD.[11]

Nowadays, besides blood and urine, hair is used as an alternative specimen for drug testing. Hair analysis has been previously proposed for retrospective detection of chronic exposure to therapeutic drugs including antiretroviral, clenbuterol, benzodiazepines, methadone and carbamazepine, and tobacco components.[12,13] Our study was based on a patient who was treated for WD and presented acceptable serum doxycycline and hydroxychloroquine levels. However, one patient told to one of us (D.R.) that she was taking her antibiotic treatment only the day before her consultation as she knew that doxycycline would be detected in the blood. As the concentration of drugs in hair reflects uptake from systemic circulation over an extended time frame (weeks to months), hair analysis provides an advantage over plasma monitoring in assessing average drug exposure over a longer period of time.[14] Our aim was to develop a suitable ultra-high performance liquid chromatography (UHPLC) based assay to investigate the levels of doxycycline in the hair of patients for whom there are doubts concerning regular treatment administration.

Methods

Patients and samples

Each patient provided written consent, and the study was performed after ethical approval by the local ethics committee (number 12–016). We tested serum samples from the outpatients of an infectious diseases unit (Hopital La Timone, Marseille, France) who were suffering from Q fever or WD and treated with doxycycline (100 mg twice per day) and hydroxychloroquine (200 mg three times per day). Additionally, for each patient, we collected several non-colored small thatches of hair (about 150–200 strands) that were cut as close as possible to the scalp at the back of the head. For each patient, we also tested at least two serum samples. Hair samples obtained from 20 control individuals without antibiotic treatment for at least 6 months (Hopital La Timone, Marseille, France) were also sampled. The exclusion criteria were an age under 18 years, a history of cancer, inflammatory bowel disease, acute or chronic diarrhea in the previous 4 weeks and the administration of another antibiotic >6 months before sampling.

Doxycycline monitoring

Chemicals and reagents

Doxycycline hyclate and oxytetracycline hydrochloride (internal standard) were high-purity standards (Sigma Aldrich, Lyon, France) prepared in water. Acetonitrile, ethyl acetate, methanol and water were HPLC-grade solvents (VWR, Fontenay sous Bois, France). Triethylamine, Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), ascorbic, acetic, formic, orthophosphoric and oxalic acids were high-purity reagents (VWR, Fontenay sous Bois, France). Lyotrol N multiparametric assayed human control serum was purchased from Biomerieux (Craponne, France). Phosphate sulfite buffer was made with 1 M NaH_2PO_4 and 1 M Na_2SO_3 in water. The EDTA extraction buffer was made of 50 mM acetic acid and 10 mM EDTA in water, as described by UCT.[15] The aqueous mobile phase was made of 1% triethylamine and 1 mM oxalic acid in water adjusted to pH 2.5 with orthophosphoric acid 85%.

Serum sample preparation

Lyotrol N human control serum was used to prepare calibration standards fortified with 1.25, 2.5, 5 and 10 μg/mL doxycycline. 100 μL of calibration standards and serum samples were all spiked at 15 μg/mL with the internal standard. Then, 50 μL of 300 mM ascorbic acid, 100 μL of phosphate sulfite buffer, followed by 500 μL of ethyl acetate were added. Tubes were vortexed then centrifuged to collect the organic supernatant containing doxycycline. The organic extract was evaporated at 75 °C under a nitrogen stream. The dry extract was dissolved in 100 μL of the initial gradient mobile phase (10% acetonitrile/90% aqueous mobile phase).

Hair sample preparation

Hair samples were cut with scissors into 1 or 2 cm segments depending on the initial quantity available, in order to work with 10–30 mg of sample. Hair segments were weighed in 2 mL hard tissue grinding MK28 tubes containing 2.8 mm stainless steel beads (Bertin technologies, Montigny le Bretonneux, France). Samples were washed with ethanol then water and the solvents removed. Hair was ground using a FastPrep-24 instrument over five 60 s cycles at 6.5 m/s (MP Biochemicals, Illkirch, France). 1 mL of EDTA extraction buffer was added to the pulverized hair, which was then spiked at 1 μg/mL with the internal standard. Blank hair was spiked with doxycycline in order to prepare calibration standards fortified with 50, 100, 250, 500,