The Detection of Vancomycin in Sweat: A Next-Generation Digital Surrogate Marker for Antibiotic Tissue Penetration: A Pilot Study

Noé Brasier^a Andreas Widmer^c Michael Osthoff^{b, g} Markus Mutke^{a, b}
Fiorangelo De Ieso^{a, b} Pascale Brasier-Lutz^f Kitty Brown^d Linxing Yao^d
Corey D. Broeckling^d Jessica Prenni^e Jens Eckstein^{a, b}

^aCMIO Research Group, Department of Digitalization and ICT, University Hospital Basel, Basel, Switzerland; ^bDivision of Internal Medicine, University Hospital Basel, Basel, Switzerland; ^cDepartment of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland; ^dAnalytical Resources Core, Bioanalysis and Omics Center, Colorado State University, Fort Collins, CO, USA; ^eDepartment of Horticulture and Landscape, Colorado State University, Fort Collins, CO, USA; ^fDepartment of Gynaecology, Kantonsspital Luzern, Standort Wolhusen, Wolhusen, Switzerland; ^gDepartment of Clinical Research, University Basel, Basel, Switzerland

Keywords

Sweat analysis · Vancomycin · Antibiotic stewardship · Skin and soft tissue infections · Clinical study · Wearable sensors

Abstract

Background: Assuring adequate antibiotic tissue concentrations at the point of infection, especially in skin and soft tissue infections, is pivotal for an effective treatment and cure. Despite the global issue, a reliable AB monitoring test is missing. Inadequate antibiotic treatment leads to the development of antimicrobial resistances and toxic side effects. β -lactam antibiotics were already detected in sweat of patients treated with the respective antibiotics intravenously before. With the emergence of smartphone-based biosensors to analyse sweat on the spot of need, next-generation molecular digital biomarkers will be increasingly available for a non-invasive pharmacotherapy monitoring. **Objective:** Here, we investigated if the glycopeptide antibiotic vancomycin is detectable in sweat samples of in-patients treated with intravenous vancomycin. **Methods:** Eccrine sweat sam-

ples were collected using the Macroduct Sweat Collector[®]. Along every sweat sample, a blood sample was taken. Biofluid analysis was performed by Ultra-high Pressure Liquid Chromatograph-Tandem Quadrupole Mass Spectrometry coupled with tandem mass spectrometry. **Results:** A total of 5 patients were included. Results demonstrate that vancomycin was detected in 5 out of 5 sweat samples. Specifically, vancomycin concentrations ranged from 0.011 to 0.118 mg/L in sweat and from 4.7 to 8.5 mg/L in blood. **Conclusion:** Our results serve as proof-of-concept that vancomycin is detectable in eccrine sweat and may serve as a surrogate marker for antibiotic tissue penetration. A targeted vancomycin treatment is crucial in patients with repetitive need for antibiotics and a variable antibiotic distribution such as in peripheral artery disease to optimize treatment effectiveness. If combined with on-skin smartphone-based biosensors and smartphone applications, the detection of antibiotic concentrations in sweat might enable a first digital, on-spot, lab-independent and non-invasive therapeutic drug monitoring in skin and soft tissue infections.

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Introduction

Emerging smart biosensors analyse molecular digital biomarkers in sweat directly on patient skin and qualify as the next-generation in point-of-care monitoring [1]. Previous studies demonstrated the detectability of β -lactam antibiotics in sweat [2]. Monitoring antibiotic concentrations at the point-of-need is pivotal to assure therapeutic antibiotic tissue concentrations. A reliable detection method has yet to be developed. The use of molecular digital biomarkers, such as antibiotic concentrations, in sweat is a novel approach to combat antimicrobial resistances, a main global issue in health-care [1, 3].

Skin and soft tissue infections are characterized by microbial invasions [4]. There has been a 40% increase of skin and soft tissue infections resulting in a 3-fold increase of direct healthcare costs in the USA between 2000 and 2012 [5]. Adapting the standard antimicrobial dosage is pivotal as tissue penetration has been demonstrated to vary significantly between patients [6]. Variation in antibiotic levels between plasma and target site typically results in insufficient antibiotic levels in tissue, which can lead to antimicrobial resistances [7]. Among other, vancomycin has a central role in treating methicillin-resistant staphylococcus aureus. While therapeutic drug monitoring for antibiotics in plasma is associated with a positive clinical outcome, it is invasive, laborious and only provides an estimation of target site concentration. Therapeutic drug monitoring in skin and soft tissue infections has so far only been utilized in research studies (skin blisters and micro-dialysis studies) [8]. A tissue targeted, personalized point-of-care monitoring for antibiotic levels is urgently needed.

Previously, the detection of β -lactam antibiotics in patients' sweat samples was demonstrated [2]. The antibiotic vancomycin has a high molecular weight and is polar, therefore its appearance in sweat remained unclear. The excretion of vancomycin into sweat is likely to be active through channels. Sweat analysis is currently lab-dependent with limited clinical utility. Smartphone-based biosensors (Fig. 1) could enable on-skin, lab-independent sweat analysis, providing "real-time" digital molecular feedback [1].

We investigated the detectability of the glycopeptide vancomycin in patients' sweat, and discussed the potential of vancomycin detection in sweat as a digital non-invasive, target site drug monitoring.

Fig. 1. A novel smart patch by Reeder et al. [10] that analyses sweat directly on the skin (photo credit: P. Gutruf, Northwestern University. Distributed under a CC BY-NC license <http://creativecommons.org/licenses/by-nc/4.0/>, re-use permission for the picture granted by the American Association for the Advancement of Science (AAAS), Science advances journals).

Materials and Methods

Trial design of this observational trial, survey of patient characteristics, Ethics Committee identification number, inclusion and exclusion criteria were previously published in Brasier et al. [2]. Sweat sampling was conducted 6 h after the last intravenous antibiotic dose using the sweat-inducing Macroduct Sweat Collector®. In short, sweat glands were stimulated using pilocarpine iontophoresis, a combination of a local acetylcholine analogue and a local current. Sweat was collected by a capillary container, samples were transported on dried ice and stored at -80°C until analysis. The ClinicalTrials.gov registration number is NCT03678142.

The sweat sample (50 μL) was combined with 50 μL of aminopterin (10 ng/mL in 50% methanol, Toronto Research Chemicals, Canada) as an internal standard and extracted with 0.4 mL of ice-cold methanol. The mixture was vortexed for 10 s and incubated at -20°C overnight, followed by centrifugation at 15,000 g for 20 min (4°C). The supernatant was then dried under a stream of nitrogen. The dried extract was re-suspended in 50 μL of 5% methanol in water and stored at -80°C until analysis. An external calibration curve was prepared by adding various amounts of vancomycin (Clearsynth, NJ, USA) to artificial sweat samples (Pickering Laboratories, CA, USA), followed by extraction as described above. Whole blood samples (50 μL) were combined with 25 μL of aminopterin (1 $\mu\text{g}/\text{mL}$ in 50% methanol) and extracted with 610 μL of cold methanol. The supernatant was used directly for UPLC-MS/MS analysis. An external calibration curve was prepared adding

Table 1. Patient characteristics and results

Patient	1	2	3	4	5
Age, years	68	63	77	67	66
Gender, f/m	F	M	F	M	M
Body weight, kg	57.7	130.0	97.5	82.7	72.0
Body temperature, °C	37.1	36.7	36.8	35.5	36.9
Creatinine, $\mu\text{mol/L}$	42	99	57	110	144
GFR, mL/min/1.73 m^2	94	70	86	59	43
Vancomycin					
Per dose, mg	1,000	1,000	750	500	750
Per day, mg	1,500	2,000	1,500	1,000	750
Amount of previous doses, <i>n</i>	1	36	4	5	12
Peripheral artery disease	No	No	No	No	Yes
Vancomycin in sweat, mg/L	0.011	0.118	0.016	0.032	0.019
Vancomycin in blood, mg/L	5.8	4.7	5.6	5.2	8.5
Vancomycin penetration, %: Vancomycin in sweat mg/L /vancomycin in blood $\text{mg/L} \times 100$	0.19	2.5	0.29	0.62	0.22

various amounts of vancomycin to drug free whole blood matrix followed by extraction. Sample extracts (2 μL) were injected into a UPLC-MS/MS system consisting of a Waters Acquity Classic UPLC coupled with a Waters Xevo TQ-S triple quadrupole mass spectrometer (Milford, MA, USA). Chromatographic separation was performed on a Waters HSS T3 UPLC column (2.1 $\times$ 50 mm, 1.8 μM). The column temperature was held at 45 °C. Mobile phases consisted of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. The solvent gradient started with 99% A and held for 1 min, increased to 99% B in 1.5 min, equilibrated at 99% B for 1 min. Total run time was 5 min. The mass detector was set in ESI+ mode with 0.6 kV of capillary voltage. The source and desolvation temperatures were 150 and 450 °C, respectively. The desolvation gas (N_2) flow was 1,000 L/h. The precursor/product ions transitions were chosen based on a previous study [9], 725 > 100, 725 > 144, 725 > 1,306 for vancomycin; 441 > 175, 441 > 294 for aminopterin. The cone voltage and collision energy were optimized using the Intellistart tool in Waters Masslynx software (online suppl. Table S1, see www.karger.com/doi/10.1159/000512947). Two analytical replicates were analyzed for each sample. A pooled quality control sample was repeatedly analyzed to monitor instrument stability (coefficient of variation of 4.8%; $n = 12$). Peak integration was completed using TargetLynx XS in MassLynx software. The peak area of vancomycin normalized to that of aminopterin was used in the determination of the vancomycin concentration.

Results

Five patients treated with intravenous vancomycin were included. Vancomycin was administered for >24 h in 4/5 patients, and 1/5 patients had an impaired renal function. The patients' ages varied from 63 to 77 years, weights varied between 57.7 and 130 kg, and the received

vancomycin doses ranged from 500 to 1,000 mg (total daily doses from 1,000 to 2,000 mg; Table 1).

Vancomycin was detectable in every sweat and blood sample. The amount of earlier applied doses were $1 \times -36 \times$ times (Table 1). Vancomycin concentrations in sweat ranged from 0.011 to 0.118 mg/L and vancomycin was detectable after first intravenous dose, whereas vancomycin concentrations in blood ranged from 4.7 to 8.5 mg/L (Table 1). Vancomycin in sweat was about 0.2–2.5% in relation to blood concentrations. Results across the five patients suggest a positive trend between applied dosage levels and sweat concentrations (Table 1).

Discussion

To the best of our knowledge, this pilot study represents the first reported detection of vancomycin in patients' sweat treated with intravenous vancomycin. Vancomycin was detected in all of the sweat samples tested. Vancomycin was detectable in sweat at low levels (0.011 mg/L) in patient 1 that received only a single dose before, in contrast to patient 2 at higher levels (0.118 mg/L) after having received 36 doses. While patient 1 weighed almost half as much as patient 2 (smaller distribution volume), both patients had an adequate renal function (>50 GFR [mL/min/1.73 m^2]) and were of a comparable age. We hypothesize that antibiotic concentrations' steady state arises after the application of multiple doses. Patient 5 (1 dose/day with 750 mg) showed a lower sweat, but a higher blood antibiotic concentration compared to patient 4

(2 doses/day, each 500 mg). We hypothesized that higher single shot doses may lead to an increase in blood concentration, but higher daily doses, applied twice a day may enable a better tissue penetration with subsequent higher sweat concentrations.

If further approved in larger trials, detecting on-spot vancomycin concentrations in sweat may allow to personalize antibiotic treatment in skin and soft tissue infections. This approach could be particularly beneficial for patients with peripheral artery diseases, as vancomycin tissue penetration varies significantly [6]. Overall, adequate antibiotic concentrations above the bacterial minimal inhibitory concentration at the point of infection is essential to prevent from development of antimicrobial resistances or overdosing with a high risk of concomitant toxic side effect [6].

In this pilot study, the sweat analysis was conducted using mass spectrometry, which is a lab-dependent and expensive technology. Clinical utility of this digital biomarker will require development of smart biosensor technology that will enable a non-invasive, on-skin detection (Fig. 1) [1]. Combined with an automated smartphone application, this would enable the dynamic integration of antibiotic concentration measurements with other data such as patient body weight, antibiotic application dose, and bacterial minimal inhibitory concentration. This personalized treatment approach could be applied in-hospital as well as in an outpatient setting, enabling a patient-centred administration of antibiotic drug treatment.

Despite describing a novel promising approach of therapeutic drug monitoring, this study had some limitations. Study sample size was small, sweat was actively induced and collected during 30 min, therefore absolute vancomycin concentrations need to be interpreted carefully. One patient was wrongly recruited despite having an impaired renal function (estimated glomerular filtration rate [eGFR] $<50 \text{ mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{[2]-1}$) and one patient was treated with the antibiotic $<24 \text{ h}$. Due to the pilot study character, the patients were not excluded retrospectively. Finally, as in patients treated with vancomycin the minimal inhibitory concentrations range in concentrations of about $>1 \text{ mg/L}$, the low antibiotic sweat concentrations need to be set in context to clinical outcome and not only to the minimal inhibitory concentration.

In summary, we see a great potential of monitoring vancomycin concentrations in sweat in skin and soft tissue infections. Especially a non-invasive and lab-independent test at the spot of need may help to prevent from

antibiotic misuse. Larger trials to proof the detectability as well as the impact on clinical outcome are needed before specific smart sweat sensors are developed.

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Statement of Ethics

This research complies with the guidelines for human studies and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. All patients provided written informed consent. The Ethics Committee of Northwest and Central Switzerland approved this study (EKNZ Reg-ID 2018-01155).

Conflict of Interest Statement

M.O. received a project grant and consulting fees from Pharming Biotechnologies B.V. with regards to a different project. J.E. is holding 0.5% of virtual shares of Preventicus. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author Contributions

N.B., P.B.-L., and J.E. contributed substantially to the conception of the trial. N.B. critically interpreted state-of-the art literature. N.B. and F.D. drafted the manuscript. N.B., A.W., M.O., M.M., F.D., P.B.-L., K.B., L.Y., C.D.B., J.P., and J.E. critically reviewed and finally approved the manuscript version to be published. N.B. and J.E. agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and reported.

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