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ORIGINAL ARTICLE

A prospective study to analyse the concentration of octenidine in hand wounds after disinfection by LC–MS/MS

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Abstract

Toxic reactions can appear after pressurised flushing of soft tissue with octenidine (OCT) containing disinfectants. Their use for surgical disinfection could complicate the diagnosis of possible contamination. In patients with open lacerations of their hand's subcutaneous tissue samples were taken before and after surgical disinfection with Octenisept® and analysed by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). In 16 out of 20 tissue samples, OCT was detected after disinfection (lower limit of quantification (LLOQ)=10 pg/mL/mg). The concentration of OCT was below the LLOQ, estimation of mean of 0.6 pg/mL/mg (0.22–0.98 pg/mL/mg, 95%-CI) before disinfection and mean of 179.4 pg/mL/mg (13.35–432.0 pg/mL/mg, 95%-CI) after disinfection. This study shows that the disinfection of open wounds with Octenisept® leads to a quantifiable concentration of OCT in open wounds. In cases of suspected OCT-mediated toxic reaction, the use of antiseptics containing OCT should be avoided.

KEYWORDS

hand injuries, octenidine, surgical disinfection, toxic reaction, ultra-high-performance liquid chromatography coupled to tandem mass spectrometry

Key Messages

- Octenidine (OCT) can be quantified in hand wounds after surgical disinfection by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry.
- The aim of this study was to develop a specific and sensitive method for quantifying OCT in the tissue of open wounds.
- The proportion of OCT-positive patients after disinfection was statistically significant compared to those prior to disinfection. Furthermore, the OCT concentration in tissue samples after disinfection was significantly higher compared to tissue samples prior to disinfection.

1 | INTRODUCTION

Octenisept® (Schülke & Mayr, Norderstedt, Germany) is a widely used antiseptic because of its low tissue irritation. It is used for disinfection of skin and mucosa as well as the treatment of chronic, traumatic and iatrogenic wounds.[1] The recommended technique for using Octenisept® is swabbing or fine spraying. Its active substances octenidine (OCT) (0.1%) and phenoxyethanol (2%) interact with polysaccharides of the cell wall of micro-microorganisms and induce cell death by destruction of the cytoplasmic membrane.[2] It covers a broad spectrum of activity against bacteria, fungi and lipophilic viruses.[1] This and its mostly painless application even in insufficiently anaesthetised wounds make it a favourable disinfectant in antiseptic wound treatment. Since OCT is not absorbed by the body there is no systemic toxicity.[3]

Toxic reactions after the application of OCT such as chemical serositis, persistent subcutaneous oedema and aseptic necrosis are described in the literature.[4,5] They predominantly occur when OCT is injected (pressurised application) into the soft tissue or when rinsing wound pockets without sufficient drainage.[3,6,7] The toxicity is explained by the interaction between the antiseptic and polysaccharides of the cell membrane, which induces cell death after destruction of the cytoplasmic membrane.[2] Histologically, cytotoxic effects were detected in keratinocytes, fibroblasts and in endothelial cells of blood vessels.[8] The observed persisting oedema which is even more pronounced in the narrow compartments of the hand[5] may indicate an increased vascular permeability and subsequently vascular damage which was also seen in-vitro.[9] The slow degradation and thus retention of OCT in the interstitium further increase the irritant-toxic effect.

OCT-mediated toxic reactions are generally characterised by severe initial pain, followed by reddening and oedematous swelling. In the further course, the oedema solidifies, and the skin becomes indurated, which leads to functional restrictions and chronic changes in the soft tissue.[4,5]

Clinically, OCT-mediated inflammatory reactions are very similar to bacterial infections, and it is extremely difficult to discriminate between the two. However, apart from taking a careful medical history, the study of medical records and eliminating other causes, no other approaches to a rapid diagnosis of this pathology are reported in the literature. The delayed correct diagnosis can lead to unnecessary antibiotic therapies as well as repeated surgical interventions and prolonged hospitalization. OCT-mediated toxic reactions can remain for up to one year after initial contact with OCT and are arduous to treat.[2]

The aim of this study was to develop a specific and sensitive method for quantifying OCT in tissue of open wounds. For this purpose, a method using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) was developed and validated. We hypothesised that Octenisept® should not be detectable in the damaged soft tissue after standardised superficial disinfection. To demonstrate this, the presence of OCT in the tissue of open wounds before and after disinfection with Octenisept® was determined in 20 patient samples as a primary outcome. The measurement of the concentration aims to allow the definition of a clinical range of OCT concentration in such cases.

2 | METHODS

The study was approved by the local ethics committee. Informed consent form (ICF) was obtained from all individual participants prior to inclusion in the study.

Patients aged ≥18 years with open injuries of their hands presenting in our department and needing surgical revision were included. Exclusion criteria were planned conservative wound therapy, disinfection of the injured hand prior to presentation to our department with either Octenisept® or any other antiseptic that was not clearly documented in the patient's history detection of OCT in wounds prior to surgical disinfection in this study and patients, who were unable to understand and follow the study procedures. Spatially separated wounds on the same patient were counted individually.

After inclusion in the study, a screening examination was carried out. At the screening visit, demographic data and medical history were recorded. A physical examination was carried out and the type and severity of injury were documented. Subsequently, patients were taken to the operating theatre.

After reaching adequate anaesthesia, a first sample (approximately 3–5 mm^3) of subcutaneous tissue was taken from the depth of the open wound prior to disinfection. The site of surgery was then disinfected three times 30 s with sterile swabs drenched with 30 mL of Octenisept®. After sterile covering, the second sample of subcutaneous tissue was harvested from the depth of the injury. The wounds were then debrided, and irrigation was carried out with 0.9% saline solution, followed by primary wound closure.

Two and six weeks after the surgery a physical examination was carried out and adverse events (AE) corresponding to an OCT-mediated toxic reaction were recorded.

The samples were temporarily stored at −20°C and then analysed by LC–MS/MS.

Calibrants and quality controls (QCs) were prepared in a 1:1 (v/v) mixture of acetonitrile: methanol (extraction solvent). The frozen tissues were gently thawed at room temperature and 1 mL of extraction solvent was added. The samples were then ground with five stainless steel balls for 5 minutes at 25 Hz. The extracts were centrifuged at 20 000 rcf (relative centrifugal force) for 5 min at 4°C. The supernatant was diluted with solvent B (see below) 100 times prior LC–MS/MS analysis. A volume of 1 µL of the diluted sample was injected on an Acquity I-Class system (Waters, Milford, MA, USA) and separated on a Cortecs UPLC C18 column (2.1 mm × 50 mm, 1.6 µm, Waters, Milford, MA, USA) maintained at 50°C. ACN:H_2O 1:1 (v/v) with 10 mM ammonium format and 0.15% (v/v) formic acid (mobile phase A) and ACN:H_2O 95:5 (v/v) with 10 mM ammonium format and 0.15% (v/v) formic acid (mobile phase B) were used as mobile phases. OCT was ionised by electrospray ionization (ESI) and detected in the positive ion mode on a triple quadrupole mass spectrometer (Xevo TQ-S, Waters, Milford, MA, USA). The parent ion was set at m/z 276.5 and the fragment ions at m/z 95.11. Both ions are double-charged.

The assay was validated following international guidelines.[10,11] Main parameters such as carryover, matrix effect, linearity, selectivity, inter- and intra-assay were defined. The lower limit of quantification (LLOQ) was 10 pg/mL/mg, and a lower limit of detection (LLOD) was estimated at 1 pg/mL/mg. No coefficient of variation (CV) and bias were defined between 10 and 1 pg/mL/mg. But the very low S/N characters of the peaks below LLOQ, increase the risk to be above specifications (>15%) regarding inaccuracy and imprecision. This led to the estimate of the results measured below LLOQ.

2.1 | Statistical and power analysis

Considering a one-sample proportion binomial test with the null hypothesis that the proportion of positive patients is 1% and 15% as the alternative hypothesis, a power of 80% is reached with a sample of 19 tissue samples and a one-sided significance level of 0.05.

Demographic data were analysed using descriptive statistics. The proportion of positive tissue samples and the mean OCT concentration were accompanied by 95% confidence intervals. The primary outcome was analysed using a one-sample binomial test with the null hypothesis (reference) proportion pre-specified to be 1%. Pre- and postoperative OCT concentrations were analysed using the Wilcoxon signed-rank test.

3 | RESULTS

In total, 23 patients with 26 wounds were eligible. Six patients had to be excluded due to OCT concentrations above the lower level of quantification (LLOQ) prior to surgical wound disinfection. Finally, tissue samples obtained in 20 wounds (17 patients) were analysed. Two patients (three tissue samples) did not attend follow-up visits, resulting in the assessment of 14 surgical sites postoperatively. Demographic data of the 17 participants and details of the individual cases are listed in Tables 1 and 2.

In 16 tissue samples, OCT was detected above LLOQ of 10/pg/mL/mg after disinfection with Octenisept®. In four tissue samples, OCT was detected below LLOQ after disinfection.

Preoperatively, no OCT was detected in 15 tissue samples. In five tissue samples, OCT was detected below the LLOQ. The proportion of OCT-positive patients above LLOQ after disinfection was statistically highly significant ($p < 0.001$, one sample proportion test) compared to prior to disinfection.

The OCT concentration in tissue samples after disinfection (mean of 179.4 pg/mL/mg, 13.35–432.0 95%-CI) was higher compared to tissue samples prior to disinfection (mean of 0.6 pg/mL/mg, 0.22–0.98 95%-CI). This difference was statistically significant ($p < 0.01$, Wilcoxon signed-rank test).

During the two follow-up visits, no OCT-mediated toxic reactions were reported. Three patients presented with prolonged wound healing of which two underwent surgical revision. In one patient, wound coverage was achieved by means of a local flap and in the other patient with a full-thickness skin graft. The microbiological

TABLE 1 Demographic data.

Characteristic	Study population
Age in years, mean (SD)	45.5 (20.3)
Female, *n* (%)	2 (11.76)
Male, *n* (%)	15 (88.24)
Profession, *n (%)*	
Administrative labour	3 (17.65)
Manual Labour	10 (58.82)
Student	1 (5.88)
Pensioner	3 (17.65)
Type of accident, *n* (%)	
Crush injury	5 (29.41)
Cutting injury	6 (35.29)
Amputation injury	1 (5.89)
Mixed	5 (29.41)

TABLE 2 Data of individual cases.

No.	Gender	Profession	Type of injury	Multiple injuries	Complication
1	Male	Manual	Circular saw injury	Yes	Surgery/antibiotic
2	Male	Admin	Cut injury		
3	Male	Manual	Cut injury		
4	Male	Manual	Crush injury		
5	Male	Manual	Crush injury		
6	Male	Retired	Cut Injury	Yes	
7	Female	Admin	Crush injury		
8	Male	Manual	Crush injury		
9	Male	Manual	Amputation		Surgery/antibiotic
10	Male	Retired	Milling machine		
11	Male	Manual	Crush injury		
12	Male	Student	Cut injury	Yes	
13	Female	Admin	Cut Injury		
14	Male	Manual	Crush injury		
15	Male	Retired	Cut injury		
16	Male	Manual	Cut injury		
17	Male	Manual	Open fracture		

analysis of the debrided tissue in both patients detected a colonization with *S. aureus*, which was treated with short-term antibiotics. One patient complained of adhesions, which could be treated conservatively with hand therapy.

4 | DISCUSSION

In 80% ($n = 16$) of tissue samples with a concentration above LLOQ, OCT was detected in soft tissue after disinfection with Octenisept®. In the remaining four samples, the concentration was detected below the LLOQ of 10 pg/mL/mg. All patients received superficial surgical disinfection with Octenisept® prior to surgery without flushing or rinsing of wound pockets. Then intraoperative irrigation of wounds was carried out with 0.9% saline solution. None of the patients with a positive tissue sample developed symptoms consistent with OCT-mediated toxic reactions.

In those wounds where OCT was detected, there is quite a large variance in OCT concentration. Although we have tried to exclude possible confounding factors as far as possible by standardised disinfection, technical differences (e.g., lighter, or coarser scrubbing, differences in exposure times with the disinfectant) or the different size and texture of the surface of the wounds could be responsible for this.

In six patients with no apparent history of prior use, OCT was detected in the obtained tissue above the LLOQ before disinfection. We were unable to determine whether prior disinfection with an agent containing OCT occurred, or whether the wound was contaminated during preoperative procedures after including the patient in this study. We therefore excluded these patients from the analysis.

Since OCT does not penetrate intact skin, it is only detectable in subcutaneous tissue if the skin barrier is damaged.[12] Disinfection of open wounds with Octenisept® leads to contamination of the subcutaneous tissue with OCT which can be detected by LC–MS/MS. According to our current knowledge, LC–MS/MS is at present not suitable for detecting OCT as the cause of a toxic reaction in cases where, as part of the surgical revision, disinfection with Octenisept® has taken place. In such clinical cases, the well-recognised sensitivity advantage of LC–MS/MS might be a disadvantage. If an OCT-mediated toxic reaction is suspected, it is therefore imperative to refrain from any further treatment with OCT-containing substances, obtain a tissue sample and send it to an appropriate laboratory for analysis.

The exact mechanism of how OCT-mediated toxic reactions develop in soft tissue is not yet fully understood, and different mechanisms are discussed.[2,8,13] The standardised disinfection with Octenisept® as performed in this study did not result in any OCT-mediated toxic

reactions. Although we were only able to collect data from a relatively small cohort, these results are consistent with data from the literature.[14] We also conclude that OCT concentrations measured in the tissue samples after disinfection are not sufficient to trigger an OCT-mediated toxic response. Since OCT-mediated toxic reactions occur mainly during pressure injection and irrigation of wound pockets without adequate drainage, a relationship with the concentration of OCT in the tissue is plausible.

The clinical manifestation of an OCT-mediated toxic reaction has been described several times in the literature. The diagnosis was then made clinically and based on the medical history and, if necessary, supported by additional examinations such as ultrasound, microbiology and histopathology.[4,6,15] The tolerability of OCT-containing antiseptics in superficial wound treatment has been evaluated in animal models as well as in clinical studies based on clinical criteria such as the influence on wound healing and additionally by histopathological analysis.[12,13,16] Furthermore, the concentration of OCT by means of high-pressure liquid chromatography (HPLC) was measured in in vitro permeability studies.[12] A comparison of our data with data in the literature is not possible due to the different technical approaches.

Attempts to detect OCT in contaminated tissue in vivo have not been documented, nor has the concentration in vivo been determined. This study therefore presents a novel approach to detecting OCT in soft tissue in vivo and is aimed to help find the correct diagnosis.

Due to the absence of appropriate data, we currently have not been able to compare our results with OCT concentrations after pressure injections or, more generally, with cases of manifest OCT-mediated toxic reactions. Therefore, aspects for subsequent investigations should include a determination of the OCT concentration after pressure irrigation, after irrigation without adequate drainage or the OCT concentration in the wound after prolonged exposure. A uniform and standardised protocol for measuring OCT concentration in tissue enables comparability and reproducibility in clinical practice.

Our study has some limitations. The method of OCT detection in tissue by LC–MS/MS is highly sensitive. Therefore, cross-contamination of the samples during extraction could be a potential source for detecting false positive samples as the ones detected before disinfection. Inadequacy of the screening method prior to inclusion might be another source for pre-operative OCT detection.

The tissue obtained was taken directly from the depth of the wound. Although it was mainly subcutaneous adipose tissue, the collection of other tissue types that were in close anatomical relationship to the wound and may have been harvested was not excluded and was not documented. In addition, the study design does not allow any conclusions to be made about how far tissue contamination with OCT extends beyond the confines of the wound.

Overall, OCT-mediated toxic reactions are a rare phenomenon. However, misapplications continue to occur where wounds are directly irrigated with disinfectants containing OCT and a rapid diagnosis should be made in case of suspicion of an OCT-induced inflammation. LC–MS/MS should be considered as a valuable diagnostic tool for the detection of OCT in treated wounds and in suspicious cases, further contamination by additional disinfection with OCT-containing agents pre-surgery should be avoided.

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study was approved by the Cantonal Ethics Committee Bern (Project ID: 2018–01242).

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