Clinical Nutrition xxx (2014) 1–6

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Microbiocidal effects of various taurolidine containing catheter lock solutions☆

E.D. Olthof[a,*,1], R. Nijland[b,1], A.F. Gülich[a], G.J.A. Wanten[a]

[a] Intestinal Failure Unit, Department of Gastroenterology and Hepatology, Radboud University Medical Centre, Nijmegen, The Netherlands
[b] Department of Medical Microbiology, University Medical Center Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 10 January 2014
Accepted 30 April 2014

Keywords:
Taurolidine
Citrate
Heparin
Catheter lock solution
Microbial growth
Biofilm

SUMMARY

Background & aims: We have recently shown that a catheter lock solution containing taurolidine dramatically decreases catheter-related bloodstream infections (CRBSI) in patients on home parenteral nutrition (HPN) when compared to heparin. Since several taurolidine formulations are commercially available, some of which also contain citrate or heparin, we were interested in the effect of these different locks on growth and biofilm formation of fungal, Gram-negative and Gram-positive pathogens that are known to impede HPN treatment.

Methods: Clinical isolates obtained during CRBSI of HPN patients were grown in the presence of catheter locks (2% taurolidine, 1.34% taurolidine–citrate, 1.34% taurolidine–citrate–heparin, citrate and heparin) or phosphate buffered saline diluted in lysogeny broth medium for bacteria and sabouraud liquid medium for yeasts. Biofilm formation, assessed by crystal violet staining, and growth of clinical isolates were determined by optical density measurements.

Results: We found that 12.5× diluted solutions of all taurolidine containing formulations completely prevented growth of *Escherichia coli*, *Staphylococcus aureus* and *Candida glabrata*. Growth of these microbes was detected earlier in 1.34% taurolidine–citrate(–heparin) than in 2% taurolidine, while citrate and heparin did not inhibit growth of clinical isolates compared to PBS. No differences in biofilm formation were found between taurolidine containing solutions.

Conclusion: Taurolidine containing lock solutions prevent growth of fungal, Gram-negative and Gram-positive pathogens. While 2% taurolidine appears to be the most potent in this respect in this *in vitro* setting, the relevance of the small differences in growth inhibition between the commercially available taurolidine containing lock solutions for clinical practice remains to be established.

1. Introduction

Catheter-related bloodstream infections (CRBSI) are the foremost threat to continuation of treatment in patients who depend on central venous catheters (CVCs) for long-term intravenous (parenteral) nutrition due to severe intestinal failure [1]. CRBSI are associated with a high risk for catheter loss, infection-associated morbidity and as such confer a considerable burden on healthcare resources. The most common microbial species that cause such infections are skin-derived Gram-positive bacteria, followed by Gram-negative bacteria and fungi [2].

Abbreviations: HPN, home parenteral nutrition; CRBSI, catheter-related bloodstream infections; CVC, central venous catheters; MIC, minimum inhibitory concentrations; *E. coli*, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; *C. glabrata*, *Candida glabrata*; LB, lysogeny broth; SLM, sabouraud liquid medium; PBS, phosphate buffered saline.

☆ **Conference presentation**
NESPEN (Netherlands Society for Parenteral and Enteral Nutrition) 2013, Veldhoven, The Netherlands: Oral presentation (4th October 2013), Microbiocidal effects of various taurolidine containing catheter lock-solutions.
ESPEN 2013, Leipzig, Germany: Poster presentation (1st September 2013), PP196-SUN Microbiocidal effects of various taurolidine containing catheter lock-solutions.
UEGW 2013, Berlin, Germany: Poster presentation (16th October 2013), P1679 Microbiocidal effects of various taurolidine containing catheter lock-solutions.

* Corresponding author. Department of Gastroenterology and Hepatology (internal code 455), Geert Grooteplein Zuid 8, 6525 GA Nijmegen, The Netherlands. Tel.: +31 (0) 243667242; fax: +31 (0) 243540103.
E-mail addresses: Evelyn.Olthof@radboudumc.nl, e.olthof@mdl.umcn.nl, evelyn_olthof@hotmail.com (E.D. Olthof), R.Nijland@umcutrecht.nl (R. Nijland), Alexandra.Guelich@gmail.com (A.F. Gülich), Geert.Wanten@radboudumc.nl (G.J.A. Wanten).
[1] EDO and RN contributed equally.

http://dx.doi.org/10.1016/j.clnu.2014.04.023
0261-5614/

CRBSI are usually preceded by catheter colonization, which implies deposition of microbes and biofilm formation on the extraluminal and intraluminal surfaces of these catheters, especially at the catheter hub [3,4]. To prevent catheter contamination as a source of infection caregivers and patients are trained in meticulous aseptic catheter maintenance. Subcutaneously cuffed single lumen catheters are recommended for HPN care in order to establish maximal barrier function [5].

Strategies to prevent intraluminal catheter colonization include use of anti-adhesive catheter biomaterials [6] and instillation of antimicrobial lock solutions to prevent bacterial attachment and minimize biofilm formation [5]. Taurolidine is such a potent broad spectrum antimicrobial agent that is used as part of catheter lock solutions. Taurolidine is non-toxic for humans and is rapidly metabolized into taurine, water and carbon dioxide [7]. The mechanism of action of taurolidine involves the chemical reaction with the microbial cell wall, endotoxins and exotoxins [8], thus inhibiting both pathogenicity and microbial adhesion [9] to inert and living surfaces. No evidence of the development of microbial adaptation to taurolidine after prolonged use of this catheter lock solution was found [10].

Clinically, the use of taurolidine as catheter lock has been shown to decrease CRBSI rates in various patient groups compared to traditional catheter locks i.e. heparin [11–15]. Several structurally different taurolidine-based solutions are commercially available, some of which contain citrate and/or heparin because these locks are used in hemodialysis practice, implying that blood is aspirated via the catheter and anticoagulants are necessary to prevent catheter clogging [14,16–18]. Although the relevance of citrate and heparin to prevent catheter-related thrombosis in this setting is undisputed because of their anticoagulant characteristics [16,19], the use of these agents for prevention of CRBSI is under debate. Especially since heparin and citrate promote biofilm formation [20], and citrate is a substrate for the growth of *Escherichia coli* [21] and *Klebsiella pneumoniae* [22], i.e. microbial strains that are known to cause CRBSI, and high-dose of 30% citrate can cause side effects, including tetany [23].

We hypothesized that when used in a setting where anticoagulants are not deemed necessary, catheter lock solutions containing taurolidine without additives are more bactericidal compared to the same volume of taurolidine formulations containing citrate and/or heparin. To determine the antimicrobial effect of these formulations, the effects on growth and biofilm formation of fungi, Gram-positive and -negative bacteria were assessed.

2. Materials and methods

2.1. Growth of clinical isolates

Clinical isolates of *E. coli*, *Staphylococcus aureus* (*S. aureus*) and *Candida glabrata* (*C. glabrata*) were previously obtained during CRBSI episodes of HPN patients and were kindly provided by the Department of Medical Microbiology of the Radboud University Medical Center, Nijmegen, The Netherlands. Clinical isolates were collected during the hospital stay and diagnosis of the CRBSI. To determine the effect of lock solutions of growth inhibition, the clinical isolates were first cultured on agar plates at 37 °C, and eventually in Lysogeny Broth (LB) medium and Sabouraud Liquid Medium (SLM), for bacteria and yeasts, respectively, at 37 °C. Subsequently, the clinical isolates were incubated in 96 well plates (Greiner® flat bottom 96 well) at an initial optical density of 0.01 at 37 °C in the presence of 7×, 10×, 12.5×, 20×, 33× and 100× diluted lock solutions of catheter locks (Table 1) or phosphate buffered saline (PBS, control) in LB-medium and SLM medium for bacteria and yeasts, respectively. Growth of clinical isolates was evaluated by optical density measurement at 660 nm using a Fluostar® Omega microplate reader continuously every 30 min for 60 h (BMG Labtech, Germany) and data were analyzed using the MARS software package (BMG Labtech, Germany), and Microsoft Office Excel (Microsoft Corp., USA) [24].

2.2. Biofilm formation

After the evaluation of microbial growth of the clinical isolates, as described above, culture medium was removed, plates were washed four times in tap water and stained with 0.5% crystal violet solution for 10 min. Subsequently, the staining solution was aspirated, the plates were rinsed with tap water until all unbound crystal violet was removed. The plates were dried to air and 150 μL of 95% ethanol with 2% acetic acid was added to dissolve the crystal violet bound to the biofilm [25]. Finally, absorption at 595 nm was measured using a Biorad iMark microplate reader (Bio-Rad Laboratories BV, Veenendaal, The Netherlands) to quantify the biofilm formation.

2.3. Statistical analyses

The effect of the catheter lock solutions on the growth of *E. coli*, *S. aureus* and *C. glabrata* was measured in duplicate, and each experiment was performed three times on separate days. A representative growth curve, presenting the optical density measurement at 660 nm every 30 min for 60 h during incubation in media at 37°, is presented in Fig. 1 with the mean of two technical replicates. The growth time required for the culture to reach 50% of the maximum optical density of the PBS control (OD50) was determined in all individual growth curves. To calculate the effect of taurolidine on the growth rate, the growth time until OD50 in taurolidine containing lock solutions was divided by their respective PBS controls to correct for inter experimental differences. Next, we calculated the multiplicative inverse to obtain a growth value. This value was expressed as a growth percentage of the PBS control and presented in Fig. 2. The experiment comparing the effect of 1.5 times diluted (to obtain similar taurolidine concentrations) pure 2% taurolidine to 1.34% taurolidine–citrate on growth of *E. coli*, *S. aureus* and *C. glabrata* was measured in quadruplicate and performed once. The mean of the in quadruplicate measured growth is presented in Fig. 3. The effect of the catheter lock solutions on the biofilm formation of *E. coli*, *S. aureus* and *C. glabrata* was measured in duplicate, and each experiment was performed twice.

3. Results

3.1. Taurolidine efficiently inhibits growth of E. coli, S. aureus and C. glabrata

The effect of different taurolidine-containing lock solutions (2% taurolidine, 1.34% taurolidine–citrate and 1.34% taurolidine–citrate–heparin), citrate, heparin and PBS on microbial growth was

Table 1
Composition of evaluated catheter lock solutions according to the manufacturers.

	Manufacturer	Taurolidine (%)	Citrate (%)	Heparin (IU/mL)
Taurosept®	Geistlich	2	–	–
Taurolock®	TauroPharm	1.34	4	–
Taurolock-Hep®	TauroPharm	1.34	4	500
Heparin®	Pharmacy Radboud University Medical Center	–	–	500
Citrate	–	–	4	–

Please cite this article in press as: Olthof ED, et al., Microbiocidal effects of various taurolidine containing catheter lock solutions, Clinical Nutrition (2014), http://dx.doi.org/10.1016/j.clnu.2014.04.023