Loss of antimicrobial effect of trisodium citrate due to ‘lock’ spillage from haemodialysis catheters

Gernot Schilcher¹, Daniel Schneditz², Werner Ribitsch¹, Joerg H. Horina¹, Martin Hoenigl³, Thomas Valentin³, Alexander R. Rosenkranz¹ and Robert Krause³

¹Clinical Division of Nephrology, Department of Internal Medicine, Medical University of Graz, Graz, Austria, ²Institute of Physiology, Medical University of Graz, Graz, Austria and ³Section of Infectious Diseases and Tropical Medicine, Department of Internal Medicine, Medical University of Graz, Graz, Austria

Correspondence and offprint requests to: Gernot Schilcher; E-mail: gernot.schilcher@medunigraz.at

ABSTRACT

Background. Due to its reported antimicrobial effects, hypertonic citrate (46.7%) is a widely used catheter lock solution, but following instillation, citrate inevitably spills into the systemic circulation. This process is mainly driven by hydraulic effects during instillation and density differences between blood and lock solution. Hence, in haemodialysis catheters, intra-luminal citrate concentration ranges from 0% (at the tip in catheters with side holes), 3% (between the side holes and the highest point of the catheter) to 46.7% (at the Luer end) with possible differences in antimicrobial effects. We investigated in vitro the antimicrobial effect of pure citrate 46.7%, citrate 46.7% diluted with saline and blood to a net concentration of 3% (=citrate 3%), and of citrate-free blood, simulating in vivo conditions in different catheter sections.

Methods. Time–kill studies measuring the antimicrobial effect of citrate 46.7%, citrate 3% and citrate-free blood were performed with overnight cultures of \textit{Escherichia coli} (\textit{E. coli}) and \textit{Staphylococcus aureus} (\textit{S. aureus}).

Results. Citrate 46.7% reduced the number of \textit{E. coli} by 2 log units but after 24 h, 10⁶ CFU/mL were still present. Citrate 3% and citrate-free blood had no antimicrobial effect on \textit{E. coli}. Citrate 46.7%, citrate 3% and citrate-free blood had scarce antimicrobial effect on \textit{S. aureus} within 24 h.

Conclusions. Spillage of catheter lock solution leading to reduced intra-luminal citrate concentrations considerably reduces the antimicrobial effect of citrate 46.7% on \textit{E. coli}. As none of the solutions tested had relevant antimicrobial effect on \textit{S. aureus}, the antimicrobial effect of 46.7% citrate lock solution in vivo has to be seriously questioned.

Keywords: catheter-related bloodstream infection, central venous catheter, lock solution, lock spillage, trisodium citrate

INTRODUCTION

Central venous catheters (CVCs) provide reliable venous access for haemodialysis and are still in common use despite the emphatic recommendations in national guidelines favouring arteriovenous fistulae [1]. Anticoagulative, fibrinolytic or antimicrobial catheter lock solutions are used as prophylaxis or therapy to maintain the intra-luminal patency of CVCs in an attempt to avoid thrombosis and infection [2, 3]. Trisodium citrate (30–46.7%) is widely used and considered effective in reducing catheter-related bloodstream infections (CRBSI) in haemodialysis patients [4, 5]. However, the use of hypertonic citrate remains controversial due to reported adverse events such as a decrease of ionized calcium due to chelation, which can cause cardiac arrhythmia, or pulmonary embolisms when plasma proteins precipitate in the lumens of the CVC [6–11].

Instillation of the listed filling volume of lock solutions, such as citrate 46.7%, is generally believed to ‘lock’ the CVC, suggesting that the entire injected volume remains inside the CVC. Recent studies report, however, that ~20% of the catheter locking volume leaks into systemic circulation at the time of instillation [12]. This spillage during instillation is a physical consequence of a parabolic flow profile within cylindrical tubes, such as CVCs. Furthermore, gravity-induced loss of lock solution into the systemic circulation should be considered when lock solutions with different densities compared with blood (e.g. trisodium citrate 46.7%) are used [13–15].
Finally, in catheters with side holes, the lock solution is completely washed out of the tip region immediately after instillation, representing additional loss of locking anticoagulant [16]. The aforementioned flow and exchange phenomena cause inhomogeneous intra-luminal dilution of lock solution which, depending on the individual design, divides the catheter into sections with different citrate concentrations. The antimicrobial effect of citrate 46.7% can therefore be expected to vary among these catheter sections. Previous in vitro studies using pure citrate lock solution showed an antimicrobial effect for concentrated citrate [17]. In vivo studies, however, reported conflicting results regarding the efficacy of concentrated citrate in reducing CRBSI in haemodialysis patients [4, 18].

The objective of this in vitro study was to investigate the antimicrobial effect of pure citrate 46.7%, citrate 46.7% diluted with blood and saline to a net concentration of 3% (=citrate 3%), and of citrate-free blood, simulating in vivo conditions in different sections of haemodialysis catheters ‘locked’ with citrate 46.7%.

**MATERIALS AND METHODS**

The methodological approach is based on recent reports regarding mechanisms and quantification of the lock spillage phenomenon [12, 13, 16, 19]. In brief, physical effects following instillation of trisodium citrate 46.7% into the lumen of a catheter dilute the lock solution and divide the catheter into different sections corresponding to the particular citrate concentrations (Figure 1). As the citrate concentration within the ‘connector section’ remains nearly unaffected by dilution, pure trisodium citrate 46.7% was used to characterize the antimicrobial effect in this catheter section. The residual trisodium citrate concentration of ~3% within the ‘core section’ arises from two successive physical effects. Firstly, the saline used to flush and clear the catheter of blood is not completely replaced when a Newtonian lock solution (aqueous solution) is used. This is due to the parabolic flow profile and incomplete saline removal from the catheter wall during injection. The magnitude of this saline dilution is in the range of 20% regardless of injection time and patient position (injection spillage). Secondly, the density difference between trisodium citrate 46.7% and blood promotes gravity-induced seepage of citrate out of the catheter, which is accompanied by blood inflow into the catheter. The end point of this process is reached after 20 min, resulting in a final citrate concentration of ~3% (gravitational spillage). The exchange of trisodium citrate 46.7% against blood continues up to the highest point of the catheter, i.e. the vertex, which is usually the venous insertion point in patients with jugular or subclavian catheters. The section boundaries in our model were set accordingly. To prepare the citrate 3% test solution, pure trisodium citrate 46.7% was consecutively diluted with saline and with blood. A mixture of four parts citrate 46.7% with one part saline 0.9%, corresponding to the initial dilution effect of 20% (injection spillage), was the source of further dilution with blood (gravitational spillage) to a net concentration of 3% trisodium citrate. In haemodialysis catheters with side holes, the region between the tip and the most distal side hole, the ‘tip section’, contains citrate-free blood following complete wash-out of lock solution within seconds after instillation. The antimicrobial effect of pure citrate 46.7%, citrate 3% and citrate-free blood thus was investigated by time–kill studies adopted from previous literature [17]. Briefly, *Staphylococcus aureus ATCC 29213* and *Escherichia coli ATCC 35218* were cultured overnight in brain heart infusion broth and diluted with each of the test solutions to a final concentration of 10⁶ CFU/mL. At the start of the microbiological tests and at 1, 3 and 24 h, 100 µL of the suspensions were plated on chocolate agar (*S. aureus*) or MacConkey blood agar plates (*E. coli*) (Biomerieux, Marcy l’Etoile, France) and incubated for 24 h at 37°C in ambient air. Colonies were then counted and recalculated to CFU/mL. All tests were performed in duplicate. Saline 0.9% (Fresenius Kabi AG, Bad Homburg, Germany) and blood from a healthy adult male donor,
provided via sterile venipuncture technique into blood collection tubes (Vacuette®, Greiner Bio-One, Austria), was used for dilution of trisodium citrate 46.7% (Department of Hospital Pharmacy, Medical University of Graz, Austria). Written informed consent was obtained from the blood donor. Haematocrit, albumin and total protein of the blood sample were measured. The study was approved by the Ethics Committee of the Medical University of Graz, Austria.

RESULTS

Compared with baseline, the pure citrate 46.7% lock solution reduced the number of *E. coli* by 2 log units but, after 24-h incubation, $10^6$ CFU/mL were still present (Figure 2). Citrate 3% and citrate-free blood had no antimicrobial effect on *E. coli* (Figure 2). Citrate 46.7%, citrate 3% and citrate-free blood barely reduced the number of *S. aureus* within 24 h of incubation (Figure 3). Sample characteristics of blood used for citrate dilution were haematocrit 0.42, albumin 4.5 g/dL and total protein 6.5 g/dL.

DISCUSSION

The current standard for *in vitro* evaluation of the antimicrobial effect of lock solutions focuses solely on the pure lock solution [20, 21]. This might be inadequate when Newtonian fluids (aqueous solutions) and fluids that differ from blood in terms of fluid density such as hypertonic citrate are investigated. Physical phenomena, i.e. sequential dilutions with saline (injection spillage) and blood (gravitational spillage), as well as the wash-out effect in catheters with side holes, considerably reduce the intra-luminal citrate concentration. For Newtonian fluids, standard tests may only be valid for the region between the Luer connector and the highest point of the catheter (vertex), where the lock solution is conserved in nearly its original concentration. Numerous factors such as lock spillage should be considered to better translate *in vitro* tests into general practice recommendations.

Recently, Polaschegg [19] published a method for the quantitative measurement of catheter lock spillage. When citrate 46.7% was used as locking anticoagulant, within 20 min a citrate concentration approaching 3% was found in the catheter section containing a blood/trisodium citrate 46.7% mixture. These results are reasonable due to the high-density difference between trisodium citrate 46.7% (1.24 g/cm$^3$) and blood ($\sim$1.05 g/cm$^3$) [10, 22]. Even a very low-density difference of 0.0094 (0.9%) causes leakage of lock solution in exchange against blood within 60 min under the influence of gravity (gravitational spillage) [13]. Subsequently, we developed a distributed model of intra-luminal citrate concentrations in standard haemodialysis catheters filled with citrate 46.7% (Figure 1). Similarly, in femoral catheters, gravity forces citrate 46.7% to leak out in any patient position, supine or head down tilt, with the tip of the catheter lowered relative to its insertion point into the vein. Hence, the same gravitational spillage process, lock dilution with blood, as occurs in jugular or subclavian catheters, can be assumed. The only exception might be patients in permanent upright position with a femoral catheter.

In previous *in vitro* studies, pure citrate 30% reduced the number of *E. coli* by 3 log units whereas no antimicrobial effect was detected in *S. aureus* time–kill studies [17]. In our study, we obtained comparable results with a 2-log unit reduction of *E. coli* but nearly unchanged colony counts of *S. aureus* after 24 h of incubation in pure citrate 46.7% (Figures 2 and 3). These results are, however, only representative for the connector section. Neither citrate 3%, representing intra-luminal conditions within the core section, nor citrate-free blood, representing the tip section in catheters with side
holes, reduced *E. coli* or *S. aureus* colony counts (Figures 2 and 3). From a clinical point of view, the antimicrobial effect of trisodium citrate catheter lock solution may therefore be considered insufficient. *Staphylococcus aureus* is one of the leading causes of CRBSIs in haemodialysis patients; however, based on this study, its intra-luminal catheter colonization is affected neither by citrate 3% (core section) nor by citrate 46.7% (connector section). Although citrate 46.7% had some effect on *E. coli* in the connector section only, intra-luminal catheter eradication cannot be expected from our experiments and previous data [17]. In addition, bacterial load in CRBSI reaches 10⁶ CFU/mL or more and so is, in fact, much higher than in previous *in vitro* tests [17, 23]. The catheter lock spillage phenomenon resulting in lower intra-luminal citrate concentrations will abolish any antimicrobial effect of citrate 46.7% on non-adherent (planktonic) organisms freely floating in liquid medium in proximal catheter sections, as shown by our experiments. Conflicting clinical data about the efficacy of concentrated citrate in reducing CRBSI in haemodialysis patients have been reported. A randomized, controlled trial by Weijmer et al. [4] comparing citrate 30% with heparin 5% showed a significant reduction of the CRBSI rate attributed to citrate use. That trial, however, included a heterogeneous group of patients with both acute and chronic renal failure using uncuffed and cuffed CVCs. In an observational retrospective analysis, there was a decrease in CRBSIs due to *S. aureus* after the standard locking anticoagulant was changed from heparin 5000 IU/mL to citrate 46.7% [5]. However, an antimicrobial effect against planktonic *S. aureus* is absent throughout all intra-luminal catheter sections, as shown by our data and previous studies [17]. It is noteworthy that CRBSI is caused only by spread of planktonic bacteria from the biofilms on CVCs into the bloodstream [24]. Power et al. [18] reported a randomized, controlled trial comparing citrate 46.7% against heparin 5% in a homogeneous cohort of chronic haemodialysis patients with a single type of cuffed jugular catheters. There was no significant difference in rates of exit-site infection or CRBSI between the two groups. The authors concluded that the use of citrate 46.7% to prevent CRBSI was not justified. Interestingly, a wide range of baseline CRBSI rates from <1 up to 17/episodes per 1000 catheter-days have been reported in clinical trials before initiation of antimicrobial or antibiotic lock solutions [4, 18, 25]. Regarding CRBSI prevention, some studies using heparin or citrate 4%, i.e. solutions that have no proven antimicrobial effect on planktonic bacteria, showed data comparable with those using antimicrobial lock solutions [26–28]. Confounding factors such as differences in adherence to catheter exit site care or line connection techniques may have considerable bearing on study results and conflicting data. A wider adoption of care standards by health personnel is desirable and might limit the use of antimicrobial lock solutions to high-risk patients [29, 30]. In *in vitro* studies have demonstrated that citrate inhibits *S. aureus* biofilm formation [31]. Biofilm formation, thought to be a key factor in development of CRBSI, is probably similarly influenced by the lock spillage phenomenon, just as is the effect on planktonic bacteria. Citrate 46.7% is not able to kill planktonic bacteria sufficiently; however, a substantial effect due to chelation of Ca²⁺ and Mg²⁺ to reduce biofilm formation and CRBSI is conceivable [32]. Even low citrate concentrations ranging from 0.5 up to 4% efficiently inhibit *S. aureus* biofilm formation; blood components present in different catheter sections might also have an influence, but this has not yet been studied *in vitro* [31, 33]. Additionally, the outer surface of the extravascular catheter segment was the most common site of bacterial growth in a study of chronic haemodialysis patients with positive blood cultures [34]. As the outer surface of catheters is not affected by lock solutions, these findings should be considered in designing further strategies to prevent CRBSI. The lock spillage effect is also important for antibiotic lock

**Figure 3.** Time–kill curves of *Staphylococcus aureus* suspended in citrate 46.7% (solid line with circles), citrate 3% (dashed line with squares) and blood without citrate (dotted line with triangles) after 0, 1, 3 and 24 h of incubation.
techniques. In catheters filled with vancomycin, there was a decreasing gradient along the catheter lumens [35]. Sequential intra-luminal dilution of antibiotic lock solutions with saline (injection spillage) and blood (gravitational spillage) may promote failure of antibiotic lock therapy and even induce bacterial resistance in prophylactic use. The blood influx into the catheter reduces the antibacterial effect of certain antibiotics due to plasma protein binding [36]. Biocompatible thixotropic gels rather than purely Newtonian fluids can be expected to completely lock the vascular access without spillage and might be a future direction for antimicrobial lock solutions but they have not yet been tested.

Our study has some limitations. Firstly, the spillage during injection also causes saline dilution within the connector section; however, the very small amount of dilution was not taken into account and can be neglected for in vitro testing. Secondly, to quantify the antimicrobial effect within the core section, time-kill studies were conducted with a single citrate concentration. Actually, due to the parabolic flow profile during lock instillation (instillation spillage) as well as during blood inflow into the catheter (gravitational spillage), the dilutional effects are not completely uniform throughout the whole catheter length. The citrate concentration is slightly higher at the most distal point compared with the most proximal point of the core section. Nevertheless, using citrate 3% is a reasonable compromise. Thirdly, also in terms of further physical influences, in vivo conditions are not completely comparable with in vitro conditions. Due to catheter compression, certain patient positions or opened catheter clamps lock spillage might exceed the in vitro quantification and shorten the connector section resulting in further reduction of antimicrobial effect [10, 19]. Finally, in vitro quantification of antimicrobial activities inevitably requires some dilution of the studied lock solution. Thus, in vitro results cannot be completely extrapolated to in vivo settings.

In conclusion, spillage of catheter lock solution leading to reduced intra-luminal citrate concentrations considerably reduces the antimicrobial effect of citrate 46.7% on E. coli.

As there was no relevant antimicrobial effect on S. aureus, even with citrate 46.7%, its antimicrobial potential to prevent infections in dialysis patients has to be seriously questioned. Furthermore, as inevitable lock spillage alters the antimicrobial effect of a given lock solution in general, additional in vitro tests accounting for dilution effects might better characterize the ‘real’ antimicrobial properties of lock solutions.

CONFLICT OF INTEREST STATEMENT

None declared.

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