Superior antimicrobial activity of trisodium citrate over heparin for catheter locking

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Abstract

Background. Haemodialysis catheters used for vascular access are frequently complicated by infection and catheter-related thrombosis. Improvement of interdialytic locking solutions could reduce these problems. Trisodium citrate (TSC) has been advocated in recent years because it might have antimicrobial qualities.

Methods. Antimicrobial efficacy of four concentrations of TSC (2.2, 7.5, 15 and 30%) was compared with three equi-osmolal sodium chloride (NaCl) concentrations, unfractionated heparin 5000 IU/ml and a solution of gentamicin 1 mg/ml in TSC 7.5%. We analysed antimicrobial properties by two classical in vitro susceptibility tests. All tests were performed in triplicate by incubation of test fluids with Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa and Candida albicans.

Results. Increasing TSC concentrations effectively killed the staphylococcal strains in both assays. For E.coli and P.aeruginosa complete killing was achieved only with TSC 30%. TSC 30% was also the only solution that significantly inhibited growth of C.albicans. Heparin manifested no antimicrobial effect of any significance. Adding gentamicin to TSC provided superior bacterial growth inhibition but had no effect on yeast growth. TSC solutions manifested superior antimicrobial activity compared with iso-osmolal NaCl solutions in both assays.

Conclusion. This in vitro study demonstrates superior antimicrobial activity of TSC, especially in higher concentrations, in contrast to heparin. The mechanism seems to differ from hyperosmolality. Ca²⁺ and Mg²⁺ chelating effects are probably more important. Adding gentamicin provided the most potent antimicrobial solution. However, for reasons concerning development of bacterial resistance and sensitization of the patient, continuous exposition to aminoglycosides seems not advisable.

Keywords: bacteraemia; catheter; haemodialysis; heparin; trisodium citrate; vascular access

Introduction

Vascular access is a major factor of concern for patients on haemodialysis treatment. Despite the recommendations of the National Kidney Foundation–Dialysis Outcome Quality Initiative Clinical Practice Guidelines for Vascular Access that recommends placement of an arteriovenous access before initiation of chronic haemodialysis treatment, the use of catheters for haemodialysis access is substantial [1]. Stehman-Breen et al. [2] reported from the United States Renal Data System 1996 that 66% of patients with end-stage renal disease started haemodialysis treatment with a catheter for access to the bloodstream. Twardowski [3] reported that 24.3% of almost 30 000 haemodialysis treatments in his outpatient facility in the period 1995–1997 were performed with a tunneled cuffed catheter. The use of haemodialysis catheters, however, is associated with an important risk for catheter-related infection and insufficient dialysis due to flow problems with or without intraluminal thrombosis [4]. Especially vascular access-related infections, mostly associated with haemodialysis catheters, have emerged as an important cause of morbidity and mortality in haemodialysis patients. From a prospective study in 796 haemodialysis patients performed in seven outpatient haemodialysis centres in 1998, Tokars et al. [5] calculated that over 92 000 episodes of vascular access infection occur annually among 220 000 prevalent haemodialysis patients in the US. A third of these patients had to be treated by hospitalization because of the infection. In addition, patients with a catheter had a relative risk for infection of 2.07
compared with patients with an arteriovenous fistula or graft.

It is recognized that microorganisms can adhere to the surface of a catheter. Contamination of the catheter hub, subsequent colonization of catheters with microbes and formation of a biofilm produced by bacteria are thought to be major risk factors for both catheter-related infections and intraluminal thrombosis [6]. It is, however, not elucidated whether the most important mechanism of catheter-related bacteremia is extraluminal or intraluminal colonization. If catheter-related blood stream infections are mainly secondary to intraluminal colonization, interdialytic locking using a solution with extensive antimicrobial effects can provide an important reduction of these complications. Traditionally, heparin 5000–10 000 IU/ml is used for interdialytic locking of haemodialysis catheters. Recently, however, trisodium citrate (TSC) has been proposed for catheter locking [7] and TSC 30% is already used in clinical practice [8]. TSC provides local anticoagulation by binding Ca2+. It can have important advantages over heparin, such as prevention of heparin-induced side-effects and unintentional systemic heparinization that can lead to bleeding complications, as was recently shown by Karaaslan et al. [9]. An additional factor in favour of TSC is its potential antimicrobial property. For these reasons TSC has been advocated for haemodialysis catheter locking and distributed temporarily by a haemodialysis catheter manufacturer (Medcomp, Medical Components Inc., Harleysville, PA). The level of hyperosmolality of the solution was considered the main explanation responsible for antimicrobial activity although binding of divalent cations was also mentioned [7].

However, very limited in vitro data on the antimicrobial properties of TSC as haemodialysis catheter locking solution are presently available. It is also not clear whether the antimicrobial potency of a solution depends on the level of hyperosmolality or not.

The purpose of this study is to evaluate the in vitro antimicrobial activity of different concentrations of TSC and to compare them with heparin and iso-osmolal sodium chloride (NaCl) solutions. We employed two classical in vitro antimicrobial susceptibility tests and used four bacterial strains and one yeast strain commonly found in catheter-related bacteremia.

Subjects and methods

Antimicrobial efficacy of four concentrations of TSC, 2.2% (300 mosmol/kg H2O), 7.5% (1020 mosmol/kg H2O), 15% (2040 mosmol/kg H2O) and 30% (4080 mosmol/kg H2O), were compared with sodium heparin 5000 IU/ml (300 mosmol/kg H2O). TSC 7.5% with gentamicin 1 mg/ml (1030 mosmol/kg H2O) was used to analyse the influence of adding an antibiotic to the solution. As a control we used NaCl 0.9%. In addition, we also compared the TSC solutions with iso-osmolal solutions of NaCl 0.9% (300 mosmol/kg H2O), NaCl 6.1% (2040 mosmol/kg H2O) and NaCl 12.2% (4080 mosmol/kg H2O). All solutions were manufactured from raw base by the Department of Pharmacy of the Vrije Universiteit Medical Center, Amsterdam, The Netherlands. The solutions were heat sterilized for 16 min at 121°C and the pH was controlled between 6.4 and 7.5. Gentamicin sulphate was obtained from commercially available vials (Gentamicin CF, Centrafarm Services, Etten-Leur, The Netherlands). All tests were performed with five standardized reference strains from the American Type Culture Collection (ATCC, Manassas, VA); Staphylococcus aureus (ATCC 25923) Staphylococcus epidermidis (ATCC 12228), Pseudomonas aeruginosa (ATCC 25922), Escherichia coli (ATCC 27853) and Candida albicans (ATCC 90028).

The antimicrobial activity of the solutions was investigated by time-kill and agar diffusion methods, essentially performed according to National Committee for Clinical Laboratory Standards guidelines [10]. Briefly, logarithmic-phase bacterial and yeast cultures were used for the final inoculum of 105 colony-forming units per ml (c.f.u./ml). Twenty microlitres of the microbial suspension was added to 2000 μl of a suspension containing a 10:1 dilution of the test solution in trypticase soy base (TSB) broth (Difco Laboratories, Sparks, MD) to achieve a final bacterial concentration of 104 c.f.u./μl. At this initial concentration, the comparison with time-kill curves of control solution was best feasible. Tubes were incubated at 37°C. At the start of the experiment (t = 0) and at 1, 2, 4 and 24 h, 50 μl of this suspension was plated on blood agar plates (BA) (Oxoid, Basingstoke, Hampshire, UK) supplemented with 7% sheep blood (Bio Trading, Mijdrecht, The Netherlands). Subsequently, plates were incubated for 24 h at 37°C. Afterwards colonies were counted and time-kill curves constructed from calculated c.f.u./μl. All tests and cultures were performed in triplicate.

The agar diffusion susceptibility test was carried out analogous to the disk diffusion test (Kirby-Bauer) [10]. BA and TSB plates were seeded with a bacterial solution with a final inoculum of 105 c.f.u./ml. Separate plates were used for each of the five microbial strains. Instead of using disks impregnated with test solution, one well with a diameter of 8 mm was punched out of the agar at the centre of the plate. The well was filled with test solution and this was repeated every 2 h for the first 6 h of incubation. A total of 0.45 ml of test solution had to be added to the well to keep it filled. Plates were incubated at 37°C for 24 h. Afterwards, zones of inhibition around the well were measured. All tests were performed in triplicate with BA and TSB plates.

Statistical analysis was performed with SPSS software package 9.0 (SPSS Inc., Chicago, IL) with repeated-measurements analysis of variance for time-kill curves. χ2 analysis was performed for means of bacterial c.f.u. at t = 24 h and for zones of inhibition achieved from the agar diffusion test. Significance of test results was based on P < 0.05 on a two-tailed test.

Results

Antimicrobial properties of different locking solutions

Time-kill studies. The time-kill curves for heparin, all concentrations of TSC and the combination of TSC
with gentamicin are presented in Figure 1. Heparin showed some growth inhibition of *S.aureus* and *S.epidermidis* compared with control (NaCl 0.9%). However, after 24 h all strains showed increasing growth (upward directed slope) when incubated with heparin. Heparin had no significant effect on growth of Gram-negative bacteria and *C.albicans* compared with control.

TSC 15% and TSC 30% reduced the number of c.f.u./ml of all strains over 24 h compared with the concentration at start of the experiment except for the yeast *C.albicans* and except for *P.aeruginosa* with TSC 15%. The citrate solutions inhibited growth of all strains compared with control (NaCl 0.9%), including Candida. The Gram-negative strains *E.coli* and *P.aeruginosa* were only adequately affected by the highest concentrations of TSC (15% and 30%) (*P* < 0.05 for *t* = 24 h). There were no statistically significant differences between TSC 30% and TSC 15%. TSC 30% was more effective in growth reduction of *E.coli*, *P.aeruginosa* and *C.albicans* than heparin (*P* < 0.05 for *t* = 24 h).

**Agar diffusion susceptibility test (Figure 2).** Studies using TSB plates and BA plates revealed similar results. The results for the zones of inhibition were therefore pooled for further analysis. Zones of inhibition are given in Figure 3. For all microbial strains no growth inhibition by the control solution (NaCl 0.9%) was found. Heparin also showed no effect at all. In general, higher concentrations of TSC demonstrated increasing inhibitory effect on all strains (Figure 3). TSC 30% was the only solution to inhibit growth of all tested microbes including *C.albicans*.
The inhibition zone was significantly larger for all strains compared with control (NaCl 0.9%) and heparin ($P < 0.01$ for all comparisons).

Addition of gentamicin to TSC potentiated the effect of TSC on all bacterial strains in both the dilution and the diffusion test. Growth of *C. albicans*, however, was not influenced.

**Antimicrobial properties of iso-osmolal solutions**

**Time–kill studies.** Comparing the results of the time–kill curves of iso-osmolal solutions, it is clear that there are major differences (Figure 1). For the iso-osmolal solutions NaCl 0.9% and TSC 2.2%, TSC 2.2% provided stronger growth inhibition in *S. epidermidis*, *S. aureus* and *C. albicans* ($P < 0.05$). The growth at 24 h was inhibited significantly better for *S. epidermidis* and *S. aureus* by TSC 15% compared with NaCl 6.1% and for *S. epidermidis* and *S. aureus* by TSC 30% compared with NaCl 12.2%. For the other strains the time–kill curves were not significantly different.

**Agar diffusion susceptibility test.** The agar diffusion test also showed larger zones of inhibition for TSC compared with iso-osmolal NaCl solutions, especially when osmolality increased (Figure 3). NaCl 6.1% and NaCl 12.2% exhibited no significant effect on microbial growth over NaCl 0.9%. NaCl 0.9% did not inhibit growth of any microbial strain. In contrast, iso-osmolal TSC 2.2% inhibited growth of *S. aureus* significantly. TSC 15% showed more antimicrobial effect compared with iso-osmolal NaCl 6.1% in all strains except for *P. aeruginosa* ($P < 0.05$). For the iso-osmolal solutions with the highest osmolality, NaCl 12.2% and TSC 30%, superior growth inhibition of TSC 30% was found in all strains ($P < 0.01$).
Antimicrobial effects of locking solutions

Discussion

In the present study we investigated the antimicrobial activity of TSC against five different microorganisms frequently encountered in catheter-related infections in haemodialysis patients using two standardized antimicrobial susceptibility tests. The antimicrobial activity was dose dependent with the highest efficacy for TSC 30%. In both tests the antimicrobial activity of TSC exceeded that of iso-osmolar NaCl concentrations, whereas heparin manifested only minimal antimicrobial activity. Thus, it can be concluded that the use of high concentrations of TSC for catheter locking could have an advantage over heparin. Adding gentamicin to TSC provided the most potent antibacterial solution. Lynn [11], however, showed that locking with a mixture containing an antibiotic, results in low systemic concentrations of the antibiotic resulting from diffusion from the tip of the catheter. The development of bacterial resistance and sensitization of the patient can be the consequence. Addition of aminoglycosides or other antibiotics to locking solutions for long-term use is therefore not advisable. Heparin revealed no relevant anti-microbial activity. This was recently also reported by Capdevila et al. [12] in vitro by means of the time-kill curves method and in vivo by implanting catheters in rabbits and inducing secondary infection but they only used one strain of S.aureus.

To investigate whether a locking solution can reduce complications, only a clinical study with large numbers of patients can provide definitive answers. The present study only provides in vitro data, but these studies have to be performed to give direction to which locking solution is most likely to reduce complications before conducting a clinical trial. No standardized methods are available for testing antimicrobial activity of catheter locking solutions. Although other in vitro methods have been advocated in the past, seldomly tests were performed using validated techniques with more than one microorganism and mainly established antibiotics were added to solutions for locking [12,13]. The methods we applied for this study consisted of two widely validated and recommended antimicrobial susceptibility tests. The tests were performed as recommended by the National Committee for Clinical Laboratory Standards [10,14]. Dilution tests are employed to provide more exact information on the concentrations of the antimicrobial solution that cause growth reduction and killing. However, the standardized disk diffusion test is the initial susceptibility test used in most laboratories because of its ease of performance, reproducibility, and proven value as a guide to antimicrobial therapy [15]. This test demonstrated the pronounced antimicrobial properties of TSC 30% most distinctly.

For the present study we selected both Gram-positive and Gram-negative bacteria frequently involved in catheter-related bacteremia. S.aureus and S.epidermidis are the most common bacteria found in catheter-related bacteremia. However, Gram-negative bacteria can be isolated in up to 45% of cultures and up to 21% of cultures reveal a polymicrobial infection [16]. We used reference microbial strains from the ATCC to minimize the variable microbial properties that may affect the results. Microorganisms were seeded on BA and TSB plates to investigate the influence of the growth medium. The results were very similar for both plates. Yeasts are not commonly involved in catheter-related infections. Nevertheless, we included a C albicans strain in our study because of the high mortality of systemic yeast infection. Inhibition of growth of Candida spp. by a locking solution could therefore be of importance.

Both susceptibility tests showed clear differences in antimicrobial properties for iso-osmolar solutions. In 18 of 30 comparisons that could be made between iso-osmolar TSC and NaCl solutions, TSC exhibited significantly greater inhibitory effects on microbial growth. Therefore, the anti-microbial properties of higher concentrations of TSC cannot be attributed to hyperosmolality. It is likely that other effects of TSC like chelation of the divalent cations Ca\(^{2+}\) and Mg\(^{2+}\) are more important. From dentistry research it is known that Ca\(^{2+}\) and Mg\(^{2+}\) chelating agents like disodium-ethylenediaminetetraacetate (EDTA) and sodium citrate exhibit similar inhibition of growth and coaggregation of microorganisms. Root et al. [17] showed in an in vitro model with catheter segments incubated with \(^{10}\)S.epidermidis that EDTA provided total killing of bacteria. They suggested that especially chelation of Mg\(^{2+}\) can interfere with cellular integrity by degradation of the bacterial cell wall membrane. Lipopolysaccharides in the bacterial cell wall are crossed-linked with divalent cations, providing stability. Lowering the concentration of these cations can lead to disruption of the cell wall and increase permeability [15,18]. Consistent with these findings is the observation that sodium citrate proved to be a potent permeabilizer of the cell wall at millimolar concentrations in a model used for permeability changes in Gram-negative bacteria. The effect was partly (P.aeruginosa, S.typhimurium) or almost totally (E.coli O137) abolished by MgCl\(_2\), suggesting that part of the action occurs by chelation [18].

Apart from Mg\(^{2+}\) binding, removal of Ca\(^{2+}\) from the surrounding milieu can be an explanation for the antimicrobial properties of TSC. Ca\(^{2+}\) may regulate several genes responsible for growth and survival of microbes. Holland et al. [19] demonstrated that cell division in E.coli in particular appears to be very sensitive to the level of cellular Ca\(^{2+}\), with the frequency of division clearly reduced by incubation with EDTA and by verapamil, a Ca\(^{2+}\)-channel inhibitor. The effect of EDTA was clearly correlated with depletion of cellular Ca\(^{2+}\). Biofilm formation, thought to be a key factor in catheter colonization and ultimately bacteremia, is probably dependent on Ca\(^{2+}\). A biofilm consists of bacteria that attach to surfaces and aggregate in a hydrated polymeric glyco-calyx matrix of their own synthesis. Formation of these sessile communities and their inherent resistance
to antimicrobial agents allows microbes to survive in a hostile environment. Even in individuals with excellent cellular and humoral immune reactions, biofilm infections are rarely resolved by the host defense mechanisms. In addition, antibiotics are not very useful because they have been shown to penetrate poorly into a biofilm [20]. Furthermore, at least some of the microbial cells in a biofilm experience nutrient limitation and therefore exist in a slow-growing state. Slow-growing or non-growing microbial cells are not very susceptible to antimicrobial agents. Until recently, the bacterial glycocalyx was regarded as being homogeneous in construction and static in its structure. It is now recognized that glycocalyces are not structurally static, but rather responsive to the chemical composition of the surrounding milieu. An increasing environmental Ca$^{2+}$ concentration dramatically enhanced the survival of Paeuruginosa in biofilms upon a 12-h exposure to tobramycin in an in vitro experiment [21]. It was suggested that Ca$^{2+}$-induced crystallization of the glycocalyx resulted in decreased permeability of the biofilm for small molecules like aminoglycosides. In summary, chelation of Ca$^{2+}$ and Mg$^{2+}$ by TSC may prevent the formation of a biofilm that consists of microbes in a firm glycocalyx. Reduction of the incidence of catheter-related bacteraemia by the intraluminal route could be the result. This hypothesis was tested in some in vitro models with catheter segments but the constructions with catheters or fragments trying to imitate the clinical situation are artificial [22,23].

As stated before, this study only provides data from in vitro antimicrobial susceptibility tests. It is not clear if the results can be translated to general practice as numerous factors have been implicated in the pathogenesis of catheter-related bacteraemia. For that reason, locking solutions must be compared in a clinical study to confirm their benefit. So far, only a few comparative studies have been published showing no clear differences between TSC and heparin [8,24]. These studies, though, only accounted about 5000 catheter-days pooled data and mostly used lower concentrations TSC. With a rate of three to five infections per 1000 catheter-days it is obvious that larger studies are needed to find a significant difference.

Ash et al. [7] reported their experience in a haemodialysis patient cohort of 70 patients with 60% tunneled cuffed catheters. After introduction of TSC 23–47% for catheter locking they observed an average decline of 4.5% of all patients per month having a bacteraemia to zero percent. Recently, Stas et al. [8] reported a study comparing heparin 5000 IU/ml and TSC 30%. Thrombus formation in the catheter was evaluated after 201 interdialytic locking periods; no significant differences could be demonstrated. In both studies no clinically relevant side effects occurred during installation of haemodialysis catheters with TSC. This is important, as concern has risen of using TSC for locking catheters after a fatal accident [25]. In this particular case, however, a large amount of TSC was injected in a previously unstable patient with severe electrolyte disturbances. It is clear that the use of these solutions should be restricted to authorized and skilled health care professionals.

We conclude that in our in vitro study using standardized antimicrobial susceptibility tests we demonstrated that TSC 30% was the most potent antimicrobial locking solution and that its hyperosmolality was of minor importance to explain the inhibitory effects of TSC on microbial growth. However, before introduction in practice, randomized clinical trials should confirm the benefit.

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References
25. FDA issues warning on triCitasol® dialysis catheter anticoagulant. *FDA Talk Paper* T00-16. 14-4-2000

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