Trisodium citrate induced protein precipitation in haemodialysis catheters might cause pulmonary embolism

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Abstract

Background. The locking anticoagulant plays a decisive role in the patency of central venous catheters (CVCs) used for haemodialysis. During injection, the hydraulic effects inevitably cause lock solution to spill into the systemic circulation. Density differences between whole blood (WB) and the lock solution cause further gravity-induced seepage of lock solution. This is followed by an influx of WB into the catheter, also described for trisodium citrate, which is a common agent for serum protein precipitation. Embolic complications from haemodialysis catheters locked with hypertonic trisodium citrate have been reported. We aimed to investigate protein precipitation in trisodium citrate locked catheters as a possible cause of pulmonary embolisms.

Methods. In vitro, WB and trisodium citrate (concentrations ranging from 4.7 to 46.7%) mixtures in a ratio of 1:4 were used to assess protein precipitation. Additionally, WB/trisodium citrate mixture was pumped through a 20-μm mesh filter, simulating pulmonary vessels, and filtrate pressure was measured. In vivo, listed filling volumes of haemodialysis catheters locked with trisodium citrate 4% (n = 10), 10% (n = 10), 20% (n = 10) or 46.7% (n = 10) were aspirated and then analysed for protein precipitation.

Results. In vitro, protein precipitation capable of causing filter occlusion was observed in test solutions containing trisodium citrate above a concentration of 12%. In vivo, protein precipitation was detected in all samples from the CVCs filled with trisodium citrate 46.7% (n = 10) and 20% (n = 10). In contrast, there were no signs of precipitation in samples from the catheters filled with trisodium citrate 4% (n = 10) or 10% (n = 10).

Conclusions. Our in vitro results demonstrate that protein precipitates inside haemodialysis catheters when trisodium citrate is used above the concentrations of 12%. Precipitated protein may have contributed to the pathophysiology of reported embolisms from haemodialysis catheters filled with hypertonic trisodium citrate. Based on our findings, we suggest that trisodium citrate lock solution up to the concentration of 10% can be used safely.

Keywords: central venous catheter; haemodialysis access; lock spillage; protein precipitation; trisodium citrate lock solution

Introduction

Central venous catheter (CVC) use, representing a major vascular access modality for haemodialysis, is steadily increasing despite recommendations favouring arteriovenous fistulae [1, 2]. Maintaining the intraluminal patency of a CVC requires instillation of a prophylactic locking anticoagulant, such as heparin [3]. Trisodium citrate (citrate) is currently used worldwide and considered a safe alternative to heparin [4, 5]. However, the use of hypertonic citrate remains controversial [6–8].

Although leakage of catheter lock solutions into the systemic circulation of ~20–25% has been repeatedly demonstrated [9, 10], in the European Union lock solutions are regarded as a ‘medical device’ rather than a systemic drug [11]. The spillage during instillation of the listed filling volume into the CVC is a consequence of laminar flow distribution within the catheter and thus cannot be avoided. Further loss of lock solution due to gravity should be considered if lock solutions (e.g. citrate 30 or 46.7%) with a density higher than blood are used [12–14]. In this case, the mentioned seepage is followed by a reverse whole blood (WB) flow into the CVC and has been observed in vivo [13].

Hence, this WB remains inside the CVC while the catheter is not in use. This is of utmost importance if the instilled lock solution has potential protein precipitating effects. Since the 19th century, ‘salting out’ of plasma proteins using high concentrations of salts (e.g. citrate) has been a common technique to precipitate a target protein [15, 16]. Reported embolisms from CVCs used with hypertonic citrate locking solution might be due to serum protein precipitation in the CVC [17].

The objective of this study was therefore to investigate whether there might be protein precipitation in citrate-locked CVCs. In vitro test solutions consisting of WB and citrate in a ratio of 1:4 (20% WB to 80% citrate) were...
used to assess precipitation. Citrate catheter locks were analysed in vivo after aspiration of the filling volume noted on each port of the CVC. Additionally, in vitro filtration experiments using a 20-μm mesh filter, representing the diameter of pulmonary arterioles [18], were performed.

Materials and methods

In vitro

WB containing 0.6% citrate from blood donors was obtained from the Department of Blood Group Serology and Transfusion Medicine (Medical University of Graz, Austria). A stock solution of 46.7% citrate (Department of Hospital Pharmacy, Medical University of Graz, Austria) was diluted with distilled water to the concentrations indicated in Table 1 (dilution series). One millilitre of WB was mixed with 4 mL of citrate (concentrations ranged from 4.7 to 46.7%). A wide range of citrate concentrations mixed with WB was studied. The tests were repeated in triplicate at room temperature (20°C). Standardized investigation for precipitation was performed as described below.

In vivo

The study was approved by the Ethics Committee of the Medical University of Graz, Austria (registration number: 21-359 ex 09/10). Written informed consent was obtained from 40 prevalent patients using a tunnelled jugular CVC for haemodialysis treatment (Medcomp Split Cath® III 14FR/32 cm with side holes). The study period, representing the time between lock installation and removal, was standardized as 48 h. Citrate 46.7%, 20%, 10% (Department of Hospital Pharmacy, Medical University of Graz, Austria) or citrate 4% (Citra-lock® with 11.7%; B7 with 9.3% and B8 with 4.7%) revealed no signs of protein precipitation.

Results

In vitro

Using different concentrations of citrate lock solutions (4.7–46.7%), protein precipitation was detected visually in test solutions containing citrate above a concentration of 12% in all test series performed (Table 1, Figure 1). The analysis of the precipitate showed that albumin was the predominant protein. Furthermore, we were able to detect IgG, beta- and pre-beta lipoproteins. There was no evidence for the presence of thrombin, fibrinogen or other clotting proteins. The precipitate could be dissolved again by the addition of WB.

In vivo

Protein precipitation was visually detected in all CVCs filled with hypertonic citrate 46.7% solution (n = 10) as well as with citrate 20% (n = 10) solution. The subsequent analysis of the precipitate revealed albumin as the main standard reagents from Roche Diagnostics. To investigate whether the precipitate might reversibly become soluble again, we added 2 mL of WB to the precipitate of the in vitro tests and gently shook the mixture for 5 min.

In vitro filtration experiments

WB from a healthy male volunteer with a haematocrit of 0.43 was mixed with hypertonic citrate 46.7% in a ratio of 1:4 (20% WB to 80% citrate) resulting in a net concentration of citrate of 37.4% and a dilution of plasma proteins to 20%. This solution, as well as undiluted WB, was pumped through a 20-μm mesh filter in a filter holder with 25 mm diameter using a syringe pump. The 20-μm mesh filter simulates pulmonary arterioles [18]. A calibrated pressure transducer (Merit Medical Systems, Inc., South Jordan, UT) positioned between syringe and filter holder allowed continuous recording of the filtration pressure using software operating under LabView 6.0 (National Instruments, Austin, TX).

Table 1. Results of in vitro protein precipitation test series

<table>
<thead>
<tr>
<th>Test solution</th>
<th>Citrate concentration of lock solution (%)</th>
<th>Visible precipitation</th>
<th>Predominant protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate/WBd</td>
<td>46.7</td>
<td>+++</td>
<td>Albumin</td>
</tr>
<tr>
<td>Citrate/WBd</td>
<td>42.0</td>
<td>+++</td>
<td>Albumin</td>
</tr>
<tr>
<td>Citrate/WBd</td>
<td>37.4</td>
<td>++</td>
<td>Albumin</td>
</tr>
<tr>
<td>Citrate/WBd</td>
<td>32.7</td>
<td>+</td>
<td>Albumin</td>
</tr>
<tr>
<td>Citrate/WBd</td>
<td>28.0</td>
<td>−</td>
<td>Albumin</td>
</tr>
<tr>
<td>Citrate/WBd</td>
<td>23.4</td>
<td>−</td>
<td>Albumin</td>
</tr>
<tr>
<td>Citrate/WBd</td>
<td>18.7</td>
<td>−</td>
<td>Albumin</td>
</tr>
<tr>
<td>Citrate/WBd</td>
<td>16.3</td>
<td>−</td>
<td>Albumin</td>
</tr>
<tr>
<td>Citrate/WBd</td>
<td>14.0</td>
<td>−</td>
<td>Albumin</td>
</tr>
<tr>
<td>Citrate/WBd</td>
<td>11.7</td>
<td>−</td>
<td>Albumin</td>
</tr>
<tr>
<td>Citrate/WBd</td>
<td>9.3</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Citrate/WBd</td>
<td>4.7</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*a*The test series was performed repeatedly, revealing the same results.

*b*Test solutions consisted of 1 mL WB and 4 mL citrate lock solution (concentrations ranged from 4.7 to 46.7%) representing the conditions inside the catheter during the interdialytic interval.

*c*Precipitation was assessed with a visual score, ranging from +++ (much) to − (none).

*d*WB sample characteristics for in vitro testing: albumin 4.6 g/dL and total protein 6.6 g/dL.

Fig. 1. The arrow indicates the precipitated protein within the test tube (B3) after centrifugation of the test solution consisting of 1 mL WB and 4 mL citrate 32.7%. The tubes with test solutions containing citrate of <12% in the dilution series (B6 with 11.7%; B7 with 9.3% and B8 with 4.7%) revealed no signs of protein precipitation.
content. In contrast, there were no visible or analytically detectable signs of precipitation in CVCs filled with citrate 4% \( (n = 10) \) or with citrate 10% \( (n = 10) \).

**In vitro filtration experiments**

The results for the WB experiment are illustrated in Figure 2. The pump was started at Time 0 with a flow of 199.5 mL/min. After 0.5 min, the pressures began to rise. With WB only, the pressure settled at 3 mmHg. For the WB plus citrate mixture, the pressure began to increase after 2.5 min up to a peak pressure of 1700 mmHg, indicating filter occlusion.

**Discussion**

Guidelines for clinical use of antimicrobial lock solutions for CVCs demand tests to exclude visual precipitation that occurs when some antibiotics are mixed with heparin or citrate [19]. Precipitation observed in a given antibiotic–anticoagulant mixture is considered to indicate pharmacological incompatibility and consequently to alter the mixture’s antimicrobial activity [20–22]. In practical application, lock solution is always split into the systemic circulation when instilled [9] or has to be completely injected if the lock solution cannot be aspirated from the catheter. Therefore, the presence of any visible precipitation or turbidity of the tested lock solution can be defined as physical incompatibility of an antibiotic lock solution [23–25].

The current standard for exclusion of precipitation of anticoagulant or antimicrobial agents used as lock solutions for CVCs focuses solely on the pure lock solution [26, 27]. This, however, is an inadequate approach if the lock solution analysed differs from WB in terms of fluid density. If such lock solutions are evaluated, these findings require *in vitro* test solutions containing a certain amount of WB to rule out protein precipitation. We therefore performed *in vitro* and *in vivo* analyses to demonstrate that there could be precipitation in CVCs filled with citrate. Our results confirm that protein, predominantly albumin, is ‘salted out’ if citrate is used at concentrations > 12% (Table 1, Figure 1).

In general, spillage of lock solution has two underlying mechanisms: unavoidable spillage due to laminar flow distribution inside the CVC during instillation [9] and, additionally, gravity-induced seepage out of the CVC if there are differences in fluid density between WB and lock solution [12–14]. The fluid density of WB is \( \sim 1.05 \) g/cm\(^3\) compared to 1.24 g/cm\(^3\) of citrate 46.7% solution. Consequently, gravity forces citrate to leak out of the CVC, to be replaced by WB (Figure 3) [13]. The catheter design may slightly affect the time course of the exchange process of citrate for WB but in any case, the region between the side holes is flushed out within seconds. Although catheters may be deformed slightly when inserted, an impact on the fluid exchange that is governed by laws of hydraulics is not expected [28]. The catheter position (jugular versus femoral) influences the lock spillage effect. While the spillage due to injection (20–25%) is unaffected by the catheter position, the density (gravity force)-induced spillage is, on the contrary, greatly affected by the relative catheter position. Assuming a vertical position of the patient, WB enters a catheter in the jugular position but not in the femoral position. However, if the point of insertion into the femoral vein is relatively higher compared to the tip of the catheter, as is to be expected in the supine position, the same process as in jugular catheters can be predicted. Therefore, hypertonic citrate lock cannot be regarded a safe option either in jugular or in femoral catheters.

The reasoning for using a ratio of 1 part WB to 4 parts citrate solution is based on the physics of the lock solution exchange process, which results in a time-dependent range of concentration products. When the high-density lock solution is injected, 20–25% will spill out immediately, resulting in a mean concentration of the lock solution of only 50% at the proximal end of the tube [9]. The less-dense WB then rises in the centre of the tube, forming a parabolic cone as is known from laminar flow. Simultaneously, the dense lock solution flows down alongside the wall of the tube. The volumes of entering WB and spilt lock solution are identical. The flow is laminar and the hydraulic cross sections behave proportionally to the viscosities. Citrate diffuses into the rising WB column. In the immediate contact zone, the concentration of citrate is close to the lock concentration. The
concentration will be decreased towards the centre of the rising WB column. This means that during this exchange process, different parts of blood will be exposed to different concentrations of citrate. Further mixing will occur when the rising WB reaches the highest point and is diverted to the wall of the lumen before succumbing to gravity because it has picked up citrate during ascension and is now denser than WB. With the catheter in a non-vertical position, the flow pattern will become asymmetric. The altered effective height increases the exchange time, resulting in increased precipitation inside the catheter.

During the exchange of lock solution for WB, the catheter plasma proteins are exposed to a range of citrate concentrations up to a maximum that is the concentration of the pure lock solution. In order to simulate the exposure to high citrate concentration, a ratio of 1 part WB to 4 parts citrate was used, this is a compromise that results in a reduced protein concentration. In reality, mixing in the catheter is by radial diffusion of citrate into WB resulting in a higher (more favourable for precipitation) citrate–protein concentration product. We questioned whether the precipitate caused by citrate is able to occlude pulmonary vessels and performed further in vitro filtration experiments to address this problem. These experiments revealed that the precipitate induced by the addition of 46.7% citrate to WB contains large numbers of particles with diameters > 20 μm that are able to occlude lung vessels. From these experiments, it cannot be excluded that the precipitate also contains particles <20 μm. Such particles may pass the lung capillaries but may occlude vessels in other tissues [29]. Additional in vitro analyses revealed that the precipitate could be re-dissolved by the addition of WB, suggesting that protein precipitation might also be reversible in vivo. However, because of the short transfer time of the precipitate from the catheter to the pulmonary circulation, dissolution is unlikely.

Chronologically and pathophysiologically, one might hypothesize that following the WB influx into the catheter, citrate induces intraluminal protein precipitation. Subsequently, some of the precipitate will leak out of the catheter. Thus, protein embolism might occur not only when the lock solution and the precipitate are injected as a bolus; subclinical protein embolisms might occur additionally following every single instillation of citrate. Possible side effects include pulmonary hypertension, which has also been claimed to be a consequence of air microbubble embolism during haemodialysis [30]. Tumour micro-embolism, when clusters of tumour cells occlude small pulmonary arterioles [31], or lipoprotein micro-embolism from microaggregates following application of unfiltered fresh frozen plasma [32], might represent entities similar to our findings. If the precipitate occludes the side holes and the tip, which might be the case for catheters with a tapered tip, this process is interrupted. Aspiration of the lock solution becomes difficult or impossible. Bolus injection might then cause pulmonary protein embolism. Our hypothesis that protein embolism is of clinical relevance is well supported by different observational studies in humans, experimental studies in animals and theoretical models. Clark et al. [33] published a model predicting that occlusion of small vessels (extra-acinar pulmonary arteries) can have a significant effect on pulmonary arterial pressures. Experimental observations have shown that small emboli (diameter < 170 μm) can have a disproportionate effect on pulmonary vascular resistance compared with larger emboli for the same tissue occlusion [34]. Our findings thus might explain why the use of hypertonic citrate locking solution resulted in symptomatic emboli in a reported series of eight patients [17]. We hypothesize that the precipitated protein might have played a crucial role in the pathophysiology of these embolisms. Although the authors pointed out that hypertonic solutions are more likely to seep out of the CVC, thus increasing risk of clotting and causing thrombo-embolism if injected, we hold the view that precipitated protein may have contributed to the pathophysiology of the reported embolisms. Clinical signs indicating protein precipitation could be catheter malfunction and difficulties in aspirating the citrate catheter lock, as occurs with thrombus formation. In these situations, especially flushing the catheter could cause life-threatening embolic complications [17].

Although no serious adverse events ‘that could be contributed to the locking solution’ were reported in a randomized controlled trial comparing heparin to 30% citrate, we believe that hypertonic citrate is not without risk for the patient [5]. We conclude that the protein precipitation observed inside the catheter, due to the density-induced WB influx, should be regarded as a physical incompatibility. We suggest that lock solutions in general should be investigated according to the criteria for solutions given intravenously. This would include testing to rule out (protein) precipitation [24, 25].

Our study has several limitations. Firstly, precipitation was examined visually and smaller amounts might have been missed. Secondly, precipitation analyses were conducted at room temperature. In fact, precipitation depends on temperature, and the quantitative difference caused by the body room temperature differential was not taken into account. Nevertheless, the salting out reaction in vitro (at room temperature) is slightly less than in vivo (body temperature). To demonstrate that plasma proteins precipitate in the vicinity of a concentrated citrate lock solution, the test setup (dilution, room temperature) has a high safety margin in the sense that it underestimates the effect quantitatively. The risk of reporting a false positive effect is remote. Although in vitro analysis revealed a limit of 12% with regard to this temperature effect, we advise a concentration limit of 10%. Thirdly, also in terms of physical influence, in vivo conditions are not completely comparable to in vitro conditions. Furthermore, WB for in vitro experiments contained 0.6% citrate, but the impact on the citrate concentration for the mixture of WB and citrate in a ratio of 1:4 is far less than 1% and therefore negligible.

In conclusion, our results demonstrate that citrate induces protein precipitation in CVCs.

To avoid severe embolic complications, we suggest 10% as the maximum admissible concentration of citrate lock solutions.
Furthermore, each lock solution containing plasma precipitating agents with density characteristics different from WB, whether less or more dense, should be evaluated for protein precipitation prior to clinical use.

Conflict of interest statement. None declared.

References


Received for publication: 23.4.2011; Accepted in revised form: 27.1.2012