The Use of Heated Citric Acid for Dialyzer Reprocessing1,2

Nathan W. Levin,3 Sandra L. Parnell, Herbert N. Prince, Frank Gotch, Hans D. Polaschegg, Robert Levin, Atthea Alto, and Allen M. Kaufman

N.W. Levin, S.L. Parnell, R. Levin, A. Alto, A.M. Kaufman, Division of Nephrology and Hypertension, Beth Israel Medical Center, New York, NY
H.N. Prince, Gibraltar Laboratories Inc., Fairfield, NJ
F. Gotch, Davies Medical Center, San Francisco, CA
H.D. Polaschegg, Medical Devices Consultant, Oberursel, Germany


ABSTRACT

Dialyzer reprocessing with heated water (100 to 105°C) for 20 h can be used safely in lieu of chemical methods for disinfection. All infective agents including spores are destroyed and depyrogenation may occur. However, these temperatures may result in structural damage to the dialyzer, limiting reuse. Dialyzer reprocessing by using 1.5% citric acid heated to 95°C for 20 h is an alternative method that produces equivalent microbiologic effects. Citric acid is well known as a disinfecting agent used for dialysis equipment. Because there is little structural damage to dialyzer components at 95°C, reuse statistics are improved (mean reuse increased to 12.8). Both small and large molecule clearances and the sieving coefficient for protein are insignificantly altered by the process. Whereas the procedure is relatively simple, quality-assurance indicators are essential. The method has appeal because it avoids the use of chemical germicides. However, at present it has only been tested thoroughly in polysulfone dialyzers with heat-resistant polycarbonate casings and polyurethane resin. The clinical experience is favorable.

Key Words: Dialyzer reuse, hemodialysis, dialyzer disinfection

1.5% citric acid to augment the sporicidal effect of temperatures lower than originally used (i.e., 100°C). Two Fresenius F80 dialyzers were filled with 1.5% citric acid solution previously contaminated with spores of B. stearothermophilus. To more precisely define the potential of 1.5% citric acid to augment the sporicidal effect of temperatures lower than originally used (i.e., <100°C), two Fresenius F80 dialyzers were filled with 1.5% citric acid solution previously contaminated with spores of B. stearothermophilus to a final concentration of at least 10⁶ CFU/mL per dialyzer. An aqueous suspension of B. stearothermophilus in saline at the

METHODS

In vitro Microbicidal Activity of the Process

In a preliminary series of experiments, the microbicidal effects of 1.5% citric acid at 95°C were determined by quantitative analysis of the suspensions by the construction of thermal death curves. Survivors were detected by standard plate count (trypticase soy agar) 0.5, 1, 3, 6, and 24 h after inoculation of 10⁵ to 10⁶ colony-forming units (CFU)/mL of a variety of organisms (Mycobacterium chelonae. Pseudomonas aeruginosa. Escherichia coli, Staphylococcus aureus, Candida albicans as vegetative species, and the spores of Bacillus stearothermophilus). To more precisely define the potential of 1.5% citric acid to augment the sporicidal effect of temperatures lower than originally used (i.e., <100°C), two Fresenius F80 dialyzers were filled with 1.5% citric acid solution previously contaminated with spores of B. stearothermophilus to a final concentration of at least 10⁶ CFU/mL per dialyzer. An aqueous suspension of B. stearothermophilus in saline at the
same concentration served as a control (i.e., citric acid was not present) in two dialyzers.

The spore-laden dialyzers were placed inside a convection oven (Baxter DX63, Baxter Healthcare Corporation, McGaw Park, IL) preheated to 95°C. Aliquots of the citric acid-spore suspension and of the saline-spore suspension were removed at 0.5, 1, 2, 4, 6, and 24 h and quantitated for survivors by membrane filtration of dilutions that were then plated on to trypticase soy agar plates and incubated for 48 h at 54 to 56°C.

A second series of *B. stearothermophilus* spore survival studies were performed at 72°C, 82°C, and 95°C using 1.5%, 2%, and 5% citric acid at each temperature.

The enumeration of survivors of vegetative organisms was accomplished by plating aliquots in tripticase soy agar for 48 h at 35°C.

Toxicity Studies

Cytotoxicity tests. To determine whether toxic leachables were extracted during the reprocessing procedure, cytotoxicity assays were performed on the effluent from two F80 dialyzers that contained 1.5% citric acid heated to 95°C for 20 h. Confluent sheets of mouse L-929 cells fed with Rosewell Park Memorial Institute 1640 (RPMI 1640, JRM Bioscience, Lenexa, KS) medium supplemented with 5% calf serum were utilized in USP dilution cytotoxicity assays (8). Effluent was compared with citric acid controls that had never been in contact with dialyzer membranes.

To determine if priming can remove toxic leachables, two F80 dialyzers containing 1.5% citric acid heated for 20 h to 95°C were primed with 500 mL 0.9% normal saline and recirculated (blood side) for 1 min. L-929 elution cytotoxicity tests were performed on the saline-rinse extracts. The positive control was a latex extract.

USP in vivo bioreactivity test. Extracts from dialyzers previously treated with 1.5% citric acid at 95°C for 20 h were studied with the USP 23 intracutaneous irritation assay (7). Extracts (0.2 mL) were administered intracutaneously to each of two rabbits and the sites were observed for erythema, edema, or necrosis at 24, 48 and 72 h.

Clearance Studies

Urea and cytochrome C clearances and protein sieving coefficients were measured in vitro in new dialyzers and after as many as 12 clinical uses. Urea clearance studies were performed utilizing a conductivity method (9), and cytochrome C clearance studies were performed with pre-and post-dialyzer blood measurements (10).

Kinetic studies

In 30 patients, urea kinetic modeling studies of hemodialysis treatments utilizing dialyzers reprocessed with heat alone were compared with treatments utilizing dialyzers reprocessed with citric acid/heat to determine whether a clinically measurable change in the fractional clearance of urea (Kt/V) occurred with citric acid/heat reuse. Kinetic measurements were performed over 6 consecutive months, with conversion of dialyzer reuse to citric acid/heat after Month 3. The dialysis prescription was unchanged during the 6-month period, with Kt/V greater than or equal to 1.2 in all patients. Standard single-pool, variable-volume kinetic formulas were used (11).

CLINICAL METHODS

Beth Israel Medical Center Institutional Review Board approval was obtained for the initial clinical studies using this process.

Procedure

USP grade citric acid (13.9 g anhydrous citric acid formulation/100 mL water wt/vol) is weighed and diluted by two staff members working together. The DRS4 automated reuse machine (Seratronics, Walnut Creek, CA) is adapted to dilute this concentration 9.27 times to produce a 1.5% citric acid solution.

At the conclusion of a dialysis treatment with adequate heparinization, residual blood is rinsed from the dialyzer with heparinized saline. If the dialyzer cannot be processed immediately, it is refrigerated. If not reprocessed within 36 h, it is discarded. The dialyzer is rinsed at the reuse room sink with water with microbiologic contamination on repeated testing of less than 20 CFUs/mL and limulus amebocyte lysate (LAL) presence of less than 0.06 endotoxin units/mL. These standards exceed AAMI microbiological and pyrogen
Figure 2. Effect of 1.5% citric acid at 95°C (upper panel), 0.9% normal saline 95°C (middle panel), and 1.5% citric acid at room temperature (lower panel) on B. stearothermophilus spore survival.

Endcaps are inspected for signs of damage and rinsed. Dialysate port caps and blood port caps are rinsed with the same quality water, and air-dried. A chemical agent is not used to disinfect the caps since they too are subsequently subjected to a temperature of 95°C for 20 h. During the rinsing process, care is taken not to leave residual dialysate on any part of the dialyzer. Before the endcaps and O-rings are replaced, the cap and dialyzer jacket threads are dried with a lint-free cloth. The dialyzer is then run through an automated dialyzer reprocessing machine (DRS4, Seratronics, Walnut Creek, CA) using the high-quality water only. After dialyzer cell volume and pressure holding tests have been performed, the dialyzer is filled with 1.5% citric acid. The dialyzer is removed from the DRS4
An integrity test is then performed at the patient station: the by manual inflation. If the pressure degrades more than from the blood side for 1 mm by using the dialysis machine orducer filter. The dialyzer is then pressurized to 400 mm Hg acid solution by using air filtered through a standard trans-

Presence of citric acid is verified by confirmation of a pH of heat label on the dialyzer jacket must have changed color. be dry on the Inside. The dialyzer must be 4/5 full and the label on the bag must have changed color and the bag must maintained Its structural Integrity.

then reprocessed, ii retesting confirms that the dialyzer has heating period. If the dialyzer has leaked, It is retested and leak of the citric acid solution out of the dialyzer during the oven. allowed to cool at room temperature, and stored for paper disc. After 20 h. the dialyzers are removed from the recorded continuously by display and automatically on a

D values (length of time to reduce the spore challenge by 1-log) were 15 mm for heated citric acid and 2 h for heated water. D = T/(log10N0-log10N), where N0 = initial CFU, N = CFU at the end of Incubation time (T).

Figure 3. Effect of 1.5% citric acid at 95°C and water at 95°C on B. stearothermophilus spore survival expressed as log-kill (y axis) over time (x axis). D values (length of time to reduce the spore challenge by 1-log) were 15 min for heated citric acid and 2 h for heated water. D = T/(log10N0-log10N), where N0 = initial CFU, N = CFU at the end of Incubation time (T).

and capped. As the dialysate side caps are being applied, citric acid solution is removed so that the dialyzer is 4/5 full (e.g., 20 to 25 mL removed from a F80 dialyzer). This solution is used to perform the conductivity test. The conductivity of the citric acid solution is measured (Myron L D5 meter, model 512M5, Myron L Company, Carlsbad, CA) to confirm that the correct concentration of citric acid is present (2875 µmho at 21°C). The conductivity meter was calibrated against a 2070 µmho reference solution (40% Na2SO4, 40% NaHCO3, 20% NaCl concentration values). A heat-sensitive label (American Sterilizer Company, Apex, NC) and the label created by the DRS4 are applied to the dialyzer. The latter contains the patient name, proof that more than 20 h has elapsed since the dialyzer was last used with the reprocessing machine, information that the dialyzer passed quality-assurance tests, and whether the dialyzer's reuse number is within unit standards (i.e., fewer than 15 reuses). The dialyzer is placed in a plastic bag, which is sealed with a second heat-sensitive label and placed in a dry-heat, forced-convection oven for 20 h at 95°C. The temperature of the oven is recorded continuously by display and automatically on a paper disc. After 20 h, the dialyzers are removed from the oven, allowed to cool at room temperature, and stored for subsequent use. The plastic bag facilitates detection of any leak of the citric acid solution out of the dialyzer during the heating period. If the dialyzer has leaked, it is retested and then reprocessed, if retesting confirms that the dialyzer has maintained its structural integrity.

Before use, the dialyzer is inspected. The heat-sensitive label on the bag must have changed color and the bag must be dry on the inside. The dialyzer must be 4/5 full and the heat label on the dialyzer jacket must have changed color. Presence of citric acid is verified by confirmation of a pH of 2.2, by using narrow-range pH paper (range, 2.0 to 2.8 pH). An integrity test is then performed at the patient station: the dialysate and blood sides of the dialyzer are emptied of citric acid solution by using air filtered through a standard transducer filter. The dialyzer is then pressurized to 400 mm Hg from the blood side for 1 min by using the dialysis machine or by manual inflation. If the pressure degrades more than 40 mm Hg during the minute, the dialyzer is not used because there may be a leak in the membrane or a fracture in the plastic or potting material. Reasons for failure are investigated and documented by reuse personnel. If the dialyzer passes this test, the extracorporeal circuit is filled with saline, and the dialysate is connected and recirculated for 5 min. The absence of citric acid is verified by using pH paper (range, 6.0 to 8.0 pH). The dialyzer is then ready for patient use.

Quality Assurance

Quality-assurance documentation allows independent review of each phase of the procedure. AAMI guidelines are followed as the minimum standard. Water quality is monitored weekly. If unit standards are not met, the water system is disinfected.

Before use, dialyzers from 3% of the patient population (or at least 6 dialyzers) are rinsed with saline weekly and cultures and endotoxin analyses are performed. The sampling procedure that is followed accesses all patient shifts and dialyzers at all phases of their reuse cycles.

Before each treatment, two staff members verify both patient identity against the name on the dialyzer label and exposure to heat for the appropriate time. This information is supplemented by the change of the heat-sensitive labels to a darker color. The labels provide confirmation only of exposure to heat and are not used quantitatively. Citric acid presence pre-prime, and absence post-prime in the dialyzer is confirmed by using narrow-range pH indicator tape. Dialyzer integrity is confirmed by the pressure-holding integrity test.

Oven temperatures are monitored by three thermometers: (1) an independent temperature recorder, which produces a continuous graphic display of the temperature (of value in monitoring during the hours when the reprocessing room is not in use); (2) a built-in oven thermometer, which displays a continuous digital reading; and (3) a mercury thermometer, which is used weekly for quality control. If the temperature of the oven deviates from 95°C for more than a few minutes, the
Figure 4. Effect of dialyzer reuse number on urea clearance in vitro. Polysulfone F80 dialyzers were studied at baseline (B) and after reprocessing up to 12 times with 1.5% citric acid at 95°C for 20 h.

Figure 5. Effect of dialyzer reuse number on clearance of cytochrome C in vitro. Polysulfone F80 dialyzers were studied at baseline (B) and after reprocessing up to 12 times with 1.5% citric acid at 95°C for 20 h.

entire contents of the oven are reprocessed in order to assure disinfection (the temperature will fall by 2 to 3°C whenever the oven door is opened for insertion or removal of the trays of dialyzers).

Other quality-assurance measures include monthly kinetic modeling, routine trending of all problems and incidents as part of an outcome-based quality assurance program, and monthly monitoring of all techniques, with review of content and timeliness.

RESULTS

In vitro Microbicidal Activity

Vegetative organisms. 1.5% citric acid heated to 95°C showed complete 5-log and 6-log kills within 30 min against all vegetative organisms tested (Figure 1, upper panel). Temperature controls of saline at 95°C without citric acid showed the same rate of kill against the organisms (Figure 1, middle panel). A 1.5% citric acid solution at room temperature showed complete kills against Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus within 3 h, and extensive but incomplete kills against the other organisms tested (up to 72 h) (Figure 1, lower panel).

Bacillus stearothermophilus spores. Spores of the heat-resistant biological indicator B. stearothermophilus required up to 3 h for complete kill with 1.5% citric acid heated to 95°C (Figure 2, upper panel). Temperature controls of saline at 95°C without citric acid showed a slower rate of kill against the spores of B. stearothermophilus (up to 6 h) (Figure 2, middle panel). 1.5% citric acid at room temperature was essentially inactive against the spores of B. stearothermophilus (Figure 2, lower panel).

The elevated temperature-citric acid system produced a 6-log kill (99.99%) in 2 h. The heated-water control produced a 1-log kill (90%) in 2 h (Figure 3).
The D values (D is the length of time in minutes or hours required to reduce the spore challenge by 1-log) were determined by inspection of 1-log intercepts and were found to be 15 and 120 min for the citric acid and water systems (both at 95°C), respectively. Thus the presence of 1.5% citric acid rendered the 95°C water eight times more efficient in killing the thermophile challenge. Extrapolation of the 15-min D-value to an unlikely (in clinical work) dialyzer contamination of 10⁶ spores would yield a sterility-assurance level (SAL) of 10⁻¹² in 180 minutes. Recognizing that any potential blood-borne contamination would harbor fewer organisms that were likely to be far less resistant to heat, an even higher degree of high-level disinfection/sterility assurance would be expected under clinical conditions.

1.5% citric acid required ≤24 h to achieve a 6-log kill of spores at 72°C, ≤4 h at 82°C, and ≤1 h at 95°C. Less time was required at higher citric acid concentrations, although even 5% citric acid required up to 8 h at 72°C (Table 1).

Toxicity Studies

Cytotoxicity tests. Tests for toxic leachables from dialyzer membranes exposed to citric acid were negative in that the results of cytotoxicity assays with USP L929 cells were identical to the scores obtained with citric acid controls without exposure to the dialyzer membrane. Specifically, the 1.5% citric acid dialysates from dialyzers heated for 20 h at 95°C and a 1.5% citric acid control at room temperature exhibited identical minimal cytotoxic doses of 1:14 and maximum tolerated doses of 1:16, indicating that the intrinsic minimal cytotoxicity of the 1.5% citric acid was not enhanced by interaction with the dialyzer. The 1.5% citric acid has a pH of 2.2, which is neutralized by bicarbonate and/or removed during priming.

Cytotoxic reactions were completely absent even when undiluted eluate from primed dialyzers was used.

USP in vivo bioreactivity test. The USP in vitro bioreactivity test was also negative, with no signs of irritation noted in skin after intracutaneous injection with saline extracts of dialyzers previously treated with 1.5% citric acid heated to 95°C for 20 h.

Clearance Studies

Urea clearance (Figure 4), cytochrome C clearance (Figure 5), and dialytic protein loss coefficients (Figure 6) (13) were not significantly changed after exposure to the procedure.

Kinetic Studies

Extracorporeal blood-flow rate (426 ± 8 versus 426 ± 8 mL/min), dialysis time (176 ± 3 versus 176 ± 3 min) and Kt/V (1.39 ± .03 versus 1.42 ± .03) were not significantly different in patients utilizing dialyzers reused with heated water or heated citric acid, respectively (data ± SE, N = 30).

Clinical Experience

In seven facilities, over 1000 patients have been treated with dialyzers reprocessed with this technique, receiving over 140,000 treatments. Subjective symptoms attributable to the process have not occurred. The mean reuse number was 12.8. Of discarded dialyzers, 48% reached maximum reuse, 42% failed either the bedside-integrity test or the pressure-holding test of the DRS4, 8% failed the fiber-bundle volume test, 2% were broken or cracked, and 0.005% had a clinical blood leak, none of which was clinically significant. No pyrogen reactions or bacteremic episodes occurred.

DISCUSSION

Dialyzer reprocessing is a necessity in the current hemodialysis reimbursement system, particularly if high-efficiency, high-flux dialysis is the objective. Re-processing methods that are safe for patients and staff, easy to use, and environmentally friendly are preferable. The citric acid/heat system appears to satisfy all these requirements. The original heat reprocessing method at 100°C to 105°C was associated with increased structural damage to the dialyzer. The use of hot (95°C) citric acid accomplishes the same bactericidal result, without the deleterious structural

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<th>Time (h)</th>
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effects that result from the use of boiling water. Citric acid is not toxic and has a long history of use in the food industry (14). In addition, citric acid has been used for the disinfection of dialysis machines (15). The choice of the 95°C temperature is based on the following: (1) 95°C is lower than the temperature of boiling water, and the dialyzer is therefore exposed to lower pressures; (2) when 1.5% citric acid is used at temperatures lower than 95°C, the destruction of spores requires longer periods of time, and the safety margin inherent in the 20-h heating procedure is reduced; and (3) depyrogenation is less efficient at lower temperatures (13). Table 1 shows a matrix of temperatures, times, and citric acid concentrations. We have not investigated lower temperatures clinically but the in vitro results suggest a smaller safety margin for sterilization. Exposure of dialyzers to citric acid and heat does not result in leachable substances that cause either cytotoxicity in vitro or intracutaneous irritation in vivo in rabbits, employing official USP tests. Although the technique does not meet the standard for sterilization as defined by the Association of Official Analytical Chemists, B. Stearothermophilus spores are destroyed by the technique. In any case, the AAMI Recommended Practice requires only high-level disinfection. The citric acid reprocessing procedure exceeds recommendation. Other techniques may claim that the agent used is a sterilant, but these claims cannot be made for the complete reprocessing procedure. Additionally, depyrogenation was reported in a recent study as occurring as the result of exposure to heat (16). Holmes suggests that it is difficult for the average user to validate heat processes for "sterilization" (17). While this may be true, it is obvious that the heat-reprocessing technique process is effective, and the heat and citric acid technique is equal or superior, in terms of microbicidal activity, to the chemical techniques widely used today.

Whereas many heated organic acids could be suitable hydrolytic agents, citric acid was chosen because of its lack of toxicity. The presence or absence of citric acid is easily detected. Even if virtually all procedural guidelines were breached, the possibility of significant quantities of citric acid remaining in the dialyzer is remarkably small, given the necessity to prime the blood side of the dialyzer with saline before use. In addition, any remaining citric acid would be buffered by the bicarbonate in the dialyzer, thereby preventing an infusion of an acid-load to the patient.

In summary, this method of dialyzer reprocessing appears to be effective, safe for patients and staff, relatively simple, and environmentally friendly. Dialyzer function as measured by small- and large-molecule clearance tests and protein loss during dialysis
Levin et al (Figure 6) are not adversely affected. The major drawback is the limited number of dialyzer types for which the process is suitable at present because of the vulnerability of membranes, and, to a lesser extent, of dialyzer jackets and potting compounds, to thermal stress. It is expected that this situation will change in the near future.

REFERENCES