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Underestimated role of alcohol at skin disinfection: lipid dissolving property when used in association with conventional antiseptic agents

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Aim: After conventional aqueous disinfectant solutions, it was shown that microorganisms were still protected in hair follicles. We hypothesized that those aqueous disinfectant solutions when used in combination with alcohol may be more effective on the inhibition of recolonization of skin and therefore catheter tip colonization.

Materials and methods: Skin surface samples were taken from epidural catheter insertion sites prior to catheterization, and before and after disinfection with different combinations of povidone-iodine, chlorhexidine, and alcohol. Before catheter removal, cultures were taken once more and tips of the catheters were cultured.

Results: Catheter tip colonization and skin culture results of 10% povidone-iodine + 70% alcohol group were significantly lower than those of other groups after disinfection.

Conclusion: Sequential use of alcohol and povidone-iodine is the most effective combination for limiting re-colonization of skin flora. Contamination of catheters appears to take place at removal or via the spread of these re-colonized bacteria along the catheter tract.

Key words: Epidural catheterization, colonization, skin disinfection, povidone-iodine, chlorhexidine, alcohol

Alkolün cilt dezenfeksiyonunda önemsenmeyen rolü: Klasik antiseptik ajanlarla birlikte kullanıldığında lipit çözücü özelliği

Amaç: Klasik su bazlı dezenfektan solüsyonlar sonrasında, kıl follikülerindeki mikroorganizmaların hala korunduğu gösterilmiştir. Bizim hipotezimiz su bazlı dezenfeksiyon solüsyonlarının alkolle kombine edilerek kullanılmasının, cilt rekolonizasyonu ve kateter uç kolonizasyonunun engellenmesinde daha etkili olabileceğidir.

Yöntem ve gereç: Epidural kateter giriş bölgesinden kateterizasyon öncesinde, povidon-iyodin, klorheksidin ve alkolün farklı kombinasyonları ile dezenfeksiyonu öncesi ve sonrasında cilt sürüntü kültürleri alındı. Kateter çıkarılmadan önce aynı yöntemle kültürler bir daha alındı ve kateterlerin ucu kültür yapıldı.

Bulgular: % 10 povidon-iyodin + % 70 alkol grubunda dezenfeksiyon sonrası kateter ucu kolonizasyonu ve cilt kültür sonuçları diğer gruplardan anlamlı olarak daha düşüktü.

Sonuç: Alkol ve povidon-iyodinin ardışık kullanımı cilt florasının rekolonizasyonun sınırlanmasında en etkili kombinasyondur. Kateter kontaminasyonun ise kateterler çıkarılırken veya rekolonize olan bakterilerin kateter yolu boyunca migrasyonu ile gerçekleştiği düşünülmektedir.

Anahtar sözcükler: Epidural kateterizasyon, kolonizasyon, cilt dezenfeksiyonu, povidon-iodin, klorheksidin, alkol

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Introduction

Disinfectant properties of chlorhexidine (CHX) and povidone iodine (PI) have been studied many times previously (1-3). However, alcoholic or aqueous properties of these 2 solutions have been neglected.

Resident organisms in skin specimens were studied after skin was prepared with disinfectants (10% povidone-iodine or 0.5% chlorhexidine in 80% ethanol) by Sato et al. (3). They observed that many resident organisms were present in the hair follicles, and viable organisms could be isolated from skin specimens in substantial proportions after strict preparations with disinfectants. Although 0.5% CHE in 80% ethanol was significantly more potent than 10% PVP-I, staphylococcal species grew after preparation of the skin with both disinfectants. This disparity between skin-surface sampling methods and skin biopsies appears to be due largely to the difference in accessibility of the disinfectants to resident organisms.

We also demonstrated in our previous study that despite efficient skin surface disinfection with aqueous solution of PI, epidural catheter contamination could not be prevented as expected (4). This result suggested the same outcome: contamination of catheters was caused by microorganisms that could not be detected by skin-surface sampling methods. In a study of cultures of skin specimens excised from cadavers and patients, Selwyn and Ellis showed that resident organisms are protected from disinfectants by lipids at the orifices of the follicles or overlying portions of the stratum corneum (5). Subsequently, Zamora and colleagues showed that the presence of organic substances in the skin decrease the bactericidal activity of PI markedly (6).

These results imply that addition of alcohol to conventional disinfectants could have more potent bactericidal activity, due to its high permeability into the hair follicles and the stratum corneum by the help of its dissolvent property on lipids in addition to its well-known bactericidal effect. To find out the role of alcohol at skin disinfection and on the re-colonization of skin flora, and consequently the possible source of catheter contamination, we combined or sequentially applied CHX or PI with alcohol.

Methods

Patients

The study was approved by the ethical committee of the university, and informed consent was obtained from each patient. One hundred and seventy five adult patients, scheduled for elective lower limb surgery with lumbar epidural anesthesia, were enrolled and randomly assigned into 5 groups via the envelope method to undergo skin decontamination. There were 35 patients in each group. Groups were defined as aqueous 10% PI, aqueous 0.5% CHX, 0.5% CHX in 70% alcohol, 2% CHX + 70% alcohol, and 10% PI + 70% alcohol. Ethyl alcohol was used in all groups containing alcohol.

The patients who had fever, preexisting skin infection, and received antibiotics within the previous 48 h were excluded from the study before randomization. Epidural needles and catheters, which required more than one attempt for placement, were also excluded. However, most of the patients received antimicrobial prophylaxis for opportunistic infections during the operative period (aminopenicillin or cephalosporin) according to international guidelines.

Insertion, maintenance and removal of catheters

Subjects in all groups had their skin prepared with 3 consecutive applications of solutions according to standard protocol (vigorously applying the antiseptic solution on an area approximately 20 cm in diameter for at least 10 s and allowing the area to dry between each application for 30 s). However, in 2% CHX + 70% alcohol and 10% PI + 70% alcohol groups, 70% alcohol solution was applied each time before the 2% CHX or 10% PI for at least 10 s and allowed to dry, then 2% CHX or 10% PI was applied. Subsequently, 60 s after the third application, remaining disinfectant was wiped dry, and a swab culture was taken from the skin just before needle placement.

Standardized preventive measures were applied, such as face masks, surgical caps, sterile gloves, and drapes at catheterization. All epidural sets (needles, catheters, and filters) used in the study were Portex-Epidural Minipac System 2 (Portex Ltd., Kent, England). The epidural space was identified by loss of resistance technique with isotonic saline, and epidural catheters were placed by the same anesthesiologist. Bacterial filters provided with the epidural sets were attached to the catheters according to aseptic guidelines.

Initial bolus dosing of local anesthetics (0.5% bupivacaine (Marcaine®- AstraZeneca, İstanbul, Turkey)) were injected in the operating room. Subsequent analgesic dosing (0.125% bupivacaine and 2 mg/mL fentanyl (Abbott laboratories, IL, USA) mixture as 5 mL bolus doses) were delivered by a patient controlled epidural pump (Abbott, Pain Management Provider, IL, USA) via single use pump sets. Catheters were kept in place for 48 h and bacterial filters were used for analgesic delivery during the entire period. Dressings were not changed until the catheters were removed.

A brief examination concerning body temperature and lower extremity strength and sensation was performed daily at least for 5 days. This period could be extended as necessitated by the culture results or clinical course.

At catheter removal, dry swabs were taken at the site surrounding catheter insertion. Then, skin was cleaned with the same disinfectant solution, using the same protocol that we used before catheterization, and a swab culture was taken once more from the insertion site just before catheter removal.

Cultures and definitions

Four skin cultures were obtained from each subject. The first swab was taken just prior to skin disinfection to determine baseline skin flora. The second was obtained from the same area immediately following antisepsis of the skin to determine efficacy of the disinfectant. The third swab was obtained just prior to removal of the epidural catheter (again before the skin flora disinfection) to determine the re-colonization rate. The fourth was obtained once again from the same area immediately following antisepsis of the skin before catheter removal to determine the possible source of catheter contamination.

Skin culture samples were taken by sterile cotton swabs, which were coded and immediately incubated in a sterile tube, and transferred to the microbiology laboratory. There they were inoculated in blood agar media and incubated under aerobic conditions at 37 °C for 72 h.

Following the collection of the fourth skin culture, all catheters were removed aseptically by the same anesthesiologist wearing sterile gloves and a mask. Distal 3 cm tip of the catheters were transected with sterile scissors and forceps, placed in sterile tubes, and submitted for culture. For the diagnosis of catheter colonization, we used both Maki's semi-quantitative method and quantitative method with vortexing catheter segments (7).

The catheter tip was rolled several times on a blood agar plate in the laboratory by aseptic technique. Then the same catheter segment was immersed in 1 mL triptic soy broth and vortexed for 60 s and 9 mL normal saline added. Thereafter, 100 µL broths were inoculated on a blood agar plate, which was incubated at 37 °C for 72 h under aerobic conditions. Colonies were enumerated on agar plates and identified by standard techniques. More than 15 CFU with a semi quantitative method and more than 103 CFU with a quantitative method were defined as catheter colonization.

Microorganisms were identified using standard techniques. In each case, the microbiologist handling the specimens in the laboratory was blinded to the disinfectant solution used.

Statistical analysis

Power analysis revealed that a sample size of 156 was required to achieve a power (ß) of 0.80, with a significance level of 95% (= 0.05). Results of cultures were tested using the Fisher's Exact Test or chi-square test as appropriate. Statistical analyses were performed using SPSS for Windows V10.0 (SPSS, Chicago, IL, USA). A value of $P < 0.05$ was considered to be significant.

Results

Subject characteristics

A total of 175 patients undergoing operation with epidural anesthesia were initially enrolled in this study. However, the skin swabs of 5 patients (laboratory problems) and the catheters of 14 patients (9 accidental catheter removal, 5 laboratory problems) could not be cultured. Finally 156 patients joined in the study.

Effectiveness of disinfectant solutions

The results of skin and catheter tip cultures of the groups are summarized in Table 1.

Table 1. Positive cultures in skin swabs and distal catheter tips.

	10% PI (#:35) n (%)	0.5% CHX (#:29) n (%)	0.5% CHX in A (#:33) n (%)	2% CHX + A (#:32) n (%)	10% PI + A (#:27) n (%)
Skin culture before disinfection	11 (31.4)	9 (31)	11 (33.3)	5 (15.6)	4 (16)
Skin culture after disinfection	7 (22.6) *	3 (10.3)	1 (3)	2 (6.3)	0 (0) *
Skin culture at catheter removal before disinfection	9 (29)	8 (32)	5 (25)	2 (6.9)	1 (4.5)
Skin culture at catheter removal after disinfection	3 (10.7)	3 (11.5)	2 (9.5)	2 (7.1)	0 (0) *
Catheter tip	5 (19.2)	4 (15.4)	3 (14.3)	2 (7.1)	1 (3.7) *

*: $P < 0.05$ when compared with other groups.
#: number of cultures
PI: povidone- iodine, CHX: chlorhexidine, A: 70% alcohol,

The groups did not differ significantly with respect to skin colonization prior to disinfection. In fact, the proportions of patients with positive skin cultures before disinfection were less in the 2 groups, 2% CHX + A and 10% PI + A (Table 1). However, these results were not statistically significant. The most common bacterial isolate was *coagulase-negative staphylococci* (CNS), found in 33 of the 40 (82.5%) positive cultures. Other isolated species included *Staphylococcus epidermidis, Enterobacter* spp*, Staphylococcus aureus,* and *Pseudomonas aeruginosa*. However, immediately after skin disinfection, there were significant differences among groups in respect to positive skin cultures ($P < 0.05$). In 10% PI + 70% alcohol group, there was no colonization. On the other hand, 10% PI group had the highest colonization (22.6%) rate comparing to the others (Table 1).

At catheter removal before disinfection, colonization rates were almost the same as before catheterization in PI, CHX, and CHX in alcohol groups. However, in 2%CHX + alcohol and 10%PI + alcohol groups, the recolonization rates were lower. Furthermore, the skin culture results of 10% PI + 70% alcohol disinfection was significantly the lowest among study groups after disinfection at catheter removal ($P < 0.05$) (Table 1).

The majority of skin cultures at catheter removal yielded growth of the same bacterial species that were present before disinfection at catheter insertion. The most common organism isolated remained CNS, and the overall distribution of species was similar to that obtained in the predisinfection at catheter insertion cultures.

CNS was also the majority of catheter cultures. Catheter tip colonization was also significantly lower in 10% PI + 70% alcohol group than the other 4 groups ($P < 0.05$).

CNS colonization was observed on the catheters of all 5 groups (interestingly even at 10% PI + 70% alcohol group at which there was no skin culture colonization after skin disinfection). CNS colonized also at these patients' skin cultures before disinfection (Table 2).

Although significant bacterial colonization was observed on lumbar epidural catheters, no patient had evidence of local or central nervous system infection during the 48-h epidural catheterization period and consecutive days.

Discussion

The results of this study demonstrate that even though aqueous PI has been found as the least effective cutaneous disinfectant, sequential application of 70% alcohol and 10% PI was the most effective method of disinfection before short-term epidural catheterization with regard to skin disinfection, recolonization, and catheter contamination.

Because each active ingredient has different modes of action and performance characteristics on microorganisms, these combination formulations act faster with a broader spectrum of antimicrobial activity than formulations containing PI or CHX alone, and more persistent than formulations

Table 2. Microorganisms cultured in skin swabs and catheter tips.

	10% PI n	0.5% CHX N	0.5% CHX in A N	2% CHX + A n	10% PI + A N
Skin culture before disinfection	CNS: 7 SE: 3 SA: 1	CNS: 8 SA: 1	CNS: 9 E: 1 PA: 1	CNS: 5	CNS: 4
Skin culture after disinfection	CNS: 4 SE: 2 SA: 1	CNS: 3	CNS: 1	CNS: 2	–
Skin culture at catheter removal before disinfection	CNS: 7 SE: 1 SA: 1	CNS: 8	CNS: 5	CNS: 2	CNS: 1
Skin culture at catheter removal after disinfection	CNS: 1 SE: 1 SA: 1	CNS: 3	CNS: 2	CNS: 2	–
Catheter tip	CNS: 4 SA: 1	CNS: 4	CNS: 3	CNS: 2	CNS: 1

PI: povidone- iodine, CHX: chlorhexidine, A: 70% alcohol,
CNS: coagulase-negative staphylococci, SE: staphylococcus epidermidis, E: enterococci, SA: staphylococcus aureus, PA: Pseudomonas aeruginosa

containing alcohol alone (8). However, our findings suggested that the potent bactericidal activity of sequential alcohol and CHX or PI applications were mostly due to priming application and consequently prior access of alcohol into the hair follicles and the stratum corneum.

In a previous study, Sota et al. showed that substantial proportions of viable organisms could be isolated from skin specimens after strict preparations with disinfectants (3). They also found that although 0.5% CHX in 80% ethanol was significantly more potent than 10% PI, microorganisms grew after preparation of the skin with both disinfectants. Furthermore, Selwyn and Ellis showed that disinfection of the skin with 1.5% iodine in 70% ethanol or with 0.5% CHX in 70% ethanol reduced colony counts by 95.5% and 87.6%, respectively. However, they still showed the presence of bacteria deep in the larger hair follicles by microscope (5). Many bacteria were viable and seen exuding from the orifices of follicles after incubation. Probably the resident organisms are protected from disinfectants by lipids at the orifices of the follicles or overlying portions of the stratum corneum. These findings were similar to 0.5% CHX in 70% alcohol group results of our study. In our 0.5% CHX in 70% alcohol group, even though the skin culture results after disinfection were significantly lower than baseline levels, recolonization rate was almost the same as before disinfection after 48 h. This finding confirms the recolonization of viable microorganisms, which were protected from disinfectants as Selwyn and Ellis suggested. However, interestingly sequential application of alcohol and CHX or PI was more potent in respect to recolonization.

Some previous studies reported that despite high colonization rates at the catheter tips, the risk of epidural space infection is low (2,9,10). These findings are consistent with our findings. Furthermore, Bevacqua reported that the majority of catheter contaminations take place during removal as the catheters traverses the unprepared exit site (11). Catheter cultures represent recolonization of the skin by the skin flora at catheter insertion sites and subsequent contamination of the catheter tip during removal (12).

Moreover, demonstration of viable organisms in large proportion of specimens by Sato et al. suggested the possibility that epidural catheter space contamination by the skin flora during insertion of an epidural needle or by subsequent spread along the epidural catheter tract may be the other mechanism of catheter contamination. The results of our study support this suggestion. In our study the colonization rates of catheters were higher than those of skin surface cultures after disinfection in 4 groups. Although there was no colonization at skin culture after disinfection by 10% PI + 70% alcohol, there was colonization at the catheter tip in which the microorganism species were identical with the predisinfection skin cultures. This can be explained by bacterial spread along the catheter tract during recolonization.

All these finding suggest that contamination of the catheter might take place at removal or by bacterial spread along the catheter tract rather than at insertion. This may explain why epidural infection rates do not correlate with the colonization rates of catheters.

One of the limitations of our study was the relatively limited number of our sample size. In order to evaluate the performance of antiseptic agents more acutely, larger scale clinical trials, especially in clinical settings, where a low contamination rate is expected, are needed. However, our clinical conditions were not appropriate for a larger sample size. Another limitation was the catheterization period, which was relatively short with a median of 48 h. Although a short duration may militate against colonization, this duration represents current practice at our hospital.

In the present study, 0.5% CHX in 70% alcohol, 2%CHX + 70% alcohol, and 10%PI + 70% alcohol are all found as effective solutions for short term continuous or single shot epidural blocks in maintaining an aseptic state of the skin surface. However, for long term catheterizations, 2%CHX + 70% alcohol and 10%PI + 70% alcohol are recommended by the authors to prevent possible bacterial spread along the catheter tract. The participants in the sequential alcohol and CHX or PI used groups were found to have much less recolonization of bacteria at insertion sites at the time of catheter removal. This markedly lower rate of recolonization may have been possibly due to higher permeability into the hair follicles and the stratum corneum due to prior alcohol application.

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