An in vitro pressure ulcer approach using High Voltage Pulsed Current (HVPC) stimulation

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D. Herminawaty, I.R. Defi, T. Prabowo, I. Parwati. An in vitro pressure ulcer approach using High Voltage Pulsed Current (HVPC) stimulation. Gerontechnology 2014;12(3):148-152; doi:10.4017/gt.2014.12.3.009.00 **Purpose** Immobilization will lead to pressure ulcers. The breadth and depth of impacts related to having a pressure ulcer on quality of life is significant in a physical, psychological, emotional, spiritual, social, and financial sense. In Indonesia, one study reported pressure ulcers in 72 of 253 patients (28%). High Voltage Pulsed Current (HVPC), an electrical stimulation current type, has been used to promote wound healing. One of its mechanisms is thought to be antimicrobial. Our study observed the effect of HVPC on extended spectrum beta-lactamase-producing Klebsiella pneumoniae (ESBLKp), commonly found in pressure ulcer cultures in Hasan Sadikin Hospital, Bandung, Indonesia **Methods** A suspension of ESBLKp was poured over an agarose-based solid medium. Immediately after inoculation HVPC (250V, 100Hz, 70µs pulse interval (current controlled) was applied for 30, 60 and 120min respectively. The experiment was done in fourfold. After 24 hrs and 48 hrs incubation the growth-inhibitory zone was measured at each pole. **Results & Discussion** Both poles showed growth inhibition after both 24 and 48 hrs incubation. Maximum inhibitory zone (27mm) was reached after 48 hrs around the cathode with 120min HVPC application. Different patterns of inhibition were found at the cathode and anode, with the anode zone being smaller but with more clearing. HVPC stimulation is a promising intervention for pressure ulcers infested with K. pneumoniae. Future studies should define parameter and schedule for HPVC stimulation in vivo.

Keywords: HVPC, Klebsiella pneumoniae, pressure ulcer

Pressure ulcer is a complication of immobilization leading to significant impacts on quality of life. Quality of life in an individual with a pressure ulcer incorporates such variables as pain and suffering, the financial costs of healthcare, the strain on personal resources, and overall impact on one's life and activities of daily living[1]. Suriadi et al.[2] reported that out of 253 patients, 72 (28%) developed pressure ulcers.

Electrical stimulation (ES) has been used for decades to promote wound healing[3-6]. Possible mechanisms that may account for enhanced wound healing include bactericidal and bacteriostatic effects, increased blood flow, orientation of new collagen formation, and retardation of edema buildup[3,7]. The bacteriostatic effect of electrical stimulation has been reported for the first time over 30 years ago by Rowley et al.[8]. Inhibition of bacterial growth has been reported by other researchers as well, such as Merriman et al.[3], Maadi et al.[4], Szuminsky et al.[9], and Petrofsky et al.[10]. There is growing evidence to support ES as adjunctive in the the treatment of chronic wounds that have not responded to conventional wound debridement, cleansing, dressing, and infection treatment, including antibiotics[3,11].

High Voltage Pulsed Current (HVPC) is a type of electrical stimulation commonly used to promote wound healing[6,7,9,12,13]. Stimulators of HVPC produce a unidirectional monophasic pulsed current with peak amplitudes of 100 to 500 volts, with a wave form that is typically twin-peak in shape and designed to last for a short period of time (5 to 100ms)[13]. The antimicrobial effect of HVPC may be produced by the direct action of the current on the organism; the electrochemical generation of antimicrobial factors, including changes in pH; localized heat generation; or the electrophoretic recruitment of antimicrobial factors already present in the body (eg, those of the immune system)[9]. This current can be delivered to the wound tissue as either a positive (anode) or negative (cathode) charge[5,11]. Typically, a cathode electrode is used during the first 3-5 days to decrease bacterial levels in the wound. After the wound is clean, polarity is reversed to anode stimulation until tissue repair is complete[5,11].

Pressure ulcers are a common and serious problem that affects acute care, nursing home, and home care populations[13]. A chronic wound is defined as one that deviates from the expected sequence of repair in terms of time, appearance, and response to aggressive and appropriate treatment. Delayed healing can result from any combination of intrinsic, extrinsic or iatrogenic factors. Intrinsic factors affecting chronic wound healing include aging, chronic ill-care, circulatory disease, malnutrition and neuropathy. Extrinsic factors include medication, immune suppression, irradiation, psycho-physiologic stress, infection, and also iatrogenic factors include ischemia and inappropriate wound care management resulting in trauma to the wound[13].

Extended Spectrum Beta-Lactamase (ESBL) producing organisms pose a unique challenge to clinicians[14]. They have spread threateningly in many regions of the world and now comprise over three hundred variants[15]. These plasmid-mediated enzymes hydrolyze broad-spectrum beta-lactams and are strongly inhibited by clavulanate[14,15]. Transmission among bacteria is accomplished by plasmids[14,15]. Furthermore, antibiotics such as trimethoprim-sulfamethoxazole, aminoglycosides and fluoroquinolone are often co-transferred on a resistance plasmid, resulting in multiple drug resistance[15]. The high rate of ESBLs among hospitalized patients is a global problem[15]. Thus clinical treatment failure occurs frequently, especially when inappropriate antimicrobial therapy is used to treat infections caused by ESBL-producing organisms[15].

Although the prevalence of ESBLs is not known, it is clearly increasing, and in many parts of the world 10-40% of strains of *Escherichia coli and Klebsiella pneumoniae* express ESBLs[14,15]. ESBL-producing *K. pneumoniae* have spread rapidly worldwide and pose a serious threat in healthcare-associated infection[15]. Infection with an ESBLs producing pathogen is associated with greater mortality, an increase in the length of hospital stay and hospitalization costs, and delays in treatment, compared with infection due to non-ESBL organisms[16]. The prevalence of ESBL producing isolates of *K. pneumoniae* varies among different countries[14,17].

The aim of this study is to observe the effect of HVPC on ESBL-producing *K. pneumoniae* (ESBLKp), commonly found in pressure ulcer culture in Hasan Sadikin Hospital, Bandung, Indonesia.

Methods

Materials

We obtained clinical isolates of ESBL-producing *K. pneumoniae* from Hasan Sadikin Hospital, Bandung. Two holes were bored through the bottom of each disposable plastic 85mm petri dish 2cm apart from the center. Two 6.5cm, 1mm diameter stainless steel wires were inserted through each hole. A 5 mm portion of the electrode inside the dish was perpendicular to the bottom of the dish, while the outside portion was fixed with glue to the bottom of the dish, with a 2cm overhang for attachment to the ES device (*Figure 1, left*) . We put double tape on the bottom of each dish to obtain a level bottom. Dishes were then ultraviolet sterilized for 24 hours.

Figure 1. Experimental set-up; Left: petri dish with stainless steel electrode; Right: working in the biosafety cabinet

Instrumentation

Electrical stimulation current was applied with an Intelect Mobile Stimulator (model 2777, Chattanooga Group, Inc.). Special cables with alligator clips were fabricated. The cables were tested for uniformity in transmission of electrical current with an oscilloscope and a multi-tester.

Procedures

Trypticase soy broth (TSB) agarose in phosphate-buffered saline (PBS) was selected as growth medium, based on preliminary clinical experiments. *K. pneumoniae* was grown in TSB until the exponential growth-phase was obtained (between 4-5 on the McFarland scale[18]). The suspension was then poured over new sterile dishes and electrical stimulation started immediately. These procedures were done in a biosafety cabinet to ensure no contamination (*Figure 1, right*). Electrical current used was HVPC 250 V, frequency 100Hz, with an interval pulse of 70μs. Electrical stimulation was applied for 30, 60 or 120min. Each test was done in fourfold. In total, 24 petri dishes were treated.

Following each ES stimulation, the petri dishes were incubated for 24 hours at 37°C, after which the width of the zone of inhibition parallel with the wire electrodes was measured with a millimeter ruler. Test results represent the average of two measurements per zone of inhibition in each dish. The dishes were then re-incubated for another 24 hours at 37°C and the diameter of zone of inhibition were measured again. Each petri dish was also examined for electrode corrosion, gas formation, and media discoloration.

Controls
Seeded dish with the organisms and wire electrodes in place were incubated without exposure to ES to determine whether the wires themselves, the media, or a combination of both would be inhibitory to bacterial growth (positive control). Seeded dish with the wire electrodes in place were incubated without exposure to ES and organisms to ensure sterility (negative control). Positive and negative control dishes were changed every day. In total 2 positive and 2 negative control dishes were used.

Data analysis
Data analysis involved the calculation of means and standard deviations (SD) and were analyzed by one-way analysis of variance (ANOVA). Confidence limit was set at 0.05.

Results
Growth inhibition of *K. pneumoniae* was represented by the measurements of the zones of inhibition. Bacterial inhibitory effect was found at both electrodes in all duration of HVPC application (*Figure 2*).

The inhibition area around the cathode is significantly larger than the one around the anode after both 24hrs and 48hrs incubation duration. Maximum inhibitory zone (27mm) was observed around the cathode of the 60min application plate on the second day. The inhibition area around the anode was small and although longer duration of electric stimulation showed an increased inhibition area, this difference was not significant.

The inhibition area around the cathode was increased significantly by a longer duration of HVPC stimulation (*Figure 2*). Differences among replicates were not significant. After 48 hours incubation, the results showed significant differences between electrical stimulation of 30min duration as compared to the other stimulation times, although there was no significant different increment between 60min and 120min duration (p=0.08).

Small amounts of gas, visible as small bubbles at the edge of electrode, were generated at the anode. Discoloration of the medium at the cathode was also observed. These occurred in the HVPC application of 120min duration.

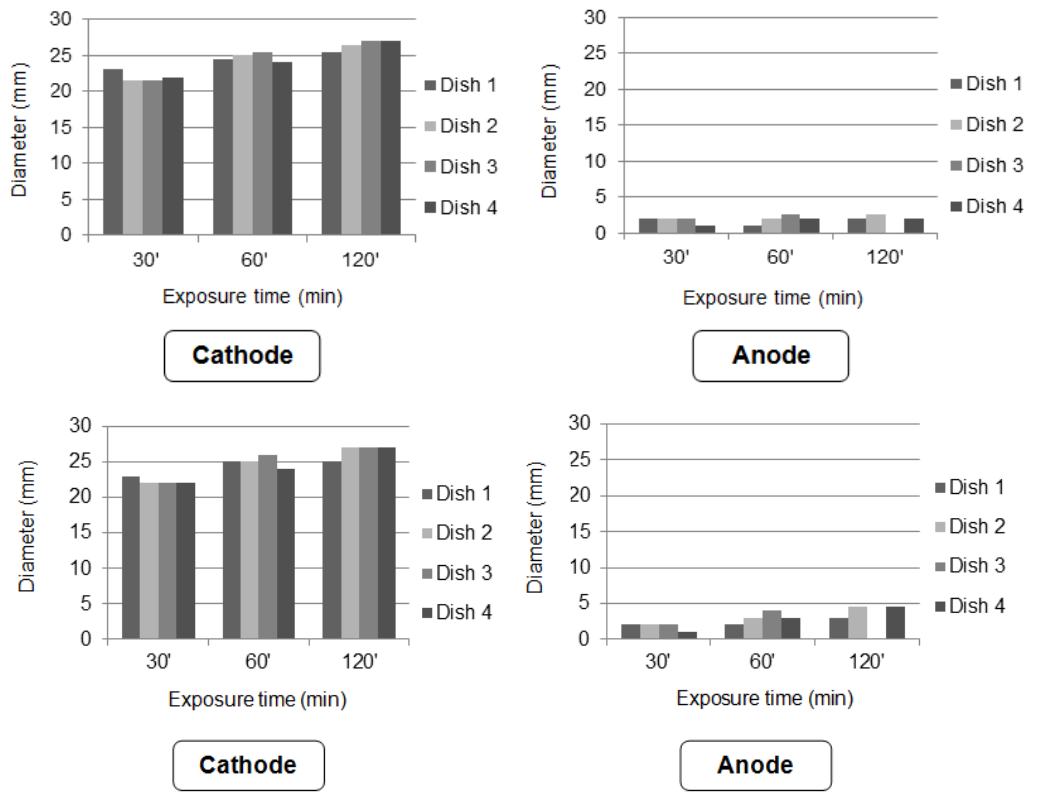

Figure 2. Inhibition zone diameter in mm after 24 hrs (upper graph) or 48 hrs (lower graph) incubation after different HVPC (High Voltage Pulsed Current) exposure times (in min)

Plates containing seeded medium that were not exposed to ES, showed no inhibition of growth, indicating that the wires themselves, the medium, or both, had no inhibitory effect. Seeded plates with the wire electrodes in place without exposure to ES and organisms revealed that the sterilization process was good and no bacteria growth was observed.

Discussion

This is the first study of electrical stimulation application on ESBLKp. The only previous study we found on *Klebsiella*, is the one of Szuminsky et al.[9]. They studied the effect of HVPC on four different species of bacteria, one of them being *Klebsiella*, but not ESBLKp. Antimicrobial effects on *Klebsiella* were observed at both the positive and the negative electrode, with varying zones of inhibition. Our study was conducted with ESBLKp and different electrical stimulation parameter that better fitted to a clinical application.

The result of our study show that HVPC possesses an antimicrobial effect on ESBLKp. It is in line with other studies on the antimicrobial effect of electrical stimulation. The antimicrobial effect appears to be due to direct action on the microbe of the current and to the generation of indirect antimicrobial factors (ie, effects that persist when the current is discontinued). A longer HVPC exposure lead to a significant enlargement of inhibition area around cathode. Longer ES application gave more superior antimicrobial effect.

We found that the inhibition area around the anode was smaller than the one around the cathode although the area was clearer. Whether this phenomenon indicates a stronger antimicrobial effect by the anode is not clear. Every method has its own limitation. A study by Petrofsky et al. [10], used a broth instead of a solid agar medium and visually approximated the concentration of microbes based on the McFarland scale[18]. Considering that the re-incubation procedure in this study was aimed to see any further indirect microbial growth inhibition, a broth would not have been ideal.

Gas formation at the anode and medium discoloration at the cathode were also observed by Merriman et al. [3] and Szuminsky et al. [9], who used the same current level. This suggests that electrochemical changes occur in the area surrounding electrodes after an ES treatment of more than 60min.

We tried to eliminate as much confounding factors as possible. Extreme pH changes have been suggested to be a factor contributing to the antimicrobial effect of electrical stimulation. Phosphate buffered saline supplemented to agarose has eliminated a pH change. Consequently, no pH changes effect will intensify the antimicrobial effect. Previous investigators have demonstrated that pH changes do not occur in human skin following 30 minutes of HVPC stimulation[17].

Temperature changes were not measured in this study due to technical limitations. However, a previous study by Szuminsky et al. [9] showed that small temperature changes between any two points on the plate do occur, but are less than 1°C. Temperature measurements subsequent to the application of the HVPC did not show more than a 1°C change, with no difference in temperature between the untreated and treated plates[9]. The other limitation of this study is the single current-type used. We have not compared the results with other current treatments commonly used in wound healing; i.e. Direct Current (DC) and Alternating Current (AC). Usage of DC in clinical practice is still limited. The electrochemical changes in the tissue applied may cause chemical burn and tissue injury. On the other hand, AC is a common current used for wound healing. Yet its antimicrobial effect is still not conclusive. Apparently, ES is beneficial in wound healing, through its antimicrobial effect, even in ESBLs.

Conclusion

The encouraging result suggests HVPC stimulation to be a promising intervention for pressure ulcers infested with ESBL producing *K. pneumonia*. Thus, it may make pressure-ulcer healing a faster process. Further study is needed to define optimal parameters and schedule.

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