BACTERICIDAL EFFECT OF DIRECT AND FILTERED ULTRAVIOLET C THROUGH TRANSPARENT PLASTIC SHEET ON GRAM NEGATIVE BACILLI – AN *IN- VITRO* STUDY

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Abstract

Wound infection (with both Gram-positive and Gram-negative organisms) is a major problem in delayed wound healing. Persistence of these organisms in the wound delays considerably the healing time and increases length of hospital stay. Ultraviolet-C has shown to effectively eliminate these organisms (bactericidal effect) in few laboratory, animal and human studies and has shown promising role in promoting wound healing. Several wound dressing methods have been used to accelerate the rate of wound healing. Among them, Limited Access Dressing (LAD) utilizes intermittent negative pressure dressing using a transparent plastic cover combined with moist wound healing principle, minimizes need for regular change of dressing. Thus, the present study was conducted to explore the in-vitro bactericidal effect of direct and filtered UVC through transparent plastic sheet on Gram-negative bacilli.

Keywords: Ultraviolet rays, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli*

1. Introduction

Ultraviolet rays (200-290nm UVC) have been shown to have bactericidal effect[1,2,3,] enhanced growth of epithelial cells and promotes granulation tissue formation which promotes wound healing[4,5]. Bactericidal effect of ultraviolet rays was studied in different in- vitro study methods[1,2] and on common wound infecting organisms like *Staphylococcus, Escherichia coli, Pseudomonas aeruginosa* and fungal strains by different researchers [2,3]. UVC produces intra–strand cyclobutane–type pyrimidine dimers in Deoxyribonucleic acid (DNA). These photoproducts can be lethal (not viable and looses the ability to replicate) and ultimate fate of irradiated cells depends upon the ability of the cells to repair the UV induced damage[6,7]. Many researchers have identified the prevalence of *Staphylococcus aureus, Klebsiella speicies, Pseudomonas aeruginosa and Escherichia coli* [8-11] in different types of wound infection. Unlike in the West, in India multi drug resistant gram-negative bacteria were found to have always been dominant in the wounds infections[12]. The widespread use of antimicrobials in the management of many infections including wound infection has led to major concern of MDR microorganisms[13]. Certain well established principles are followed in wound care like debridement and cleansing, maintenance of a moist environment, providing topical agents to reduce bacterial load and promote healing, prevention of further injury along with systemic antibiotics. Long term use of topical antimicrobials like iodine, hydrogen peroxide, silver preparations even though reduces the bacterial burden but have many side effects[14,15]. The widespread use of negative pressure wound therapy (NPWT) is a relatively a new trend in wound care[16]. However, in extensive open wounds it may be difficult to use NPWT. In these instances, a modification using sterilized plastic covers and two drainage tubes- a technique called Limited Access dressing (LAD)[17,18] has been used successfully in more than 1000 patients in 2009 at Kasturba Hospital (Manipal, INDIA). LAD uses intermittent negative pressure (-30 mmHg for 30 minutes) through the tube(s) and maintains moisture at the wound site (for 3 hours 30 minutes without negative pressure) with the use of transparent polythene material (a total of 21 hours of moist dressing and 3 hours of negative pressure dressing in a 24- hour period, case report[19],case series[20]).

Previous studies have shown that UVC is possessing bactericidal effect against multi drug resistant bacteria in in-vitro 21 and in

human studies[21,22]. In recent years, increase in the multi drug resistant bacteria[13-15] and understanding the benefits of both LAD (cost effectiveness, transparency, reduced chances of anaerobic infection and reduced smell) and UVC on bacterial growth have increased. In an empirical study[23], it was reported inconclusive results regarding the bactericidal effects of UV light on a wound covered with the transparent dressing. Though UVC has proven as definitive adjunct in wound healing, of late, not many studies have been directed in this regards. Using this information, authors designed this prospective in -vitro study to ascertain the bactericidal effect of UVC, direct and filtered through a transparent plastic sheet, on gram-negative bacilli.

2. Materials and methods:

2.1: An In-vitro study was conducted on following Gram-negative bacilli: Following organisms (clinical isolate) were collected from the pus section of Department of Microbiology KMC Manipal. Organisms were identified according to standard microbiological methods24 and tested for antibiotic sensitivity by disk diffusion method and sensitivity pattern recorded. *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* (Sensitive, moderately sensitive and resistant pandrug resistant pattern, designated as 1, 2, 3, 4) were used.

***2.2: Preparation of bacterial cultures*:** A standard inoculum for the study was prepared from the isolated bacterial colony as follows: A single bacterial colony was inoculated into peptone water and incubated for six hours at 37^0C. After six hours, turbidity of the peptone water was standardized to McFarland's standard tube to get final concentration of 10^5 organisms /ml. One standard loop (4mm size, holding 0.01ml of inoculum) of the broth culture was inoculated to MacConkey agar plate by semi quantitative method by continuous streaking without intermittent heating on four quadrants[25-27].

2.3: UVC dosimetry: Ultraviolet violet rays (UVR) source Endolamp 47428 (Enraf Nonius Holland Figure1) which emits 74%of UVC 254nm (Spectrum analysis, Figure 2), UVB: 5%, UVA 2.5% and visible light 18.5% (percentage of emission was obtained from the company manual). Unit was regularly monitored for the output (5mJ/s/cm2 at 10 cm of distance) by using Broadband power energy meter (13PEM001 Melles Griot).

***2.4:UVC exposure protocol*:** During the study each culture medium was exposed to direct and filtered UVC through plastic sheet (thickness 0.15mm) kept as interface between plate and UV source being regularly used for limited access dressing for 5,10,15,20,25,30 seconds. The distance between the source and plate for the in vitro study was maintained at 10 cms (as per the instruction manual of machine) by use of indigenously designed (by investigator using thermocol piece) rigid box and the applicator was kept on the box before irradiation. One culture plate with bacterial colony streaked was kept as sham control (was not exposed to UVC) while 18 plates with bacterial growth were exposed to UVC (3 plates containing same bacteria for each duration). Experiment was repeated thrice with different organisms with each antibiotic susceptibility patterns. (Sensitive, moderately sensitive, resistant and pan drug resistant, designated as 1, 2, 3, 4).

2.5: Incubation and bacterial growth: All plates (control and UVC exposed) were incubated at 37° C for 24 hours and observed for growth of bacteria. The duration of the exposure required to cause no growth of the organism was noted. Media growth patterns were assessed. No growth was considered as zero. Scanty growth (first quadrant only), colonies were counted and then multiplied by 10029,30. Scanty growth (first quadrant only) and <10 colonies or <1,000 CFU/mL. Scanty growth and approximately 50 colonies or 5,000 CFU/mL. Scanty growth and 50–100 colonies or 10,000 CFU/mL; 25,000 CFU/mL growth or difficult to count in first quadrant. Growth in first and second quadrant and 50,000 CFU/mL; first, second and third quadrant growth and 75,000 CFU/mL; and >105 CFU/mL and growth in all 4 quadrants.

2.6: Data collection and analysis: Specimen collection and preparation of bacterial culture was conducted at Department of Microbiology (JK, PGR) and BKR conducted UVC exposure to the inoculated agar plate and, PS provided interpretations of the results.

3. Results

Using direct UVC, the exposure time required to achieve no growth (complete eradication) ranged from 15 seconds for *Escherichia coli* to 25 seconds for *Klebsiella pneumoniae* .Filtered UVC even until 30 seconds had no effect on bacterial growth(full growth/105 CFU/ml). This shows that direct UVC has good bactericidal effect on the organisms studied and plastic sheet of 0.15 mm effectively blocks UVC (Table 1).

Table 2, 3, 4 shows effect of different duration of exposure and the growth of organisms for different sensitivity patterns of *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli*. Different antibiotic susceptibility patterns of *Pseudomonas aeruginosa* did not have any influence on UVC sensitivity. Antibiotic resistant *Klebsiella pneumoniae and Escherichia coli* showed increased UVC resistance.

4. Discussion

We studied the in vitro direct and filtered effect of ultraviolet rays (UVC) on growth of Gram-negative organisms. There are currently no recommendations for safe doses for human skin[31]. The National Health and Medical Research Council (NHMRC Canberra)[32] standard provides UVR exposure limit values for exposure of the eye or skin in the spectral region between 180 and 400 nm.

Exposure time limit for germicidal (UVC) lamps is 1-3 minutes[33]. All the in vitro and in vivo studies have used considerable longer duration of exposure (180 second) which may lead to unnecessary exposure to UVA and UVB produced along with UVC source. Considering the benefits and exposure limit risk associated with UVR on humans, we intended to explore the shortest effective exposure time (Inverse square law). Also, LAD is a new method of moist wound dressing that uses transparent polyethylene material, also the increase in the multi antibiotic resistance of bacteria in recent years and understanding the benefits of both LAD and UVC on bacterial growth, hence it is worth to explore the synergistic/any other combined effect of UVC and LAD.

Our study demonstrated excellent bactericidal effect (100% eradication of bacteria inoculated) of ultraviolet rays (254nm) on eight common wound infecting bacteria by 15-25seconds. Two studies using UVR (254nm, 15.54mW/cm2) exposure from 1-inch distance have shown 99-100% bactericidal efficacy with exposure duration ranging from 4-120 seconds[2,34]. In our study, 5mJ/s/cm^2 (=5mW/cm^2) of the UVC energy was delivered from a distance of 10 cm.

Our study on exposure duration for 100% eradication of gram- negative bacilli revealed that *Escherichia coli* were most sensitive to UVC exposure. *(Escherichia coli* -15 seconds > *Pseudomonas aeruginosa* -20 seconds >*Klebsiella pneumoniae* -25 seconds*)*. Effect of UVC on *Escherichia coli and Pseudomonas aeruginosa* was similar to the previous study[35].

Our study on *Pseudomonas aeruginosa*, 100% eradication was achieved (20seconds) with source at 10 cm distance and 5mW/cm2 UVC energy. Previous study3 reported 100% eradication of *Pseudomonas aeruginosa* by 30 seconds (average output, 15.54mW/ cm^2 at 1-inch distance). Different antibiotic susceptibility patterns of *Pseudomonas aeruginosa* did not have any influence on UVC sensitivity.

The time required to obtain 100 % bactericidal effect on *Klebsiella pneumoniae* was 25 seconds. Our study showed that longer duration of exposure was required for 100% eradication of pan drug resistant *Klebsiella pneumoniae* than their sensitive strain. This indicated increased UVC resistance of the antibiotic resistant *Klebsiella pneumoniae*. Similar finding was reported in a study[36] which showed that UV radiation (exposure duration of 20 seconds) reducing the number of viable cells was 30%-62% for resistant *K. pneumoniae* and 43%-66% reduction for the parent drug-susceptible strains. There was no mention about the wavelength and intensity of UVR in their study.

We have obtained bactericidal effect for *Escherichia coli*, which varied, from 5-15 seconds for different antibiotic sensitivity patterns. Our results indicate that the antibiotic resistant organisms were also resistant to UVC exposure. This is similar to study[36], which reported with 20 seconds of irradiation with UV light, a 14-69% reduction in the number of viable cells for resistant E. coli strains, compared with 56- 79% reduction for susceptible strains. Another in- vitro study1 showed the effect of Kromayer (UVR) lamp model 10, (254-436nm) on *Pseudomonas aeruginosa* and *Escherichia coli* with dosage varying from E2, E3, E4 at 10 cm distance. Authors concluded these bacterial strains were not viable beyond E4 dose. However, in this study authors have not described duration of exposure and percentage emission of different wavelengths of UVR.

In previous in vitro-study conducted by our team on gram-positive cocci showed, higher UVC sensitivity compared to the results of present study on gram-negative bacilli (*Klebsiella pneumoniae and Pseudomonas aeruginosa)*[37]. This observation is not consistent with results of a previous study38 .The probable reason for the different UVC sensitivity between Gram-positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms. Gram-negative cells have a complex additional outer barrier that

may be responsible for increased resistance to UVR[38].

In vitro filtered effect of UVC through plastic sheet on common wound infecting organisms: In our study, there was no bactericidal effect of filtered UVC. Then we conducted an experiment to see the amount of radiation passing through the plastic sheet used in this study by UV photometer (UV 1700 series) at Department of Biotechnology, Manipal University. This showed highest optical density of 0.079 at 292 nm. Optical densities showed a reduction from 296 nm till 400nm. This indicates that complete absorption of the rays were seen below 296nm.This may be the reason for filtered UVC not having any bactericidal effect. In 1984 a study[23] reported UVR can be applied through the transparent dressing. Then in 2001 a research group16 reported UVR did not show any filtered effect, our results were consistent with reports of this group.

5. Conclusion

Direct UVC (Endolamp 474) has bactericidal effect in short duration (15 seconds) of exposure for Gram- negative bacilli but filtered UVC is ineffective. This beneficial effect of direct UVC in short duration minimizes the bacterial load and simultaneously reduces side effect related to exposure duration. *Escherichia coli* is more sensitive to the effects of UVC than Pseudomonas aeruginosa and *Klebsiella pneumoniae*. More resistant patterns of *Klebsiella pneumoniae* and *Escherichia coli* was also UVC resistant, but similar effect was not observed with *Pseudomonas aeruginosa*. Also plastic sheet of 0.15 mm thickness may be used to protect the surrounding skin to protect from exposure during therapy.

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Table-1 shows effective time to cause no growth for Gram-negative bacilli

Pathogen	Time in seconds (Direct)	Time in seconds (filtered)
Pseudomonas aeruginosa	20 (no growth)	105 CFU/ml till 30 seconds of exposure
Klebsiella pneumoniae	25 (no growth)	105 CFU/ml till 30 seconds of exposure
Escherichia Coli	15 (no growth)	105 CFU/ml till 30 seconds of exposure

Table-2: Effect of direct UVR (254nm) on *Pseudomonas aeruginosa*

Pathogen	Control	5 seconds			10 seconds			15 seconds			20 seconds	25 seconds	30 seconds
P.Aeruginosa (Sensitive)	105	25x103	25x103	25x103	25x103	25x103	25x103	-	104	-	-	-	-
P.Aeruginosa (moderately sensitive)	105	-	-	-	-	-	-	-	-	-	-	-	-
P.Aeruginosa (resistant)	105	6	4	5	3	5	4	6	1	7	-	-	-
P.Aeruginosa (pan drug resistant)	105	104	104	104	5	6	2	1	3	6	-	-	-

*No growth in the agar plate is shown as (-)

Table-3: Effect of direct UVR (254nm) on *Klebsiella pneumoniae.*

Pathogen	Control	5 seconds			10 seconds			15 seconds			20 seconds			25 & 30s
Klebsiella pneumoniae (Sensitive)	105	-	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumonia (moderately sensitive)	105	50x103	50x103	50x103	25 x103	25 x103	25 x103	104	104	104	-	-	-	-
Klebsiella pneumonia (resistant)	105	104	104	104	3	5	6	1	2	2	-	-	-	-
Klebsiella pneumonia (pan drug resistant)	105	105	75x103	75x103	25 x103	25 x103	25 x103	104	104	104	4	6	6	-

*No growth in the agar plate is shown as (-)

Table4: *In vitro* direct effect of Endolamp 474 ultraviolet rays on *Escherichia coli* with different antibiotic susceptibility pattern

Pathogen	Control	5s			10s			15s	20s	25s	30s
Escherichia coli (Sensitive)	105	-	-	-	-	-	-	-	-	-	-
Escherichia coli (moderately sensitive)	105	104	104	104	-	-	-	-	-	-	-
Escherichia coli (resistant)	105	25x103	25x103	25x103	-	-	-	-	-	-	-
Escherichia coli (pan drug resistant)	105	104	104	104	5x103	-	-	-	-	-	-

*No growth in the agar plate is shown as (-)