Wound Medicine 7 (2014) 14–17

Contents lists available at ScienceDirect

Wound Medicine

journal homepage: www.elsevier.com/locate/wndm

In vitro study on endotoxin release of gram-negative bacteria after contact with silver releasing compared to DACC coated wound dressings

Horst Braunwarth [b], Dietmar Becher [c], Florian H.H. Brill [a,*]

[a] Dr. Brill + Partner GmbH – Institute for Hygiene and Microbiology, Hamburg, Germany
[b] Coloplast GmbH, Hamburg, Germany
[c] Micromun GmbH, Greifswald, Germany

ARTICLE INFO

Article history:
Received 14 January 2015
Accepted 12 March 2015
Available online 8 April 2015

Keywords:
Wound dressing
Endotoxin
Pseudomonas aeruginosa
Silver
Bactericidal effect
Hydrophobic effect

ABSTRACT

The treatment of critically colonised or locally infected wounds with local antimicrobial agents is a standard of care. The destruction of especially gram-negative bacteria potentially increases the endotoxin level in the wound. This in vitro study aims to answer the question of whether and to what extent endotoxin release caused by the destruction of gram-negative bacteria is influenced by different wound dressing. Silver ion releasing dressings were compared to wound dressings with hydrophobic effect coated with dialkyl carbamoyl chloride (DACC). In addition, the bactericidal efficacy was measured. The $\log_{10}$ reduction factors (RF) against *Pseudomonas aeruginosa* were between 0 and 0.9 for the hydrophobic dressings and 8.7 for the silver releasing dressing. The bacterial endotoxin content of the agar located under the dressing after contamination with *P. aeruginosa* was >300 <3000 IU/ml in the case of a cotton gauze (control), >3000 <30,000 IU/ml for DACC coated distance grid, >30 <300 IU/ml in for the DACC coated foam dressing and >0.3 <3 IU/ml in the case of the silver ion releasing dressing. The content of bacterial endotoxins which could be extracted from the wound dressing after contact with *P. aeruginosa* was >30,000 <300,000 IU/ml for the control dressing, >30,000 <300,000 IU/ml in the case of Cutimed Sorbact, >3000 <30,000 IU/ml for the DACC coated foam dressing and >3 <30 IU/ml for the silver-releasing dressing. According to these findings, the silver ion releasing dressing has a higher antibacterial effect than wound dressings coated with DACC and it also releases a significantly lower amount of bacterial endotoxins.

1. Introduction

Nowadays, the treatment of critically colonised or locally infected wounds with local antimicrobial agents is a standard of care. The destruction of bacteria potentially increases the release of endotoxins, especially from the cell membrane of gram-negative bacteria, as demonstrated in in vitro studies involving antibiotic agents [1].

Endotoxin release may trigger a number of local and systemic reactions. It only takes 100 ng of purified endotoxins to prompt fever in human beings; doses of several milligrams can lead to death. With regard to wound care, contamination caused by endotoxins released from bacteria can delay the healing process [9–11].

In the past, it was therefore demanded that no silver ions were released into the wound and/or that evidence be provided showing that the release of silver ions did not lead to a release of endotoxins in the wound [2]. However, this issue has not played such a significant role in recent discussions. These discussions have instead focussed on the fact that this requirement is met by using wound dressings which eliminate bacteria from the wound by means of adsorption based on the hydrophobic surface of dressings. Wound dressings coated with DACC play a key role due to their hydrophobic effect [3].

The in vitro data presented in this paper aim to clarifying whether and to what extent endotoxin release caused by the destruction of gram-negative bacteria, in turn achieved by released silver ions, differs from a reduction in the number of bacteria prompted by the hydrophobic effect of wound dressings coated with dialkyl carbamoyl chloride (DACC).

* Corresponding author. Tel.: +49 40 557 631x0; fax: +49 40 557 631x11.
E-mail address: florian.b@brillhygiene.com (Florian H.H. Brill).

http://dx.doi.org/10.1016/j.wndm.2015.03.004

2. Materials and methods

The following wound dressings were tested:

- Biatain Ag (foam dressing with silver ion release, Coloplast GmbH).
- Biatain Super (hydrocapillary dressing, Coloplast GmbH).
- Cutimed Sorbact (distance grid with DACC coating, BSN Medical).
- Cutimed Siltec Sorbact (foam dressing with DACC coating, BSN Medical).
- Sterile cotton gauze as a negative control.

The antimicrobial effect was determined in a qualitative and quantitative wound assay involving *Pseudomonas aeruginosa* ATCC 15442 as test organisms [4].

The endotoxin concentration was determined through a limulus amebocyte lysate (LAL) assay using a testing kit produced by BioWhittaker (Lonza) in accordance with Pharm. Eur. No. 7 [5]. The data were determined in a total of nine independent tests and specified using international units (IU).

The endotoxin content in and on the agar was determined after the test piece of a wound dressing came into contact with *P. aeruginosa*. The agar surface was contaminated with approx. 10^7 bacteria. After drying for 2 min circular test specimens of the dressing with a diameter of 20 mm were applied onto the agar and incubated for 24 h at 36 °C. After removing the wound dressing, the agar which had been directly underneath the dressing was removed and homogenised in 10 ml of neutraliser solution before the colony forming units (CFU/ml) were determined. This solution was autoclaved before endotoxin determination so as to prevent the surviving bacteria from possibly multiplying and increasing the endotoxin amount. The autoclave procedure does not influence the test results as endotoxins are considered as heat stable [6,7].

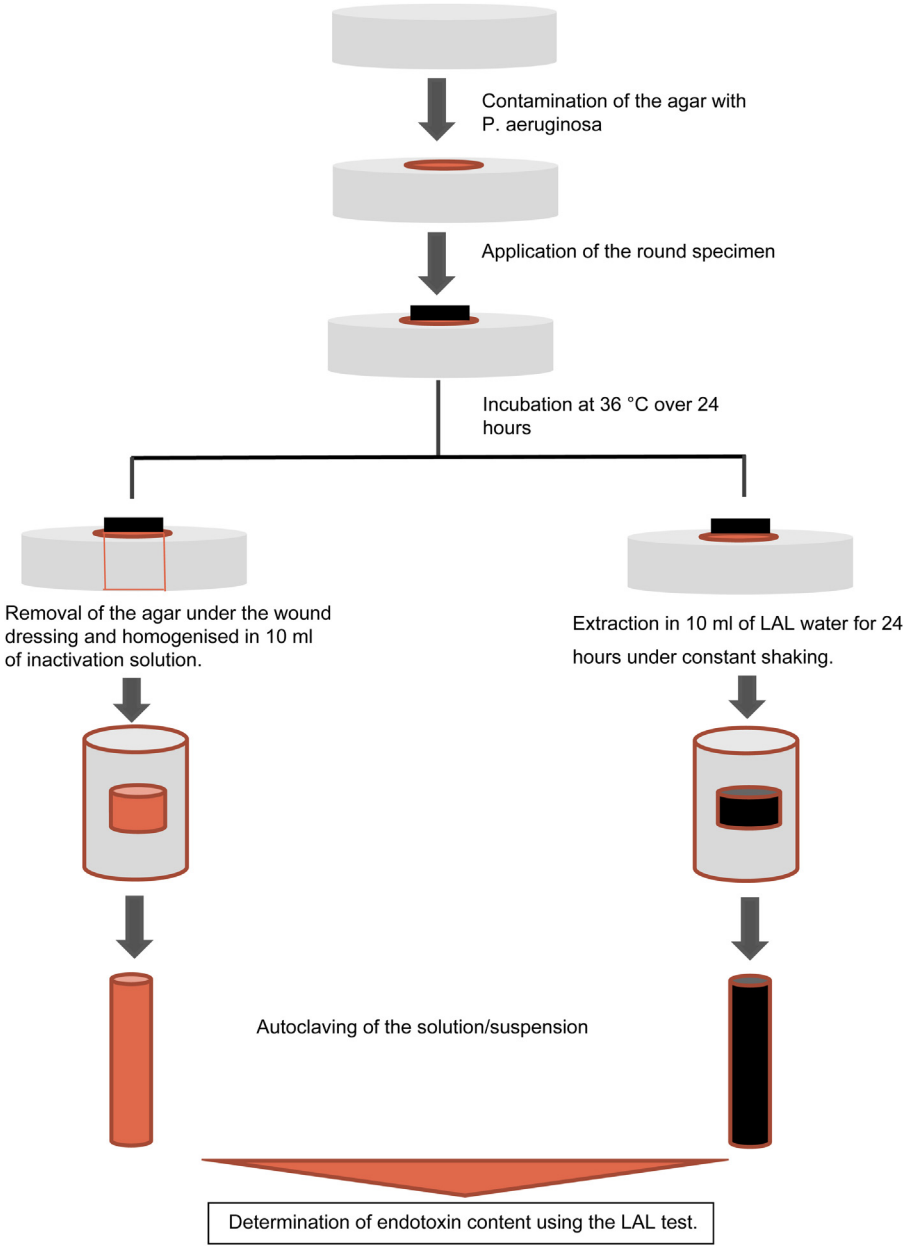

Fig. 1. Schematic study sequence. *Abbreviations.* LAL water: water with an endotoxin content: <0.001 EE/ml and a content of (1 → 3)-β-D-glucan content: <1.56 pg/ml. LAL test: limulus amebocyte lysate test.

In addition, the endotoxins were determined by extraction from the wound dressing after coming into contact with *P. aeruginosa*. The wound dressing specimens with a diameter of 20 mm were autoclaved first to avoid any proliferation of surviving bacteria after the efficacy testing. After that the dressings were extracted with 10 ml LAL water over 24 h while being constantly shaken. They were then concentrated down to 1 ml before the endotoxin content was determined using the LAL test (see Fig. 1).

The overall surface of the wound dressings was determined by means of gas adsorption in line with the static-volumetric principle for the characterisation of surfaces, pore systems and gas/solid interactions using the multiple measuring point technique [8]. This involves exploiting the interactions of a clean and inert gas with the solid, which results in the gas under analysis being adsorbed on the surface and thus consumed (adsorption–desorption isotherm). The consumption of the gas can be verified and converted to apply to the entire surface of the respective solid. Krypton (at 77.35 K) was used as the gas under analysis. The ASAP 2020 Accelerated Surface Area and Porosimetry System (by Micrometrics) were used for analysis purposes. The software "DataMaster™" was used to analyse the data.

3. Results

With regard to antibacterial effect, a $\log_{10}$ reduction factor (RF) of 0 was found for Cutimed Sorbact and an RF of 0.9 for Cutimed Siltec Sorbact. An RF of 8.7 was determined for Biatain Ag (Tables 1 and 2). The bacterial endotoxin content in the agar located under the dressing following contamination with *P. aeruginosa* amounted to >300 <3000 IU/ml with the control dressing, >3000 <30,000 IU/ml with Cutimed Sorbact (distance grid with DACC coating), >30 <300 IU/ml with Cutimed Siltec Sorbact (foam dressing with DACC coating) and >0.3 <3 IU/ml with Biatain Ag (foam dressing with silver ion release) (Fig. 2).

The extractable bacterial endotoxin content determined in the wound dressing following contamination with *P. aeruginosa* amounted to >30,000 <300,000 IU/ml with the cotton gauze (control dressing), >30,000 <300,000 IU/ml with Cutimed Sorbact, >3000 <30,000 IU/ml with Cutimed Siltec Sorbact and >3 <30 IU/ml with Biatain Ag (Fig. 3).

The size of the wound dressing surfaces was found to differ significantly due to the different structures, despite the specimens having the same diameter (Table 3). The largest surface area was provided by Biatain Super at approx. 305 cm^2/20 mm specimen, whereby the lowest was provided by the cotton gauze (control dressing) at approx. 32 cm^2/20 mm specimen. The following surface areas were determined for the other dressings: 98 cm^2/20 mm for Biatain/Biatain Ag, 177 cm^2/20 mm for Cutimed Sorbact and 37 cm^2/20 mm for Cutimed Siltec Sorbact.

Table 1
Micro-biostatistical effect in the agar diffusion test DIN 58940:2007 and microbiocidal effect in the quantitative agar diffusion test [1] involving *P. aeruginosa*. The average values from three parallels are specified.

	Average value, diameter of zone of inhibition (mm)	SD	Average value, log RF
Biatain Ag	3.7	±0.3	8.7
Cutimed Sorbact	0.0	±0.0	0.0
Cutimed Siltec Sorbact	0.0	±0.0	0.9
Control dressing (gauze)	0.0	±0.0	–

Table 2
$\log_{10}$ reduction factors (RF) in the quantitative agar diffusion test involving *P. aeruginosa*. The average values of colony-forming units (CFU) and standard deviation (SD) are specified.

	$\log_{10}$ CFU	SD	$\log_{10}$ RF
Biatain	6.6	0.27	2.1
Biatain Super	8.1	0.55	0.6
Cutimed Sorbact	8.8	0.37	−0.1
Cutimed Siltec Sorbact	7.5	0.69	1.2

Modified in line with [10].

4. Discussion

Different local and systemic reactions can be triggered upon coming into contact with endotoxins. Clinically dependent on the immune status, concentration and portal of entry, these reactions can range from affecting the impact on wound healing through to septic shock entailing multiple organ failure [9–11]. It only takes 100 ng of purified endotoxins to prompt fever in human beings; doses of several milligrams can lead to death. In terms of wound care, contamination with bacteria can delay the healing process.

Our previous studies show that advanced wound dressings, including those which are not coated in DACC, are able to bind bacteria to foam materials and also demonstrate a hydrophobic effect. Based on these findings, the hydrophobic effect by DACC appears to have no boosting effects on the binding of bacteria [12]. The results on endotoxin release in the agar are not as expected: despite a considerably greater antimicrobial effect, the wound dressing which releases silver ions demonstrates a lower

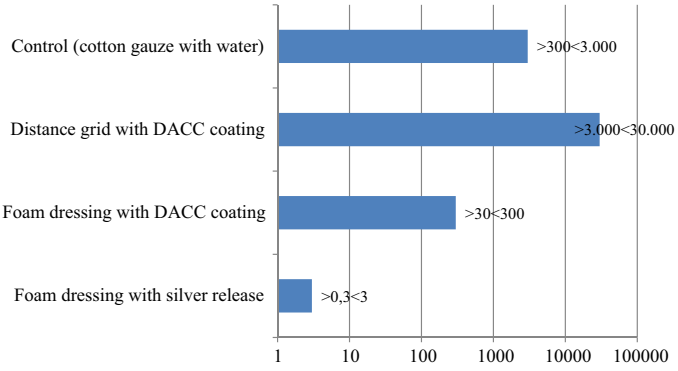

Fig. 2. Content of bacterial endotoxins (IU) in the agar located under the wound dressing following contamination with *P. aeruginosa*, application of the specimens with subsequent incubation for 24 h and removal of the wound dressing (specimen). Distance grid with DACC coating = Cutimed Sorbact, foam dressing with DACC coating = Cutimed Siltec Sorbact, foam dressing with silver ion release = Biatain Ag.

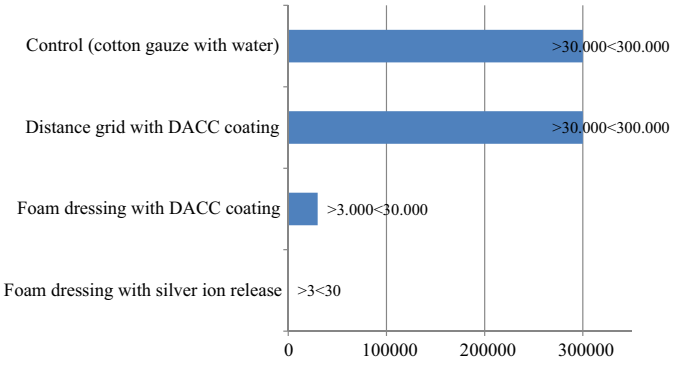

Fig. 3. Content of bacterial endotoxins which can be extracted from wound dressings, which were incubated for 24 h on agar surfaces contaminated with *P. aeruginosa*. Distance grid with DACC coating = Cutimed Sorbact, foam dressing with DACC coating = Cutimed Siltec Sorbact, foam dressing with silver ion release = Biatain Ag.

Table 3
Available surface area of the various wound dressings for the binding of bacteria in cm^2 per specimen.

Wound dressing	Surface pursuant to BET (m^2/g)	Weight of wound dressing (g/20 mm)	Specimen surface (m^2/20 mm)	Specimen surface (m^2/20 mm)
Biatain/Biatain Ag	0.0411	0.238	0.0097818	97.82
Biatain Super	0.2778	0.11	0.030558	305.58
Cutimed Sorbact	0.0973	0.182	0.0177086	177.09
Cutimed Siltec Sorbact	0.0196	0.188	0.0036848	36.85
Control dressing (gauze)	0.101	0.032	0.003232	32.32

release of endotoxins: 100–10,000 times lower than the cotton gauze, 10,000 times lower than Cutimed Sorbact (DACC-coated distance grid) and 10–100 times lower than Cutimed Siltec Sorbact (DACC-coated foam dressing) (Fig. 2).

This argues in favour of the fact that when bacteria are bound to the DACC coating at least their outer cell membrane is destroyed and the lipopolysaccharide structures measures as endotoxins contained in the membrane are released. The silver ions undoubtedly kill the bacteria. The release and thus the destruction of the bacteria, however, does not happen immediately, but successively, which means that it takes a while before the full effect is achieved: in the quantitative suspension test and in the disc carrier model, an almost complete destruction of the test organisms, *P. aeruginosa*, was verified within 30 min [13,14]. The endotoxins released into the agar during this time were likely bound by the foam material straight away, as the proportion of extractable endotoxins is low in the case of the foam dressing with silver ion release (Fig. 3).

The results in Tables 1–3 show that there is no direct correlation between the surface area, the reduction factor and both the released and extractable endotoxins. It is therefore to be expected that bacteria are only bound to wound dressings if there is a large enough surface area. It is thus conceivable that the available surface area of wound dressings which are not antimicrobial has an influence on binding capacity. This could explain the different results related to binding when comparing standard foam dressings with DACC-coated dressings [12].

The results argue in favour of the fact that the adsorption and binding capacity factor of the wound dressings has a greater influence on endotoxin release than the active principle of destruction and/or passive bacteria elimination.

5. Conclusion

The results show that there is a release of endotoxins through antimicrobial agents and also through binding processes of gram-negative bacteria. The tested silver wound dressing is evidently able to bind a larger proportion of these endotoxins than the DACC wound dressings. It can therefore not be concluded that antimicrobial agents more strongly burden the wound with endotoxins than in the case of wound dressings which are coated in DACC.

These in vitro results cannot be directly transferred to clinical practice. Despite this, they give clear indications that using the tested silver wound dressing is not restricted by an increased release of endotoxins. Further studies are needed to demonstrate whether this is the case for the dressings coated in DACC.

Conflict of interest

The study was partially sponsored by Coloplast GmbH, Germany. Horst Braunwarth is an employee of Coloplast GmbH, Germany.

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