Pharmaceutical Microbiology

88
In vitro efficacy of a non-alcoholic antibacterial cleansing lotion as a potential routine hand disinfectant

R. Turner, S. Lim, B. Forbes and M. B. Brown

MedPharm, 150 Stamford St, London SE1 9NH, UK marc.brown@medpharm.co.uk

Hand dermatitis among healthcare workers often results from the frequent use of hand wash products containing anionic detergents. Furthermore, healthcare workers are frequently asked to use medicated preparations containing high levels of alcohols and/or disinfectants, which result in skin irritation and dryness. Consequently, hand washing compliance is low, averaging only 40% (Larson & Kretzer 1995). One approach to avoid this problem is to use a dermatologically acceptable, non-alcoholic, antibacterial product containing non-ionic surfactants. Dermol 500 lotion is one such antiseptic preparation, specifically formulated for the management of dry skin conditions such as eczema and atopic dermatitis. The active ingredients incorporated in Dermol 500 are chlorhexidine hydrochloride 0.1%, benzalkonium chloride 0.1%, liquid paraffin 2.5% and isopropyl myristate 2.5%. This product also contains cetostearyl alcohol, cetomacrogol 1000, phenoxyethanol and water. The aim of this study was to examine the *in-vitro* efficacy of Dermol 500 lotion against *E.coli* (a microorganism commonly present on the hands of healthcare professionals acquired by contact with contaminated surfaces) when used for routine hand wash infection control. Initially, after obtaining a baseline of natural flora, the hands of 10 volunteers were washed in 70 ml of ethanol and dried. In the next stage, the left hands of the volunteers were contaminated with 20 μl of a viable *E coli* suspension (approx 10^8 CFU/ml). The hands were then rubbed together for 20 s and air-dried for a further 100 s. After this period, 3 ml of Dermol 500 lotion was applied to the cupped hands and the opposing hands rubbed together for one minute. The hands were then rinsed gently under 500 ml sterile Ringer solution. Organisms were recovered by rubbing the hands vigorously for 1 min in a 100 ml of neutralising broth and using 100 ml of 3 mm diameter glass beads. The viable bacteria were enumerated using a standard plate count method. Microbial recovery was measured after hand washing with ethanol (negative control), after application of *E coli* with no treatment applied (positive control) and after application of *E coli* followed by hand washing with Dermol 500 Lotion. The hand washing efficacy was calculated as a $\log_{10}$ reduction factor (RF) when compared with the positive control. The results indicated that the average $\log_{10}$ reduction factor of the treatment group was 3.02, after a single wash of *E.coli*-contaminated hands with Dermol 500 lotion. In the negative control group, no viable counts of *E.coli* were detected. It is widely accepted that the removal of bacteria from the hands using an alternative Glove Juice Recovery Method should result in a 3 $\log_{10}$ reduction after 10 washes with a medicated soap. In this study, Dermol 500 lotion showed an average reduction of 3 $\log_{10}$ after just one single wash. These results confirm that this non-alcoholic, antibacterial and non-ionic emollient/cleanser is effective in removing *E.coli* from the surface of hands and might be employed for routine hand disinfection by healthcare workers.

Larson, E. L, Kretzer E. K (1995) *J. Hosp. Inf.* **30** (Suppl): 88–106

89
Microbial skin flora under adhesive dressings

D. G. Chapman, S. J. McClay and P. M. Wilson

The School of Pharmacy, The Robert Gordon University, Schoolhill, Aberdeen, AB10 1FR, UK d.g.chapman@rgu.ac.uk

It is widely recognised that staphylococci are the principal microbial component of the micro-environment of human skin with *Staphylococcus epidermidis* being the predominant species on the skin surface of the upper areas of the body surface and *Staphylococcus hominis* on the lower body surfaces. Although restrictive on their growth, staphylococci are able to withstand a high level of desiccation and osmotic stress. However, other adverse factors on the skin also restrict their growth while hydration of the skin surface has been shown to encourage growth. Following application to the forearm, occlusive dressings have been shown to promote the growth of large numbers of organisms on the skin of male prisoners (Marples & Kligman 1969). These dressings did not contain an antimicrobial antiseptic. In this study the change in the number of skin flora under commercially available occlusive and porous ("breathable") adhesive dressings used by male and female subjects was determined. The procedure was also performed on dressings containing benzalkonium chloride (BKC) as an antiseptic. The adhesive dressings were applied to the skin of the forearm of eight young healthy male and female adults over a period of four days. The skin surface was sampled before and after removal of the dressings using contact plates containing tryptone soya agar supplemented with 0.5% polysorbate 80 to support the growth of lipophilic organisms. The plates were incubated at 30–35°C for 48 h when the colony forming units were counted. The results were then subjected to statistical analysis using a two tailed Mann-Whitney non parametric independent two group comparison test. Interestingly, the female subjects exhibited a statistically significant ($P<0.05$) lower microbial count on the skin than the male subjects before and after application of the dressings. This result correlates well with previous findings of microbial dispersal into environmental air (Noble et al (1976). Microbial growth on skin under the occlusive dressings was statistically greater ($P<0.05$) than was evident with the "breathable" dressings. This result was equally applicable to both male and female subjects and would seem to be due to the more hydrated skin present under occlusive dressings. Inclusion of benzalkonium chloride as an antiseptic component of dressings reduced the microbial growth on skin under the dressings for both male and female subjects ($P<0.01$). These results would seem to indicate that from a microbiological viewpoint the preferred adhesive dressing would be porous and contain an antiseptic.

Table 1 Microbial count on skin under dressings

	Female Initial count*	Final count	Male Initial count	Final count
Occlusive dressing	7.9×10^2	6.1×10^3	2.1×10^3	4.6×10^4
Permeable dressing	6.2×10^2	1.3×10^3	1.8×10^3	9.1×10^3
Occlusive dressing plus 960 mg m^{-3} BKC	7.3×10^2	2.1×10^3	2.0×10^3	1.8×10^4
Permeable dressing plus 960 mg m^{-3} BKC	8.4×10^2	1.2×10^3	1.9×10^3	6.2×10^3

*Colony forming units per cm^2 median of 8 samples.

Marples, R. R. Kligman, A. M. (1969) *Arch. Dermatol.* **99**: 107–110
Noble, W. C. et al (1976) *J. Med. Microbiol.* **9**: 53–61

90

Development of novel porphyrin-impregnated biomaterials for the prevention of intraocular lens-associated infectious endophthalmitis

C. Parsons, S. P. Gorman, D. S. Jones and C. P. McCoy

School of Pharmacy, The Queen's University of Belfast, 97 Lisburn Road, Belfast, BT9 7BL, UK c.parsons@qub.ac.uk

Photodynamic therapy (PDT) has been utilised in antimicrobial applications using photosensitising agents in solution (Merchat et al 1996). Infectious endophthalmitis is a rare but potentially sight-threatening condition often arising following cataract removal and insertion of an intraocular lens (IOL). This study examined the use of porphyrins, well established photosensitisers, not in solution but attached to the surface of the biomaterial to prevent bacterial colonisation of the IOL. Light activation causes the porphyrin to generate highly reactive singlet oxygen, $^1O_2$, a cytotoxic species, leading to cell death due to peroxidative damage (Taylor et al 2002). In this study, the cationic porphyrin tetra-4-N methylpyridinium porphyrin (TMPyP) was bound via electrostatic attraction to the surface of an anionic copolymer comprising 2-hydroxyethyl methacrylate (HEMA) (90% w/w) and methacrylic acid (MAA) (10% w/w) via direct adsorption from solution, by dipping prehydrated samples in a 100 mcg/mL TMPyP solution for 60 seconds, blotting with medical tissue to remove excess porphyrin solution, and repeating until the samples were dipped a total of five times. 90% HEMA 10% MAA was selected as the most promising candidate for microbiological testing from photochemical investigations. Bacterial adherence was tested using two microorganisms; however, as 70% of cases of infectious endophthalmitis are caused by Gram-positive coagulase-negative staphylococci, mostly *S. epidermidis*, this organism was our principal focus. An inoculum of approximately 10^3 colony forming units was added onto the surface of a disc of the material being tested and incubated for 10 min at 37°C. Non-adhered bacteria were removed and the discs exposed to laboratory light (1260 lux) or to a more intense light source (4300 lux), or kept in the dark, for one hour. Adhered bacteria were removed via sonication and vortexing and then enumerated. Materials containing no porphyrin served as controls. These results showed that there was a significant reduction (ANOVA, $P < 0.05$) in bacterial adherence to porphyrin-impregnated materials compared to controls in the different light conditions (Table 1). There appeared to be no significant difference (ANOVA, $P < 0.05$) between the reduction in adherence to materials exposed to laboratory light and those kept in the dark, indicating perhaps a light-independent activity of the porphyrin or an alteration in the surface of the material on impregnation with porphyrin rendering it less adherent. However, on exposing the materials to the more intense light source, reduction in adherence was significantly enhanced (ANOVA, $P < 0.05$). Initial studies with a Gram-negative organism, *Proteus mirabilis*, have shown similar, although less pronounced, reductions. The study has shown that impregnation of 90% HEMA 10% MAA with TMPyP significantly reduced bacterial adherence and that increasing the intensity of the light improved the anti-adherent effect. Studies are ongoing to define whether this is an anti-adherent effect or whether the organisms are killed.

Table 1 Percentage reduction in adherence of *S. epidermidis* for porphyrin-impregnated materials

Light conditions	% reduction (±s.d.) in organisms adhered vs control *S. epidermidis*
Intense light	99.022 ± 0.423
Laboratory light	91.059 ± 5.971
Dark	90.259 ± 5.680

(n = 5, ±s.d.).

Merchat, M. et al (1996) *J. Photochem. Photobiol. B: Biol*. **32**: 153–157
Taylor, P. W. et al (2002) *Drug Discovery Today* **7**: 1086–1091

91

Effect of lecithin on viability and stability of probiotics

A. R. Donthidi, F. Al-Ghazzewi, K. Aidoo and R. F. Tester

Dept. of Biological Sciences, Glasgow Caledonian University, 70 Cowcaddens Road, Glasgow, G4 0BA, UK E-mail: amar.donthidi@gcal.ac.uk

Probiotics (Füller 1989) means "for life" and this name is now mostly used to refer to concentrated supplements of beneficial bacteria taken by humans and animals. *Bifidobacterium* and *Lactobacillus* species have been the focus of probiotic interest since a large population of these bacteria in the intestinal tract is generally considered to be indicative of a healthy microbiota (Mercenier et al 2002). To benefit from probiotic bacteria the concentration of dosage forms should exceed 1×10^6 live bacteria per ml (Lee & Salminen 1995). In this study a range of *Lactobacillus* and *Bifidobacterium* species were encapsulated using alginate (2% w/v)/starch (2% w/v) and different concentrations of lecithin (1, 2, 3 and 4% w/v) and the effect of lecithin on viability of the probiotics was determined. The beads were prepared by an extrusion method. The feed suspensions were prepared with viable bacterial strains (1% culture of *Lactobacillus casei* NCFB 161) above 10^{10} CFU/ml and a carrier solid (alginate, starch and lecithin) to coat the cells. The feed suspension was extruded through tubes with an internal diameter 0.64 mm into a 0.1 M $CaCl_2$ solution (Smidsrod & Skjak-Braek 1990). The $CaCl_2$ solution was stirred at a uniform rate with a magnetic stirrer. After 30 min the beads were removed and washed with sterile water. The beads were freeze dried and stored in sealed containers at 4, 25 and 37°C. Incorporation of lecithin improved the viability of bacteria compared to when the bacteria were encapsulated without lecithin ($P = 0.02$). The inclusion of 1% lecithin with alginate mix was found to be the optimum concentration for increasing the survival of probiotic bacteria during the freeze drying process. However, increasing the concentration of lecithin above 1% did not show any significant improvement ($P > 0.05$) in the survival of bacteria during the freeze drying process. The encapsulation method used in this study resulted in a uniform bead size. A further study was carried out to monitor the effect of lecithin on the survival of *Lactobacillus* and *Bifidobacterium* spp. at different temperatures over a period of 24 weeks. This study showed that the survival of encapsulated cultures of *L. acidophilus* and *Bifidobacterium* spp. was above 10^6 CFU/ml when stored at 4°C during 6 months. Freeze dried beads containing 1% lecithin had good stability (> 10^6 CFU/ml) of *Lactobacillus* species and *Lactococcus lactis* when stored at 25°C for 12 weeks. The inclusion of lecithin with alginate and starch for encapsulation has shown significant improvement on the viability of probiotics from the point of encapsulation to storage.

Füller, R. (1989) *J. Appl. Bacteriol*. **66**: 365–378
Lee, Y. K., Salminen, S. (1995) *Trends in Food Sci. Technol*. **6**: 241–245
Mercenier, A. et al (2002) *Curr. Pharm. Design* **8**: 99–110
Smidsrod, O., Skjak-Braek, G. (1990) *Trends Biotechnol*. **8**:71–78

92

Solid phase synthesis and biological screening of N-mercaptoamide template-based *Pseudomonas* elastase (LasB) inhibitors as putative antibiofilm compounds

G. Cathcart[1], B. Gilmore and B. Walker

School of Pharmacy, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK gcathcart@qub.ac.uk

Pseudolysin or LasB is a metalloprotease virulence factor of *Pseudomonas aeruginosa*, which degrades or inactivates host tissues or immune system components in the various modalities of *P. aeruginosa* respiratory tract infection. Neutropenic or mechanically ventilated patients are at particular risk of acute infection, and it features as the major pathogen associated with chronic infective exacerbations of cystic fibrosis (CF). Mucoid strains of *P. aeruginosa* form mucopolysaccharide biofilms under certain conditions through secretion of alginate. Once the biofilm has formed, secretion of LasB is reduced, but the bacteria within a biofilm are many orders more resistant to the immune system or to antibiotic treatment than their planktonic counterparts, and so chronic infection ensues. LasB has been implicated in a number of important pathophysiological processes involving *P. aeruginosa* infection, most interestingly perhaps is its role in alginate production during biofilm formation. The reduced secretion of LasB during biofilm growth has been attributed to its intracellular retention for its involvement in the generation of secreted alginate. This occurs through LasB proteolytic processing of nucleotide diphosphate kinase (Ndk) within the cell, and so it has thus been demonstrated that *Pseudomonas* elastase is essential for alginate synthesis, and hence biofilm formation (Kamath et al 1998). LasB, therefore, represents a novel therapeutic target for the reduction of pseudomonal biofilm formation on both biotic and abiotic surfaces. We now report the solid phase synthesis, kinetic characterisation and biological evaluation of a series of N-mercaptoamide dipeptide inhibitors of LasB as putative anti-biofilm compounds utilising the high throughput screening capabilities of the Calgary biofilm device. In accordance with results presented by Nishino and Powers, LasB has shown preference for large aromatic side groups in the P1' position (Nishino & Powers 1980). These findings have been used to optimise inhibition, supported within the context of a library of inhibitors modified at the P1' position, some of which have been included in

Table 1 Synthesised inhibitors and corresponding K_i values

Inhibitor	K_i (μM)
HSCH2CO-Phe-Ala	0.583
HSCH2CO-Leu-Leu	5.4
HSCH2CO-Ala-Tyr	NI

Table 1. Antibiofilm properties of synthesised inhibitors will be tested using the high-throughput screening capabilities of the Calgary biofilm device (Ceri et al 1998). We envisage that these inhibitors will have utility in prevention of *Pseudomonas* biofilm formation on indwelling medical devices either by direct incorporation or surface modification of the biomaterial.

Ceri, H. et al (1998) *J. Clin. Microbiol*. **37**: 1771–1776
Kamath, S. et al (1998) *Mol. Microbiol*. **30**: 933–941
Nishino, N., Powers, J. (1980) *J. Biol. Chem*. **255**: 3482–3486