Activity of histone H1.2 in infected burn wounds

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Objectives: Infections with multidrug-resistant microorganisms (e.g. Pseudomonas aeruginosa and Staphylococcus aureus) cause immense complications in wound care and in the treatment of immunosuppressed patients. Like most antimicrobial peptides, histones are relatively small polycationic proteins located in each eukaryotic nucleus, which naturally supercoil DNA. The aim of this study was to investigate the in vitro and in vivo activity of histone H1.2 in infected burn wounds and its potential toxicity.

Methods: To characterize the antimicrobial properties of histone H1.2 against potential causative organisms of burn wound infections, the in vitro radial diffusion assay and modified NCCLS microbroth dilution MIC assay were carried out. Haemolytic and cytototoxic properties were determined in human red blood cells and primary human keratinocytes. In vivo antimicrobial activity was tested in an infected rat burn model with P. aeruginosa (ATCC 27853). All results were compared with the naturally occurring broad-spectrum antimicrobial peptide protegrin-1 and with antibiotics clinically used against the corresponding bacteria.

Results: Human histone H1.2 exerted good antimicrobial activity against all tested microorganisms without significant haemolytic activity. Surprisingly, histone H1.2 showed cytotoxicity with an LD50 of 7.91 mg/L in primary human keratinocytes. The in vivo burn model data revealed a significant threefold higher reduction in bacterial counts within 4 h compared with carrier control.

Conclusions: These findings indicate that histone H1.2 is a potential candidate for use as a local and, because of its low haemolytic activity, systemic antimicrobial agent. However, further investigations are needed to specify the cytotoxicity and the dose–response relationship for histone H1.2.

Keywords: skin infections, wound healing, rat burn model, host defence peptides, innate immunity, antimicrobial peptides

Introduction

Infections, especially those caused by antibiotic-resistant Gram-positive and Gram-negative bacteria, such as multidrug-resistant Pseudomonas aeruginosa, methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci, are a growing concern according to the data of the National Nosocomial Infections Surveillance system report from 2003. They especially affect patients with burn wounds, whose innate immune system is locally impaired if the protective barrier function of the epithelia is damaged. Of equal concern are immunosuppressed patients, whose acquired immune system is impaired either intentionally for transplantation or as a result of a disease such as acquired immune deficiency syndrome. As an effect, the increasing resistance of bacteria to antibiotics decreases our ability to control the spread of multidrug-resistant bacteria with clinically available antibiotics. This spread is an alarming and globally growing problem. Thus, there is an urgent need to find new therapeutic alternatives to antagonize multidrug resistance.

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Within the past decade, research on new antibiotics has stagnated because of the development costs and the increasing number of multidrug-resistant microorganisms caused by the exaggerated and irresponsible application of antibiotics. Increased attention has been paid to host defence peptides. Protegrin-1 is a potent, broad-spectrum antimicrobial agent with high haemolytic activity. Another antimicrobial peptide is buforin-1, which results from a cleavage of one of the histone proteins, histone H2A. This study focuses on another histone, H1.2, which has a potential role in innate antimicrobial defence in the human gastrointestinal tract, as indicated by Rose et al. Except for histone H4, where only one member has as yet been identified, other histone classes (H1, H2A, H2B and H3) consist of several subtypes.

The H1 histones represent the most heterogeneous class of histone proteins. So far, six H1 subtypes (H1.1–H1.6) have been identified in mammalian somatic cells. The central globular domain of five of these subtypes is highly conserved. The C- and N-termini exhibit microheterogeneity, which could contribute to the multifunctionality of these peptides.

Another intranuclear function of histone H1, apart from structural features, is the regulation of gene expression. There is growing evidence that histones belong to a group of proteins taking part in multiple biological processes, such as apoptosis, both extranuclearly and extracellularly. Because histone H1 has different toxicity profiles in human leukaemia cell lines, bovine histone H1 has been used for tumour growth suppression in vivo. Extracellular histone H1 serves as a thyroglobulin-binding protein on cellsurface of macrophages, mediates thyroglobulin endocytosis, serves as a potential target for autoantibodies, e.g. in patients with systemic lupus erythematosus, and is involved in other autoimmune diseases such as multiple sclerosis, diabetes and rheumatoid arthritis. Further functions of histones are the stabilization of sea urchin flagellar microtubules, and protection against infection of mice with Leishmania major by histone H1. The binding of lipopolysaccharide, the stimulation of tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) expression and other inflammatory mediators confirm the hypothesis that histone has a central role in immune modulation. These facts imply that histone H1 functions as an effector molecule of the immune system in a fashion similar to other antimicrobial peptides. Furthermore, histone H1 is up-regulated during scrapie and Alzheimer’s disease.

The aim of this study was to obtain detailed information about the antimicrobial potency of histone H1.2 against burn wound pathogens, its haemolytic and cytotoxic activity, and its bioactivity.

**Materials and methods**

**Peptide**

The recombinant human histone H1.2 (mol. wt 21.5 kDa) was produced as previously described. The antimicrobial peptide protegrin-1, which was naturally derived from porcine leucocytes, was described in earlier publications to be highly efficient against bacterial strains in vitro and in vivo. Dr Peter Henklein produced protegrin-1 (mol. wt 2.0 kDa) synthetically (Charité, Berlin, Germany).

**Bacteria**

Eight bacterial strains were used in the in vitro tests. Four strains are ATCC listed: S. aureus ATCC 25923; Staphylococcus epidermidis ATCC 12228; Enterococcus faecalis ATCC 29212; and P. aeruginosa ATCC 27853. Four clinical isolates such as S. aureus (MRSA) and clinical strains of P. aeruginosa were isolated from human burn patients. The clinical isolate of S. aureus (MRSA) was resistant to ampicillin and tetracycline but susceptible to vancomycin. The isolates from human burn patients were resistant to ampicillin but susceptible to imipenem and tobramycin. P. aeruginosa (ATCC 27853) was used in the infected rat burn model.

**Bacterial growth inhibition assays**

The radial diffusion (RD) assay has been described previously. This sensitive assay was used to evaluate the antibacterial activity of histone H1.2 against microorganisms responsible for most of the infections of human burn wounds. The results were compared with those obtained with the naturally occurring antimicrobial peptide protegrin-1, and commonly used clinical antibiotics. The following antibiotics were used: imipenem, ampicillin and vancomycin. Imipenem (Merck, Darmstadt, Germany) was utilized against P. aeruginosa (ATCC 27853) and against S. aureus isolated from human burn wounds. Ampicillin (Roth, Karlsruhe, Germany) was used against S. aureus and E. faecalis. S. epidermidis and S. aureus (MRSA) were treated with vancomycin (Merck, Darmstadt, Germany). Bacteria were grown overnight in trypticase soy broth (TSB; Unipath United, Hampshire, UK). An overnight culture was diluted into fresh TSB and incubated for 2.5 h at 37°C. The subculture was centrifuged (10 min, 4°C, 3350 g). The bacterial pellet was washed once and re-suspended in cold sodium phosphate buffer (J. T. Baker, Deventer, Holland, pH 7.4). The optical density was measured at 620 nm (UV-VIS-Spektrometer, Perkin Elmer 555). cfu were calculated by OD620 measurement (UV-VIS-Spektrometer, Perkin Elmer 555). Approximately 2 x 10^7 cfu/mL was incorporated in an underlay agar (sodium phosphate buffered to pH 7.4). After the agar solidified, a series of wells were punched. The wells were filled with the prepared diluted peptide. The peptide was serially diluted to the following concentrations: 250, 79.1, 25, 7.91, 2.5, 0.791 and 0.25 mg/L. After incubation for 3 h at 37°C, a nutrient-rich agar (sodium phosphate buffered to pH 7.4) was overlaid and the plates were incubated for 18 h at 37°C. Afterwards, the plates were stained with 0.001% Coomassie Blue for 10 h. The clear zones were converted into units by subtracting the well diameter and multiplying the difference by 10. Results were plotted using a semi-log scale, and correlation coefficients and x-axis intercepts were obtained from linear regression analysis. The minimum effective concentration (MEC) corresponds to the x-axis intercept value. MEC was defined as the lowest concentration (mg/L) of the drug that represented a clear zone of growth inhibition.

Modified NCCLS microbroth dilution (MNMD) assays were performed according to the NCCLS guidelines. The bacterial solutions were prepared by transferring a single colony into Mueller–Hinton broth (Merck GmbH, Hohenbrunn, Germany) and incubating overnight at 37°C. Afterwards, the cultures were diluted and the absorbance was measured at OD620 to provide a bacterial concentration of 4 x 10^7 cfu/mL in the microplate wells. One hundred microlitres of the freshly diluted cultures and 11 μL of the serially diluted peptide samples, in the range 1000–1 mg/L, were dispensed into 96-well polypropylene tissue culture plates (Ushape; Greiner, Solingen, Germany). The plates were covered and incubated at 37°C...
for 16–18 h (Heraeus, Heraeus Holding GmbH, Germany). To determine the minimal inhibitory concentration (MIC), the last well in the series without any visible growth was read. Subcultures were prepared out of each well without any visible growth. The first well in the series without any visible growth indicated the corresponding minimal bactericidal concentration (MBC).

**Haemolytic activity**

The assay was prepared in a modified way as described previously.34 Human blood was extracted, centrifuged to separate erythrocytes from plasma, and washed with 0.9% isotonic NaCl solution (Delta-Pharma, Pfullingen, Germany). A 2.8% (v/v) erythrocyte solution in PBS (PAA Laboratories GmbH, Linz, Austria) was incubated with different concentrations of substances to be tested in triplicate. The peptide was serially diluted to the following concentrations: 250, 79.1, 25, 7.91, 2.5, 0.791 and 0.25 mg/L. After 30 min at 37°C, the tubes were centrifuged, the OD 450 was measured and the haemolytic activity calculated. A 0.1% Triton X-100/PBS solution was added to determine 100% effective lysis.

**Cell culture**

Freshly received human skin was washed in PBS. Skin was digested overnight with 0.2% dispase solution (4.7 U/mL; Gibco, UK). Epidermis was gently peeled off, collected directly into trypsin/EDTA solution (0.05%/0.02%; Gibco, 35400-027) and incubated at 37°C for 5 min in a gently shaking water bath. After trypsin digestion was stopped by adding FBS (HyClone, Logan, USA) the cell suspension was filtered through a 100 μm cell strainer (Becton Dickinson, Heidelberg, Germany) and centrifuged at 400 g at 4°C for 5 min. Cells were re-suspended in 5 mL of complete keratinocyte medium [containing 3:1 DMEM (Gibco) Ham’s F12 (Gibco), 10% FBS, 1% penicillin/streptomycin (ICN, USA), 4 mM L-glutamine (ICN), 24.3 mg/L adenine (Calbiochem, Germany), 5 mg/L insulin (Sigma, USA), 0.4 mg/L hydrocortisone (Calbiochem), 1.36 μg/L triiodothyronine (Sigma), 10⁻¹⁰ M cholera toxin (Sigma), 10 μg/L EGF (Sigma)] and counted by CASY-1 (Schäfer-System, Reutlingen, Germany). Cells were seeded at 75000 cells/cm² into collagen type I (Becton Dickinson Falcon, Germany) pre-coated culture flasks. Medium was changed every second day.

**Cytotoxicity**

To analyse the cytotoxic effect of histone H1.2 on primary human keratinocytes, the BrDU cell proliferation ELISA with the chemiluminescent detection system (Roche Diagnostics, Germany) was performed. For that purpose, primary human keratinocytes were seeded in a 96-well microplate at a density of 85000 cells/cm². After 24 h of incubation at 37°C with 5% CO₂ in a humidified atmosphere, the primary cells were further incubated for 6, 12 or 24 h with different concentrations of histone H1.2 and protegrin-1 (250, 79.1, 25, 7.91, 2.5, 0.791 and 0.25 mg/L) in serum-free medium. Every step was performed according to the manufacturer’s instructions. Chemiluminescence was measured after automatic injection of substrate solution with a microplate-luminometer (Orion, Berthold Detection Systems, Pforzheim, Germany).

**Animal model**

The research protocol described below complied with all regulations relating to animal use and other federal statutes. It was conducted in compliance with the principles in the ‘Guide for the Care and Use of Laboratory Animals’ from the German Animal Welfare Act.

The animals were housed at the animal facility of the Berufsgenossenschaftliche Forschungsanstalt BGFA, Bergmannsheil, Ruhr-University, Bochum. The infected rat burn model was modified as described previously.31 In this experiment, 34 adult Sprague–Dawley rats weighing 220–260 g were used. The rats were kept at a constant temperature of 22°C, on a 12 h light cycle, in a 65% humidified atmosphere. Before debilitation, the following anaesthetics were used: ketamine (100 mg/kg; Ratiopharm, Ulm, Germany) and xylazine (20 mg/kg; Bayer, Leverkusen, Germany). Both anaesthetics were diluted in 0.9% isotonic NaCl solution (Delta-Pharma, Pfullingen, Germany) and applied intraperitoneally. The entire back region of the rat was depilated by electrical clippers and depilatory cream (Veet; Reckitt Benckiser, Mannheim, Germany).

Twenty-four hours after debilitation, the rats were anaesthetized. Thirty minutes before the beginning of the experiments and every 12 h during the study, 0.04 mL (2 units) of buprenorphine HCl (Temgesic; Essex Pharma, München, Germany) was applied subcutaneously. The rats were transferred in an insulating tube barion only two skin areas to be burned. Each rat in the protective mould with a 1 cm² surface was isolated and anaesthetized at 60°C water for 20 s. After 20 s, both burned areas were dried, marked and thoroughly disinfected with Softaspet (Braun, Melsungen, Germany). Each burn wound was inoculated with 1 x 10⁸ P. aeruginosa (ATCC 27853). To avoid cross-contamination, and to improve growth conditions for the used bacteria, all sterile dressing materials [Tegaderm (6 × 7 cm, 3M) Health Care, Borken, Germany] were applied immediately after the inoculation. The wound was bandaged with Peha-haft (Hartmann, Heidenheim, Germany) for stabilization and protection clipped with Visistat (Weck Closure Systems, North Carolina, USA). Afterwards rats were single caged.

Two days post-infection, animals were randomized and subdivided into the following groups: (1) 25 μg histone H1.2 (n = 7); (2) 250 μg histone H1.2 (n = 7); (3) 2500 μg histone H1.2 (n = 7); (4) 250 μg protegrin-1 (n = 7); and (5) PBS as carrier control (n = 6). Each peptide dose was suspended in PBS to reach a final volume of 500 μL per treated wound. Infected burned wounds were subdivided into definite squares (1.5 cm × 1.5 cm); 250 μL of the prepared solution containing histone H1.2, protegrin-1 or PBS was intradermally injected into each square. A further 250 μL was added topically after sterile gauze was applied. After the application, the wounded areas were bandaged with the occlusive dressings Tegaderm, Peha-haft and clipped with Visistat. Rats were kept single caged.

Four hours later, a lethal dose of 1 mL of pentobarbital sodium (Narcoren, Athen, USA) was applied intraperitoneally. The infected wound tissues were harvested aseptically, weighed and homogenized in PBS with a rotor stator homogenizer Polytron (Braun, Melsungen, Germany). Each burn wound was inoculated into isolation agar (Becton Dickinson, USA) and on P. aeruginosa isolation agar (Becton Dickinson, USA) and on Mueller–Hinton agar with 5% sheep blood (Becton Dickinson, Germany). The plates were incubated for 18 h at 37°C. Colonies were counted and multiplied with the corresponding dilution factor. Evaluation of results was done calculating the number of cfu/g of tissue.

**Statistical analysis**

All assays were performed in triplicate. Results of the RD assay, the MNMD assay and the investigation of the haemolytic activity were analysed using the program Stat View 5.0 (SAS, Cary, USA). Data from the rat burn model and the cytotoxic assay were evaluated with the program SPSS 11.0 (SPSS Inc., Chicago, USA). Results were regarded as significant with P < 0.05.
Results

**RD assay**

The results derived from RD assay against Gram-positive bacteria are presented in Figure 1. Against the most frequently found bacterial strains in human burn wound infections, *S. aureus* and *E. faecalis*, histone H1.2 demonstrated significantly (*P* < 0.05) higher antimicrobial activity than the corresponding antibiotics (MEC of H1.2 3.2 mg/L and 3.1 mg/L compared with MEC of ampicillin 10.5 mg/L and 28.9 mg/L). Compared with protegrin-1, histone H1.2 revealed a significantly (*P* < 0.05) higher activity at a lower MEC against *S. epidermidis* and *E. faecalis*—H1.2 MECs of 3.1 mg/L and 3.1 mg/L compared with protegrin-1 MECs of 4.5 mg/L and 4.5 mg/L. RD assay results for the bacterial isolates from human burn wounds are shown in Figure 2. The MEC of histone H1.2 for all bacterial isolates was significantly lower compared with the MEC of the corresponding antibiotics.

Histone H1.2 had almost the same antimicrobial activity against reference strains and clinical isolates from burn wounds.

**MNMD assay**

The antimicrobial activity of histone H1.2 was analysed against the same microorganisms tested in the RD assay. The MIC and MBC of histone H1.2 against Gram-positive bacteria (Table 1) were striking because of the equal values (MIC 12.5 mg/L and MBC 50 mg/L). Similar results were possessed by testing histone H1.2 against the *P. aeruginosa* reference strain and the bacterial isolates from human burn wounds (Table 1). Tests revealed a constant MBC (100 mg/L). The MIC varied between 50 mg/L and 100 mg/L.

### Table 1. Antibacterial activity of the histone H1.2

<table>
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<th>MBC (mg/L)</th>
<th>MEC (mg/L)</th>
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<tr>
<td><em>S. epidermidis</em></td>
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<td>50</td>
<td>3.1</td>
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<tr>
<td><em>E. faecalis</em></td>
<td>12.5</td>
<td>50</td>
<td>3.1</td>
</tr>
<tr>
<td>MRSA</td>
<td>12.5</td>
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<td>3.2</td>
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<tr>
<td><em>P. aeruginosa</em></td>
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<td>100</td>
<td>3.1</td>
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**Haemolytic properties**

Histone H1.2 showed almost the same haemolytic properties as the three tested clinically applied antibiotics (Figure 3). An increase to 250 mg/L did not lead to any significant increase in haemolytic activity. Compared with human histone H1.2, the porcine-derived antimicrobial peptide protegrin-1 possessed a considerable haemolytic effect, which achieved 100% haemolysis at 79.1 mg/L. The haemolytic activity of histone H1.2 was seen in low concentrations, but averaged at 2% and was concentration independent.

**Cytotoxicity of histone H1.2**

The cytotoxicity tests measured on isolated primary human keratinocytes demonstrated that the effect of histone H1.2 and protegrin-1 is reversed compared with the results of the haemolytic assay. At a concentration of 7.91 mg/L of histone H1.2, 50% of

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**Figure 1.** Activity of histone H1.2 against Gram-positive bacteria. MEC (mg/L) = minimal effective concentration. Data are shown as means ± S.E.M.

**Figure 2.** Activity of histone H1.2 against *Pseudomonas aeruginosa*. MEC (mg/L) = minimal effective concentration. Data are shown as means ± S.E.M.

**Figure 3.** Haemolytic activity of histone H1.2 and comparators. Human histone H1.2 (diamonds) and the porcine-derived protegrin-1 (squares) were compared with clinically applied antibiotics [ampicillin (triangles), imipenem (stars) and vancomycin (crosses)]. Total values were normalized to isotonic salt solution and 0.1% Triton X-100. Data are shown as means ± S.E.M.
Histone H1.2 in infected burns

Figure 4. Cytotoxicity activity of histone H1.2. Primary human keratinocytes were incubated with histone H1.2 for 6 h (filled diamonds), 12 h (filled squares) or 24 h (open triangles) and compared with protegrin-1 (6 h, open circles). Data are shown as means ± S.E.M.

Figure 5. Bacterial clearance in an infected rat burn wound model. *P < 0.05: histone H1.2 (25 µg), histone H1.2 (2500 µg) and protegrin-1 (250 µg) versus carrier control. Data are shown as means ± S.E.M.

the seeded cells showed no further proliferation (Figure 4), whereas protegrin-1 showed the same ratio of non-proliferating cells at a concentration of 0.791 mg/L.

In vivo activity of histone H1.2

The rat burn model was used to investigate the antibacterial activity of histone H1.2 in vivo. In order to simulate an infection, P. aeruginosa (ATCC 27853) was applied. The sensitivity of this bacterial strain, related to histone H1.2 and protegrin-1, was proven in both in vitro assays. Three different amounts of histone H1.2 (25, 250 and 2500 µg) were used and compared with the antibacterial activity of protegrin-1 (positive control, 250 µg). PBS served as carrier control.

Results of the evaluation of the counted plates are shown in Figure 5. The lowest concentration of histone H1.2 exerted no significant effect on clearance. The results matched with the results of the negative control [histone H1.2 (25 µg) 2.91 × 10^7; negative control 2.94 × 10^7 cfu/g tissue]. The application of 250 µg histone H1.2 achieved the highest antimicrobial activity. The number of cfu/g tissue treated with histone H1.2 250 µg was similar to the tissue treated with protegrin-1 250 µg (8.93 × 10^6 versus 7.40 × 10^6). In contrast, the highest concentration of histone H1.2 (2500 µg) had a lower antimicrobial activity (1.68 × 10^7 cfu/g tissue). In conclusion, 250 µg of histone H1.2 seems to be the optimal concentration for developing an effective antibacterial activity.

Discussion

Evidence that endogenous antibiotics play a key role in host defence has been demonstrated. These host defence peptides are essential components of the innate immune system. They act as effector substances for the innate immune response in fighting microbes.

We have demonstrated that histone H1.2 has good in vitro and in vivo activity against a small number of burn wound infection pathogens.

The results of the in vitro assays revealed that the human histone H1.2 had significantly higher antimicrobial activity compared with antibiotics that are clinically applied against the corresponding bacteria, except for vancomycin against MRSA. The MEC was similar against all tested microorganisms, regardless of use against Gram-positive bacteria or P. aeruginosa, each tested by the RD assay. Almost equal MIC and MBC values arose from the MNMD assay. In vitro and in vivo data proved that histone, in comparison with the clinically tested antibiotics, is much more effective against almost every tested microorganism. Another advantage of histone H1.2 is that it is a physiologically occurring agent within human cells.

In investigations examining haemolytic activity, histone H1.2 and the clinically used antibiotics demonstrated minimal damage to human erythrocytes (2% average). In contrast, protegrin-1 revealed considerable haemolytic effects at the effective antimicrobial concentration (100% damage). On the other hand, histone H1.2 was cytotoxic at low concentrations against human keratinocytes. It is difficult to understand why histone H1.2 destroys settled keratinocytes while it spares the rather fragile erythrocytes. One possibility is the apoptotic activity of histone H1.2, which is induced at very low concentrations and can occur with keratinocytes but not erythrocytes. It may also be possible that the physiological concentrations of endogenous extracellular histone are considerably higher in blood than in peripheral tissues, which results in active protection of floating blood cells like erythrocytes.

Our results of the tests with protegrin-1 confirm the data from previous studies. The results of the in vivo and in vitro tests are similar in principle but there are also some differences in MEC and MIC or the total bacterial killing in vivo, which could be explained by the usage of different bacterial strains for both the in vivo and in vitro tests. In addition, our in vivo investigations differ from those previously described, in application of a different number of cfu per wound (10^6 instead of 10^7), in a different location and in different animals.

In the burn infection model, histone H1.2 at 2500 µg was only half as effective as histone H1.2 or protegrin-1 at 250 µg, whereas the lowest concentration of 25 µg showed no effect compared with carrier control. We do not know why the higher concentration is less effective. It is possible that histone forms aggregates in tissue at higher concentrations and is therefore less effective against bacteria. A further explanation may be that the disruption of the bacterial cell wall induced by histone H1.2 is also concentration dependent and decreases at higher
concentrations when it binds, but also stabilizes the bacterial cell wall. Further studies are needed to gain a better understanding of the concentration dependency of the microbial activity of histone H1.2.

Another interesting property of histone H1.2 is its ability to bind bacterial lipopolysaccharide, which stimulates the production of TNF-α, IL-6 and other inflammatory mediators. Therefore, histones may function as immune modulators to prevent fatal septic shock. Histones exert an approximately two-fold stronger antimicrobial activity at pH 5.6 than at pH 7.34 This property could contribute to the development of histone’s activity in inflamed tissues with a lower pH compared with healthy tissues.

The mechanism of histone H1.2 killing of bacteria has yet to be investigated in detail. Both histone H1 and most of the known antimicrobial peptides are positively charged polycationic peptides, containing an amphipathic structure. The structure of histone H1, which provides potent binding to DNA, could be responsible for additional binding to bacterial membranes. On the other hand, antimicrobial peptides, which are able to associate with bacterial membranes, can bind to DNA: Yonezawa et al. demonstrated the binding of antimicrobial peptide tachyplesin I to DNA. Thus, we hypothesize that histone H1 and antimicrobial peptides exhibit a similar mechanism of action. Antimicrobial peptides associate with the negatively charged microbial membrane, mediated by their cationic hydrophilic part, and form transmembrane ion-permeable channels with their apolar part. These channels lead to the breakdown of the microorganism by the collapse of membrane potential, the increase in permeability and the loss of ions.

The results of this study reinforce the assumption that human histone H1.2 has been involved in protecting mammals against infection for millions of years. In the near future, histone H1.2 could also be used in the therapy of wound infections as an alternative or adjunct to known antibiotics. Because of its minor haemolytic effect it may also be a potential substrate for systematic application. However, particularly with regard to the cytotoxicity observed against primary human keratinocytes in vitro, further studies are clearly needed to better define the therapeutic potential of histone H1.2.

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