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Diluted sodium hypochlorite (bleach) in dogs: antiseptic efficacy, local tolerability and *in vitro* effect on skin barrier function and inflammation

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Background – Diluted sodium hypochlorite represents an inexpensive and widely available topical antiseptic, but there are no tolerability and efficacy data in veterinary dermatology.

Objectives – To determine the *in vivo* antibacterial effect and tolerability of topical diluted bleach application and to assess its *in vitro* effect on skin barrier lipids and anti-inflammatory properties on keratinocytes.

Methods – Topical hypochlorite at 0.05% and tap water were applied to both sides of the thorax of four healthy dogs. The anti-inflammatory effect on canine keratinocytes was determined by real-time polymerase chain reaction; skin barrier integrity was assessed by evaluating stratum corneum lipid changes in canine stratified epidermal constructs.

Results – The cell viability of primary keratinocytes treated with water and diluted hypochlorite at 0.005 and 0.01%, reduced the percentage of viable cells by 10%. The exposure of primary keratinocytes to 0.005% diluted hypochlorite significantly reduced the induction of inflammatory genes chemokine ligand-2 (CCL2; $P = 0.015$) and thymus and activation-regulated chemokine (TARC/CCL17, $P = 0.032$). There were no changes in skin lipid ceramide and nonceramide fractions in stratified epidermal constructs cultured for 17 days with 0.05% hypochlorite. Topical hypochlorite at 0.05% and tap water were well-tolerated without signs of skin irritation. Although a marked reduction in bacterial counts was seen within 20 min of diluted bleach application compared to the tap water control, this was only marginally significant ($P = 0.06$).

Conclusions and clinical importance – The results indicate that a topical diluted bleach solution, at either 0.05 or 0.005% hypochlorite concentrations, is a well-tolerated antiseptic that also exhibits anti-inflammatory properties.

Introduction

With the emergence of multidrug-resistant bacteria, antiseptics have gained popularity as an alternative to antibiotics. Diluted bleach (sodium hypochlorite, hereafter referred to as hypochlorite) represents an inexpensive and widely available topical antiseptic. It is commonly used as part of the treatment regimens for recurrent skin and soft tissue infections in human dermatology, with recommended therapeutic concentrations varying between 0.005 and 0.016% of hypochlorite.[1,2] For the treatment of skin infections due to meticillin-resistant *Staphylococcus aureus* (MRSA), household bleach (8.15% sodium hypochlorite) diluted to 0.008% hypochlorite has been recommended for application for 15 min twice weekly.[1] One review proposed use of 0.016% hypochlorite for the treatment of human patients with atopic dermatitis and recurrent MRSA skin infections.[3] Dilute bleach baths (approximate concentration of 0.005% hypochlorite) have been shown to remarkably reduce the severity of infected atopic dermatitis (AD) in children.[4]

Dogs with AD frequently develop recurrent staphylococcal infections, particularly due to *Staphylococcus pseudintermedius*,[5] topical therapy using antimicrobial shampoos remains an essential component in the long-

Abbreviations: AD, atopic dermatitis; cfu, colony forming unit; FBS, fetal bovine serum; MRSA, meticillin-resistant Staphylococcus aureus; RT-PCR, real-time PCR; TARC, thymus and activation-regulated chemokine; TNF-α, tumour necrosis factor-alpha.

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term management of AD in dogs.[6] The emergence of multidrug-resistant *S. pseudintermedius* has furthered the interest in targeted topical antimicrobial therapy, as sole or adjuvant therapy, in dogs with bacterial skin infections.[7] In one review on the therapeutic approach for canine superficial pyoderma, the application of 0.06–0.12% diluted hypochlorite solution, two to four times weekly, as an adjunctive topical therapy for this disease was recommended; however, the dilution range appeared solely based on personal clinical experience.[8] Although there is evidence of diluted bleach efficacy against common canine pathogenic micro-organisms affecting the skin *in vitro*,[9,10] there are, to the best of the authors' knowledge, no objective studies using diluted bleach solutions as antiseptics in veterinary dermatology.

A study of acute radiation dermatitis revealed the anti-inflammatory properties of topical diluted bleach (0.005% hypochlorite).[11] *In vitro* testing showed the reversible attenuation of tumour necrosis factor-alpha (TNF-α)-stimulated nuclear factor-kappa B (NF-κB)-dependent gene induction, such as chemokine ligand-2 (*CCL2*) and superoxide dismutase 2 (*SOD2),* in primary human keratinocytes exposed to diluted bleach.[11]

The primary goals of this study were to determine the optimal concentration of topical diluted bleach for antibacterial effect on healthy dog skin, and to assess the clinical tolerance by monitoring the effect of diluted bleach on the skin itself. In addition, as diluted bleach is reported to exhibit anti-inflammatory properties, the effect of diluted bleach on primary canine keratinocytes stimulated with pro-inflammatory cytokines was determined by quantitative real-time PCR (RT-qPCR). Finally, we evaluated the effect of hypochlorite on skin barrier integrity by assessing stratum corneum lipid changes in a canine epidermal keratinocyte progenitor cell line (CPEK) that forms a stratified epidermal structure.

Material and methods

Diluted bleach effect on primary keratinocyte cell viability

Preparation of cells

Canine primary keratinocytes were isolated and cultured as described previously, with slight modifications (Supporting Information Materials and Methods).[12] Each well of a prepared 96 well culture plate (Thermo Fischer Scientific; Waltham, MA, USA) was inoculated with 3×10^4 primary keratinocytes in 100 μL of medium. Plates were incubated at 37°C with 5% CO_2 for 5 h to allow the cells to have time to attach to the wells. Then, the medium in each well was replaced with 100 μL of maintenance medium, 10% fetal bovine serum (FBS). The plates were incubated and at 37°C with 5% CO_2.

Diluted bleach treatment

Treatment consisted of diluted hypochlorite in a sterile-filtered maintenance medium at concentrations of 0.0025, 0.005, 0.05, 0.1 and 0.5%; the untreated medium served as a negative control. The treatment concentrations were selected to be consistent with the proposed concentrations used in AD in children.[1–4] After keratinocytes reached 80% confluence in the 96 well culture plates, 100 μL of each treatment was added to each designated well. Control wells were treated with 100 μL of maintenance medium without any bleach. The plates were then incubated for 24 h at 37°C with 5% CO_2. All treatments were run in triplicate with three independent experiments performed.

Cell viability assay

The reduction of tetrazolium salt, MTS [3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium], was used as a parameter for cytotoxicity assessment as described previously (Supporting Information, Materials and Methods).[13] Cells that were treated with the medium only served as the controls for 100% viability (positive control) and cells that were treated with the lactate dehydrogenase (LDH) lysis buffer solution served as the controls for 0% viability (negative control). Cell viability results for the cells in each of the treated wells were calculated as the percentage of light absorbance compared with that for the positive control. All treatments were run in triplicate with three independent experiments performed; for each treatment the mean cell viability was calculated.

Effects on chemokine ligand-2 (CCL2) and thymus and activation-regulated chemokine (TARC)/CCL17 mRNA expression in primary keratinocytes after stimulation with TNF-α

Chemokine ligand-2 represents a typical NF-κB-dependent inflammatory gene;[11] CCL17 is secreted by epidermal keratinocytes and plays an important role in AD of humans and dogs.[14,15] The primary keratinocyte cultures were transferred into 24 well plates (Thermo Fischer Scientific) at a density of approximately 1×10^5/well and subcultured until 80% confluence in maintenance medium (10% FBS). All cells at around 80% confluence were starved for 12 h with medium containing 1% FBS and then exposed to diluted sodium hypochlorite at the concentration of 0.005% for 1 h. The cells treated with the water in medium and medium alone served as controls.

Following exposure, treatments were removed and cells were stimulated with canine recombinant TNF-α (R&D systems; Minneapolis, MN, USA) at a concentration of 10 ng/mL in maintenance medium for 3 h and 6 h. Recombinant TNF-α has been shown to induce gene expression of CCL2 and CCL17 in keratinocytes[11,16]; the concentrations of diluted hypochlorite, TNF-α cytokine and exposure times were based on previous canine[16] and human studies.[11] Each experiment was repeated on three separate occasions and all treatments were run in triplicates.

Total RNA was isolated from the cultured primary keratinocytes using the RNeasy Mini Kit (Qiagen; Valencia, CA, USA) according to the manufacturer's manual and the quantification of CCL2 and CCL17 mRNA by real-time PCR was performed as described previously.[16] Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a reference gene.[16] The primers sequences for CCL2, CCL17 and GAPDH were designed as previously described (Supporting Information Table S1).[16,17] We selected GAPDH as the internal control, because the transcription of GAPDH was not affected by TNF-α treatment as shown previously.[16]

Alteration of skin lipids in a CPEK line cultured at the air–liquid interface

For the purpose of evaluation of the diluted bleach effect on skin lipids, canine epidermal (CPEK) cells in medium (CnT-09, CELLnTEC Advanced Cell Systems; Bern, Switzerland) were cultured as described previously with minor modifications.[18] Briefly, CPEK cells were maintained in medium for seven days and then exposed to air, which led to stratification of the cells and formation of an enucleated cell layer (stratum corneum-like layer) by Day 17.[18] The cells were exposed to 0.05% diluted hypochlorite and a commercial 0.11% hypochlorous acid (HOCl) product (Vetericyn VF, Innovacyn; Rialto, CA, USA); cell cultures with water only served as controls. Although 0.05% diluted hypochlorite induced *in vitro* reduction in cell viability of primary nonstratified keratinocytes, we chose to test this higher concentration directly on a stratified CPEK epidermal construct in order to evaluate the tolerability of higher concentration for the purpose of topical application in a clinical setting. Lipids were extracted on Day 17. For this, 3 mL ethyl-acetate/methanol (20:80) were added for 1 h. The supernatant was removed into another vial and 3 mL chloroform/methanol (2:1) were applied onto the construct for 1 h.

Afterwards, the supernatant was removed as well and was dried under liquid nitrogen together with the first supernatant. Chloroform/methanol (2:1) was used to dissolve the lipids for lipid extraction analysis according to a study published previously.[19] The following lipids were quantified: cholesterol sulfate, galactocerebrosides, ceramide AP, ceramide AS, ceramide NP, ceramide NS, ceramide EOS, cholesterol, free fatty acids, triglycerides and cholesterol ester.

Antimicrobial effect of the topical diluted bleach on skin of healthy dogs

Study subjects

Four atopic, neutered male, Maltese terrier–beagle cross-bred dogs without clinical skin lesions were entered in this study; their age was 6.5 years. Withdrawal times from previous medications were two weeks for systemic and/or topical antibiotics, antifungals, nonsteroidal anti-inflammatory medications and topical (skin and ear) and oral glucocorticoids. None of the dogs were bathed with any type of shampoo for two weeks before and during the study. This study was approved by the NCSU Institutional Animal Care and Use Committee.

Testing products

The sodium hypochlorite formulation [Chlorox regular bleach (8.15% sodium hypochlorite), The Clorox Company; Oakland, CA, USA) was diluted with sterile water to a final concentration of 0.05% hypochlorite. We elected to test this higher hypochlorite concentration directly on the normal skin of atopic dogs outside an active AD flare, because it did not induce significant ceramide changes in the CPEK-derived epidermal construct and we wished to better approximate the concentration proposed in a review on the therapeutic approach to canine pyoderma.[8] Furthermore, a stronger *in vitro* antimicrobial effectiveness of diluted sodium hypochlorite against common canine pathogenic micro-organisms affecting the skin has been reported at higher concentrations.[9,10] Sterile water was used as a negative control. All testing products were freshly prepared for each application.

Intervention

In all dogs, both sides of the dorsal thorax (left and right side of the body) were clipped and divided into five areas corresponding to the size of a contact plate. To minimize any traumatic effect on the skin, clipping was performed two to three days before test product application, and different disinfected blades were used for each dog for clipping. The contact plate areas were marked with a black marker on the skin. The five test areas on both sides of the body were defined as test area Dog X 1a/1b to 5a/5b counting cranio-caudally. The test areas 2a, 3a, 4a and 5a on the right side of the thorax were treated with diluted bleach, whereas areas 2b, 3b, 4b and 5b on the left side of the thorax were soaked with water. All testing products were applied using a Professional Compounding Centers of America (PCCA) Bottle spray 2 oz. (PCCA; Houston, TX, USA), calibrated to deliver 0.8 mL per actuation. To ensure the appropriate distribution of the solution onto the relevant skin areas, the products (diluted bleach and water) were applied to five different sites within the marked testing area in a standardized manner: a full trigger pressed at a distance of 5 cm from the skin (a total of 4 mL) was applied per each 20 cm^2 testing area. To prevent the dissemination of droplets between testing areas, adjacent areas were protected with sterile diaper pads; different diaper pads were used between dogs. The sprayed areas were allowed to dry passively (around 3–5 min) as assessed by visual inspection.

Plating method sampling

Contact plates with neutralization medium (Rodac contact plates with Dey/Engley neutralization medium, BD; Sparks Glencoe, MD, USA) were pressed directly on the skin of the treated areas for 15 s at the time. This neutralization medium had been shown to deactivate a broad range of antiseptic and disinfectant chemicals, including chlorine preparations.[20] The validated neutralization step was included because the antiseptic (here, diluted bleach) could continue to damage bacterial cells after plate sampling, thereby leading to an overestimation of the antiseptic antimicrobial efficacy.[20] Because *S. pseudintermedius* is the most common pathogen isolated from the dog's skin, the ability of varying *S. pseudintermedius* strains to grow on the contact agar plates with neutralization medium was determined prior to use on dogs. Each test area was sampled only once, because the neutralization agar residue after contact with the skin may deactivate and influence any remaining sodium hypochlorite antimicrobial activity. For baseline values, we collected contact plate samples from the dogs at test areas 1a/1b (baseline values pre-application) 10 min before applying the tested products. After 10 min, test areas 2a, 3a, 4a and 5a were sprayed with diluted bleach and 2b, 3b, 4b and 5b with tap water. Contact plate samples from bleach- and water-treated areas were collected 30 min (test area 2a/2b), 24 h (test area 3a/3b), three days (test area 4a/4b) and seven days (test area 5a/5b) after application. After the final application, the content of each spray bottle used for each dog was sprayed directly on the agar to verify their sterility.

Enumeration of microbial reduction and bacterial isolation

After incubation of the plates at 37°C and 5% CO_2 for 24 h, all colonies present on the plates were counted manually twice by the same investigator who was blinded to all treatments. The mean number of colony forming units (cfu) per plate (20 cm^2) was recorded and converted to $\log_{10}$ cfu/cm^2.[21]

In order to evaluate the effect of treatment on the diversity of bacterial population, ten morphologically different colonies per contact plate were selected and streaked for isolation onto blood agar plates. For consistency purposes, up to two morphologically different colonies were picked always from the five identical quadrants of each plate (centre, upper left and right, lower left and right), transferred to blood agar plates, incubated at 37°C overnight and stored at −80°C using preservation vials for further identification.

Bacterial species identification

Isolated colonies recovered from contact plates were subjected to matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) according to the manufacturer's protocol. The extraction method was performed as described previously on overnight colonies grown on blood agar at 37°C.[22] The data were analysed by the software programme (VITEK MS system, bioMérieux, Marcy-l'Étoile, France); the mass peak profiles were matched to the reference database and a score generated based on similarity.

Local tolerability assessment

Local tolerability assessment was performed for each test area by an investigator immediately after the test product application and at the same time when contact plates were sampled. Erythema and scaling were each evaluated using a four point rating scale (0 none, 1 mild, 2 moderate, 3 severe).

Outcome measures for antimicrobial efficacy and local tolerability

Our primary outcome measure was the comparison of antiseptic efficacy (i.e. a reduction in cfu) between diluted bleach at 0.05% concentration and control areas. Secondary outcome measures of interest included the comparison of residual activity between diluted bleach at different concentrations and the grading of local tolerability (lesional score) of the test products.

Statistical analysis

All statistical analysis was performed using Graphpad Prism v6.0 (Graphpad Inc.; San Diego, CA, USA). All results for cell viability were reported as the percentage of the respective results for controls. For mRNA analysis we used the double delta Ct analysis (or $\Delta\Delta$Ct method). All samples were examined in duplicate and the mean value of Ct was calculated. Each PCR reaction included a no template control with sterile distilled water instead of cDNA templates to test for contamination of assay reagents or primer dimers. The

expression of CCL2 and CCL17 in primary keratinocytes was compared among treatments by means of ANOVA followed by a Dunnett's multicomparison *post hoc* test. Logs of the total bacterial count (log_{10} cfu/cm^2) were compared between sampling times (pre-application, post-application: 20 min, 24 h, three and seven days) using the Friedman rank sum test with Dunn's multiple comparisons and the Wilcoxon signed rank test. Use of nonparametric procedures was necessary because the assumption of normality was not satisfied. For all statistical analyses, a value of $P < 0.05$ was considered statistically significant.

Results

Cell viability in primary keratinocytes

The viability (expressed as a percentage of that of untreated control cells) of cells treated with diluted bleach at each of the six concentrations (0.0025, 0.005, 0.01, 0.05, 0.1 and 0.5%) and water is summarized in Figure 1. Following a 24 h incubation, the viability of keratinocytes treated with diluted bleach at the concentrations 0.5% ($P = 0.0018$), 0.01% ($P = 0.0008$) and 0.05% ($P = 0.0013$) differed significantly from that of untreated control cells, and it was identical to that of cells treated with the LDH lysis ($P = 0.0004$). Treatment with water and diluted bleach at 0.0025, 0.005 and 0.01% reduced the percentage of viable cells by 10%, but this difference was not found to be significant. As a result, the mid-concentration of 0.005% was selected for the subsequent *in vitro* studies.

The effects of TNF-α on CCL2 and CCL17 mRNA transcription

As shown in Figure 2a, the transcription level of *CCL2* mRNA in primary keratinocytes exposed to media only was significantly augmented after addition of recombinant canine TNF-α stimulation. The exposure of keratinocytes to 0.005% diluted hypochlorite 1 h prior to TNF-α stimulation significantly reduced the induction of *CCL2* at 3 h ($P = 0.002$) and 6 h ($P = 0.015$). Surprisingly, sterile water in media also reduced the induction of *CCL2* at 3 h ($P = 0.047$) but not at 6 h ($P = 0.071$). After TNF-α stimulation, the transcription level of *CCL17* mRNA increased five-fold at 6 h compared to the unstimulated keratinocytes (Figure 2b). Treatments with diluted hypochlorite decreased *CCL17* mRNA expression only at 6 h ($P = 0.032$); all other treatments did not significantly influence *CCL17* expression at both time points. Altogether, these results suggest that a 0.005% hypochlorite concentration reduces the transcriptions of two chemokine genes affected by the transcription factor NF-κB.

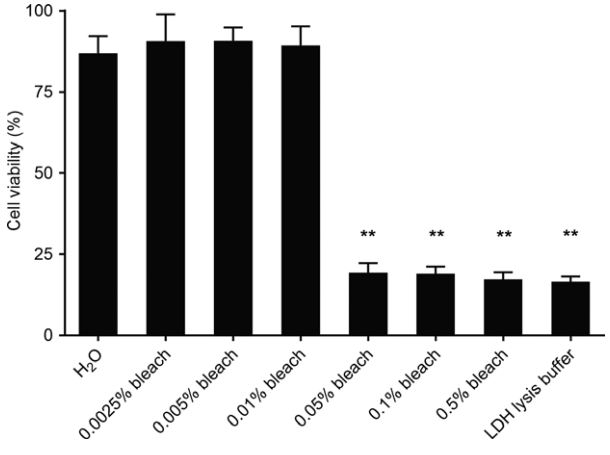

Figure 1. Effect on primary keratinocytes cell viability after treatment for 24 h with different concentrations of the diluted bleach (0.0025, 0.005, 0.01, 0.05, 0.1 or 0.5%) and tap water.
Viability/proliferation was evaluated by MTS assay and compared to control. Results are expressed as means ± S.D. of three independent experiments. The significant differences were determined by Dunnett multiple comparison test following one-way ANOVA (**$P < 0.01$).

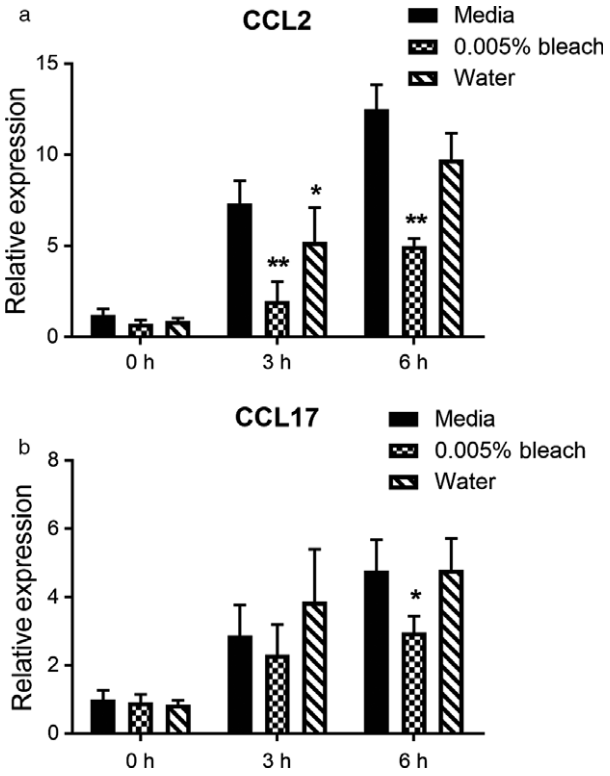

Figure 2. The inhibitory effects of diluted bleach on CCL2 and CCL17 mRNA transcription in primary keratinocytes after stimulation with TNF-α.
Primary keratinocytes were cultured with medium alone or with 10 ng/mL each of TNF-α for 3 h and 6 h. The transcription levels of CCL17 mRNA were measured by real-time PCR. The relative transcription levels of CCL17 mRNA were corrected with GAPDH and then compared with an unstimulated control sample. The results represent the means ± S.D. of three independent experiments (**$P < 0.01$, *$P < 0.05$).

Skin lipids changes in the CPEK cell line

After 17 days of culture of the epidermal construct there were no changes in skin lipids, either in the ceramide or nonceramide fractions, between epidermis cultured with 0.05% hypochlorite and 0.11% hypochlorous acid (Veterycin) compared to controls. (Figure 3; nonceramide data not shown). There were nonsignificant reductions in ceramide AP, ceramide NP and galactocerebroside.

Antimicrobial effect of topical diluted bleach application

The efficacy of topical diluted bleach at 0.05% and tap water on skin bacteria are summarized in Table 1. The control plates inoculated with diluted bleach and tap water spray did not reveal any bacterial growth, thereby

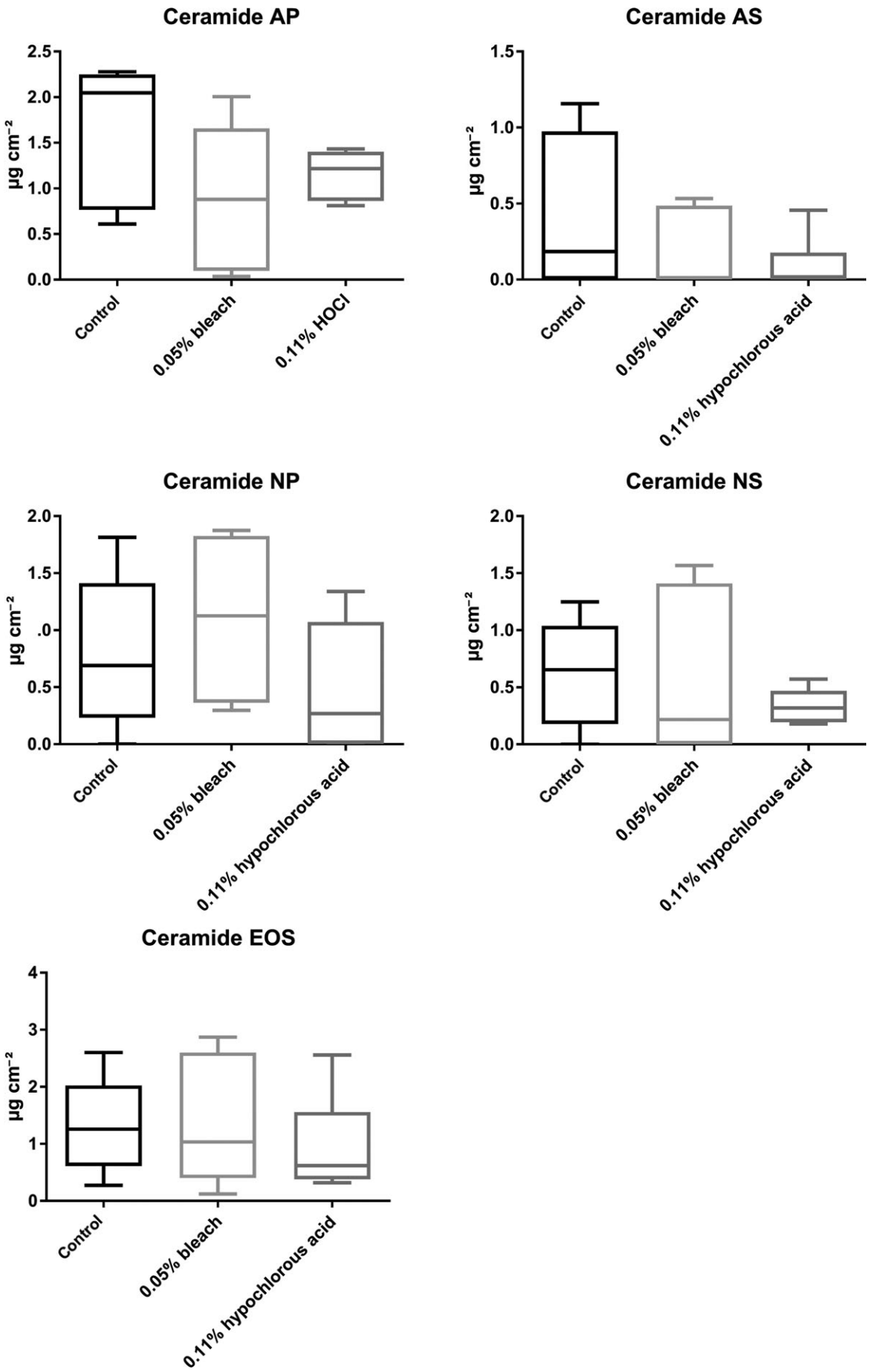

Figure 3. Effect of diluted sodium hypochlorite at 0.05% concentration and hypochlorous acid 0.011% on the surface concentration (micrograms per square cm) for selected skin ceramides of stratified epidermal construct in CPEK cultured keratinocytes.

Table 1. Efficacy of diluted bleach and tap water in reducing or eliminating skin bacteria in four dogs

	Number of cfus				
	Before treatment	20 min after application	24 h after application	Three days after application	Seven days after application
Diluted bleach	108 (16–512)	35 (1–182)	69 (24–201)	58 (8–216)	78 (4–326)
Tap water	123 (15–219)	132 (9–295)	146 (12–246)	216 (17–500)	191 (25–500)

Results expressed as median (range) of colony forming units (cfus) at different sampling times.

ensuring that the application of sprays could not have caused any bacterial contamination of treated sites. At the baseline pre-treatment skin areas, bacterial counts ranged from 16 to 512 cfus (median 108, mean log count 1.87 for diluted bleach side and 15–219 cfus (median 123, mean log count 1.92) at the tap water side; this difference was not significant ($P = 0.07$). The effect of diluted bleach and tap water on post-application bacterial cfus for each dog is shown in Figure 4. There was no significant difference in the total log count of bacteria cultured before and after treatment application for both diluted bleach (20 min versus baseline $P = 0.24$; 24 h versus baseline $P = 0.99$; three days versus baseline $P = 0.99$; seven days versus baseline $P = 0.99$) and the tap water group (20 min versus baseline $P = 0.99$; 24 h versus baseline $P = 0.99$; three days versus baseline $P = 0.99$; seven days versus baseline $P = 0.99$), respectively. Although a marked reduction in bacterial counts was seen within 20 min of diluted bleach application compared to the tap water control, this was not significantly different (Figure S1; $P = 0.06$). Diluted bleach reduced the cfu number in all dogs with a complete elimination of skin bacteria occurring in a single dog (Dog 4), whereas the number of cfus increased after tap water application in two dogs (dogs 1 and 2).

Table 2 summarizes the prevalence of the most common bacteria isolated at the various sampling stages during the study. Six additional species of bacteria were isolated on one or two occasions. The sampling method of up to ten morphologically different colonies per plate yielded numerous coagulase-negative *Staphylococcus* (CoNS) and *Enterococcus* species in both bleach- and tap water-treated skin areas. Among the CoNS, *S. cohnii* ssp. *urealyticus* and *S. epidermidis* were the predominant species. A total of five *S. pseudintermedius* isolates were identified in water-treated skin areas at four different sampling times (20 min, 24 h, three and seven days) from different dogs (dogs 1, 2, 4) whereas only a single isolate was cultured from any of the topical bleach areas (20 min; Dog 4).

Local tolerability assessment

There was no development of skin erythema or scaling after application of diluted bleach or water on the thorax of any dogs (scores of "0" at all times). After clipping, a small erythematous ventral area that extended beyond the contact plate sampling was noted before diluted bleach application on the right cranial sampling area 1a in one dog. Spraying with diluted bleach did not cause any immediate skin irritation or hypersensitivity and the erythematous skin area resolved over the following days.

Discussion

The *in vivo* part of the present study demonstrated the antibacterial efficacy and safety of diluted bleach for use in dogs. A single application of topical hypochlorite at 0.05% acutely reduced bacterial numbers on the skin of dogs without signs of skin irritation; however, the reduction was only marginally significant ($P = 0.06$), which might be explained by the small number of dogs tested. Furthermore, in this study dilute hypochlorite was not rinsed with water as recommended in real life and, despite our methodology having a higher potential for

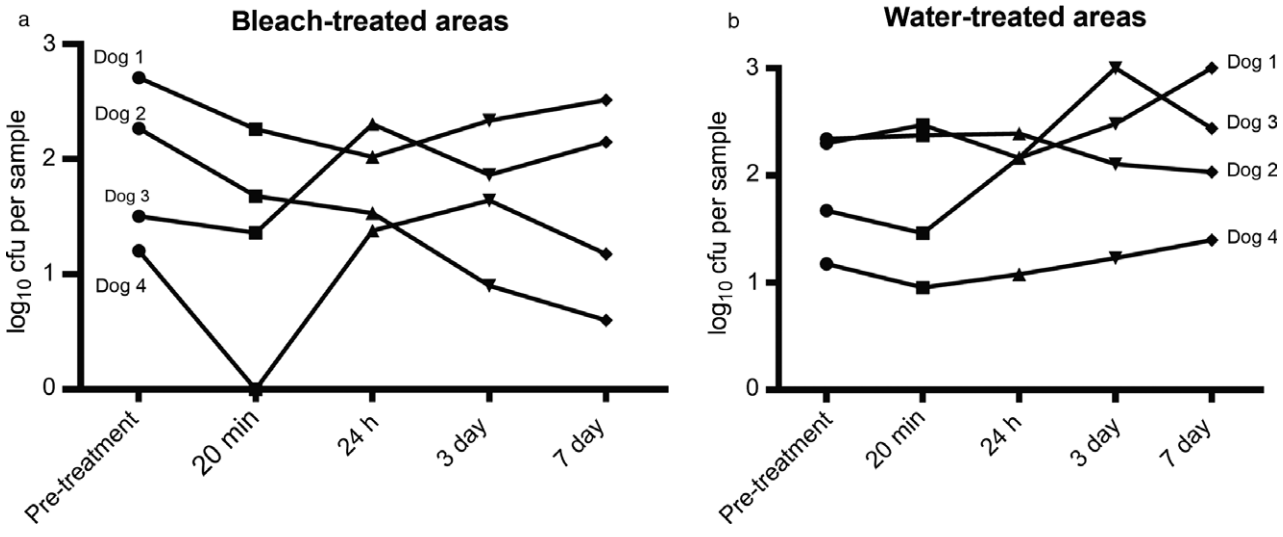

Figure 4. Mean log counts of bacterial growth on plates expressed as $\log_{10}$ cfu/cm^2 by treatment group, bleach (a) and tap water (b), at pre-treatment and at 20 min, 24 h, three and seven day sampling points.

Table 2. Prevalence of the most common bacteria isolated at various sampling times after topical skin application with diluted bleach solution and tap water in four dogs

	Sampling time									
Bacterium identification	Bleach Baseline	Water Baseline	Bleach 20 min	Water 20 min	Bleach 24 h	Water 24 h	Bleach 3 day	Water 3 day	Bleach 7 day	Water 7 day
Staphylococcus pseudintermedius			1 (7%)	4 (14%)		1 (4%)		5 (26%)		3 (9%)
Coagulase-negative *Staphylococcus* spp.	12 (48%)	6 (30%)	7 (53%)	11 (40%)	11 (47%)	10 (40%)	8 (36%)	6 (31%)	10 (40%)	19 (55%)
Bacillus spp.					1 (4%)	1 (4%)		3 (15%)		1 (2%)
Acinetobacter spp.		4 (20%)	3 (23%)		3 (13%)	3 (12%)	3 (13%)		1 (4%)	
Enterococcus spp.	12 (48%)	7 (35%)	1 (7%)	2 (7%)	4 (17%)	8 (32%)	3 (13%)		2 (8%)	1 (2%)

irritation, the applications of dilute hypochlorite on the multiple skin areas were well-tolerated in all dogs. An acute reduction in bacterial colonies using contact plates was not observed at 24 h, supporting the evidence that the short-term use of skin antiseptics might not have a noticeable long-term effect on the composition and abundance of the bacterial microbiome.[23]

Limited data are available on the microbiological efficacy of dilute bleach on methicillin-susceptible (MSSP) and -resistant *S. pseudintermedius* (MRSP).[9,10] One study recommended a 15 min exposure of household bleach dilution at 0.19% for killing MRSP isolates.[9] This recommendation suggests that higher hypochlorite concentrations are necessary for killing MRSP,[9] in comparison to MRSA isolates where isolates were killed after *in vitro* incubation with diluted bleach concentration at 0.06%.[24,25] The reason for the discrepancy in susceptibility between MRSP and MRSA may be explained by the modified *in vitro* broth microdilution method performed. A 10 times higher standardized bacterial MRSP inoculum (1 mL) was applied to hypochlorite wells (100 μL) diluting the hypochlorite 1:10 in the wells.[9] The Clinical and Laboratory Standards Institute guidance recommends adding an equal amount (e.g. 100 μL) of standardized bacterial inoculum and the antimicrobial tested (e.g. 100 μL) to achieve a 1:2 dilution of each antimicrobial concentration and a 1:2 dilution of the inoculum for broth microdilution antimicrobial evaluation.[26]

In the *in vivo* part of our study, the dilute hypochlorite at 0.05% was sprayed directly on the skin and this led to a contact time of a few minutes before completely drying. Although dogs could have licked those areas, none developed any sign of oral toxicity. Oral toxicity is a matter of debate because at 0.005% hypochlorite resembles chlorinated pools and dogs swim in certain households daily without any toxicity; at higher concentrations of hypochlorite, such as 0.05%, the hypochlorite application on dogs should be followed by a normal shampoo or a moisturizing mousse. Interestingly, *S. pseudintermedius* was isolated frequently from tap water-treated areas whereas only one isolate was found from plates applied to hypochlorite areas. This observation suggests that, even though the entire bacterial population might not have decreased after hypochlorite contact, this antiseptic might have a more specific anti-staphylococcal effect.

Rare adverse effects limited to mild itching and skin dryness have been reported after repeated bleach baths in humans. Bleach contains small amount of sodium hydroxide that has been shown to affect skin barrier integrity by increasing transepidermal water loss.[27] Ceramides, essential stratum corneum lipids and intercellular adhesion molecules are crucial for maintaining an effective skin barrier function. Changes in ceramide homeostasis generally lead to increased transepidermal loss and decreased barrier function, particularly in human patients with AD.[28] The CPEK cells cultured at the air–liquid interface after 12 days became stratified and formed a stratum corneum-like layer; this organotypic culture construct was used to investigate changes in barrier function of the stratum corneum in dogs.[18] During the *in vitro* work of the present study, the 0.11% hypochlorous acid and 0.05% hypochlorite solutions had no effect on skin lipid ceramide or nonceramide fractions of the epidermal construct, and the topical application of the 0.05% hypochlorite solution on the skin did not induce any visible skin scaling. To allow for a better understanding of the effect on skin barrier integrity, future studies should assess stratum corneum ceramide content of the skin after long-term use of twice weekly bleach baths at a contact time of 15 min.

A study evaluating acute radiation dermatitis revealed the anti-inflammatory properties of topical diluted bleach baths (0.005% hypochlorite);[11] this intervention attenuated TNF-α-stimulated NF-κB-dependent gene induction and markedly ameliorated the severity of acute radiation-induced dermatitis in a mouse model. Furthermore, the use of bleach baths prevented skin ulceration in comparison to tap water-treated mice. Application of dilute hypochlorite inhibited the expression of two inflammatory chemokine genes, CCL2 and CCL17, in primary canine keratinocytes after stimulation with proinflammatory cytokine TNF-α. Thymus and activation-regulated chemokine/CCL17 is a key chemokine involved in lymphocyte migration to skin with chemotactic activity specific to type 2 helper T (Th2) cells. TARC is overexpressed in the lesional skin of humans and dogs affected with AD and a meta-analysis revealed that serum CCL17 appeared to be the most reliable biomarker of AD.[14,15,29] Previous observations in an acute radiation dermatitis model, as well as results in this study, suggest that diluted bleach could be of value beyond that of an antiseptic in other skin diseases involving the NF-κB pathway, such as AD.

In conclusion, we report that topical diluted bleach solutions, at 0.05 and 0.005% hypochlorite concentrations, are well-tolerated antiseptics that exhibit anti-inflammatory properties. Interestingly, during the bleach treatment

the coagulase-negative staphylococci predominated the microbial contact plates, implicating a change in skin microbiome. These observations warrant additional controlled safety, efficacy and skin microbiome studies using inexpensive diluted bleach baths, at 0.05 and 0.005% concentration for a 10–15 min duration, as antiseptics. This treatment modality should be investigated not only for superficial bacterial pyoderma, but also for dogs with *S. pseudintermedius*-colonized AD.

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References

1. Liu C, Bayer A, Cosgrove SE et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children: executive summary. *Clin Infect Dis* 2011; 52: 285–292.
2. Fritz SA, Camins BC, Eisenstein KA et al. Effectiveness of measures to eradicate *Staphylococcus aureus* carriage in patients with community-associated skin and soft-tissue infections: a randomized trial. *Infect Control Hosp Epidemiol* 2011; 32: 872–880.
3. Ong PY. Recurrent MRSA skin infections in atopic dermatitis. *J Allergy Clin Immunol Pract* 2014; 2: 396–399.
4. Huang JT, Rademaker A, Paller AS. Dilute bleach baths for *Staphylococcus aureus* colonization in atopic dermatitis to decrease disease severity. *Arch Dermatol* 2011; 147: 246–247.
5. Bradley CW, Morris DO, Rankin SC et al. Longitudinal evaluation of the skin microbiome and association with microenvironment and treatment in canine atopic dermatitis. *J Invest Dermatol* 2016; 136: 1,182–1,190.
6. Olivry T, DeBoer DJ, Favrot C et al. Treatment of canine atopic dermatitis: 2010 clinical practice guidelines from the International Task Force on Canine Atopic Dermatitis. *Vet Dermatol* 2010; 21: 233–248.
7. Bryan J, Frank LA, Rohrbach BW et al. Treatment outcome of dogs with meticillin-resistant and meticillin-susceptible *Staphylococcus pseudintermedius* pyoderma. *Vet Dermatol* 2012; 23: 361–369.
8. Bloom P. Canine superficial bacterial folliculitis: current understanding of its etiology, diagnosis and treatment. *Vet J* 2014; 199: 217–222.
9. Pariser M, Gard S, Gram D et al. An *in vitro* study to determine the minimal bactericidal concentration of sodium hypochlorite (bleach) required to inhibit meticillin-resistant *Staphylococcus pseudintermedius* strains isolated from canine skin. *Vet Dermatol* 2013; 24: 632–634.
10. Banovic F, Lemo N. *In vitro* evaluation of the use of diluted sodium hypochlorite (bleach) *against Staphylococcus pseudintermedius*, *Pseudomonas aeruginosa* and *Malassezia pachydermatis*. *Vet Dermatol* 2014; 25: 233–234 (Letter).
11. Leung TH, Zhang LF, Wang J et al. Topical hypochlorite ameliorates NF-κB–mediated skin diseases in mice. *J Clin Invest* 2013; 123: 5,361–5,370.
12. Bäumer W, Kietzmann M. Effects of cyclosporin A and cilomilast on activated canine, murine and human keratinocytes. *Vet Dermatol* 2007; 18: 107–114.
13. LaDuke KE, Ehling S, Cullen JM et al. Effects of azathioprine, 6-mercaptopurine, and 6-thioguanine on canine primary hepatocytes. *Am J Vet Res* 2015; 76: 649–655.
14. Olivry T, Mayhew D, Paps JS et al. Early activation of Th2/Th22 inflammatory and pruritogenic pathways in acute canine atopic dermatitis skin lesions. *J Invest Dermatol* 2016; 136: 1,961–1,969.
15. Ungar B, Garcet S, Gonzalez J et al. An integrated model of atopic dermatitis biomarkers highlights the systemic nature of the disease. *J Invest Dermatol* 2017; 137: 603–613.
16. Shibata S, Maeda S, Maeda S et al. Augmentation of CCL17 and CCL28 gene expression by TNF-alpha, IL-1beta, or IFN-gamma in cultured canine keratinocytes. *Res Vet Sci* 2010; 88: 422–426.
17. Menezes-Souza D, Guerra-Sá R, Carneiro CM et al. Higher Expression of CCL2, CCL4, CCL5, CCL21, and CXCL8 Chemokines in the Skin Associated with Parasite Density in Canine Visceral Leishmaniasis. *PLoS Negl Trop Dis* 2012; 6: e1566.
18. Yagihara H, Okumura T, Shiomi E et al. Reconstruction of stratum corneum in organotypically cultured canine keratinocyte-derived CPEK cells. *Vet Res Commun* 2011; 35: 433–437.
19. Stahl J, Paps J, Bäumer W et al. *Dermatophagoides farinae* house dust mite allergen challenges reduce stratum corneum ceramides in an experimental dog model of acute atopic dermatitis. *Vet Dermatol* 2012; 23: 497–e97.
20. Reichel M, Heisig P, Kampf G. Pitfalls in efficacy testing-how important is the validation of neutralization of chlorhexidine digluconate? *Ann Clin Microbiol Antimicrob* 2008; 7: 20.
21. Bashir MH, Olson LK, Walters SA. Suppression of regrowth of normal skin flora under chlorhexidine gluconate dressings applied to chlorhexidine gluconate-prepped skin. *Am J Infect Control* 2012; 40: 344–348.
22. Decristophoris P, Fasola A, Benagli C et al. Identification of *Staphylococcus intermedius* group by MALDI-TOF MS. *Syst Appl Microbiol* 2011; 34: 45–51.
23. Two AM, Nakatsuji T, Kotol PF et al. The cutaneous microbiome and aspects of skin antimicrobial defense system resist acute treatment with topical skin cleansers. *J Invest Dermatol* 2016; 136: 1,950–1,954.
24. Fisher RG, Chain RL, Hair PS et al. Hypochlorite killing of community-associated methicillin-resistant *Staphylococcus aureus*. *Pediatr Infect Dis J* 2008; 27: 934–935.
25. Chang B, Nerandzic MM, Kundrapu S et al. Efficacy of dilute hypochlorite solutions and an electrochemically activated saline solution containing hypochlorous acid for disinfection of methicillin-resistant *Staphylococcus aureus* in a pig skin model. *Infect Control Hosp Epidemiol* 2013; 34: 1,231–1,233.
26. CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: approved standards. – 3rd ed. CLSI document M31-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
27. Fluhr JW, Bankova L, Fuchs S et al. Fruit acids and sodium hydroxide in the food industry and their combined effect with sodium lauryl sulphate: controlled *in vivo* tandem irritation study. *Br J Dermatol* 2004; 151: 1,039–1,048.
28. Chiang C, Eichenfield LF. Quantitative assessment of combination bathing and moisturizing regimens on skin hydration in atopic dermatitis. *Pediatr Dermatol* 2009; 26: 273–278.
29. Thijs J, Krastev T, Weidinger S et al. Biomarkers for atopic dermatitis: a systematic review and meta-analysis. *Curr Opin Allergy Clin Immunol* 2015; 15: 453–460.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Contact plates with colony forming units in Dog 2 at pre-treatment (a, d) and at post-treatment with bleach (20 min – b; 1 day – c) and tap water (20 min – e; 1 day – f).

Appendix S1. Materials and Methods

Résumé

Contexte – L'hypochlorite de sodium dilué est un antiseptique topique largement disponible et peu coûteux mais nous ne disposons pas de donnée de tolérance et d'efficacité en dermatologie vétérinaire.

Objectifs – Déterminer l'effet antibactérien *in vivo* et la tolérance de la javel diluée topique et déterminer ses effets *in vitro* sur les lipides de la barrière cutanée et les propriétés anti-inflammatoires sur les kératinocytes.

Méthodes – L'hypochlorite topique à 0,05% et l'eau du robinet ont été appliqués sur les deux côtés du thorax des chiens sains. L'effet anti-inflammatoire sur les kératinocytes canins a été déterminé par PCR en temps réel; l'intégrité de la barrière cutanée a été évaluée par estimation des modifications des lipides du *stratum corneum* sur les constructions épidermiques stratifiées.

Résultats – La viabilité cellulaire des kératinocytes primaires traités à l'eau et l'hypochlorite dilué à 0,005 et 0,01%, réduit le pourcentage de cellules viables de 10%. L'exposition des kératinocytes primaires à 0.005% d'hypochlorite dilué a diminué significativement l'induction des gènes inflammatoires CCL2 (chemokine ligand-2; $P = 0.015$) et TARC/CCL17 (activation-regulated chemokine, $P = 0.032$). Il n'y avait aucun changement dans les céramides cutanés et les fractions non-céramides des éléments de l'épiderme stratifié mis en culture pendant 17 jours à l'hypochlorite à 0,05%. L'hypochlorite topique à 0,05% et l'eau du robinet ont été bien toléré sans signe d'irritation cutanée. Bien qu'une diminution marquée des comptages bactériens a été vue en 20 minutes d'application de javel diluée comparé à l'eau du robinet, ceci n'était que marginalement significatif (P=0.06).

Conclusions et importance clinique – Les résultats indiquent qu'une application topique de javel diluée, soit à 0,05 soit à 0,005% de concentration d'hypochlorite, est un antiseptique bien toléré qui montre également des effets anti-inflammatoires.

RESUMEN

Introducción – El hipoclorito de sodio diluido representa un antiséptico tópico de bajo costo y ampliamente disponible, pero no hay datos de tolerabilidad y eficacia en dermatología veterinaria.

Objetivos – Determinar el efecto antibacteriano *in vivo* y la tolerabilidad de la aplicación tópica de lejía diluida y evaluar su efecto *in vitro* sobre los lípidos de la barrera cutánea y las propiedades antiinflamatorias de los queratinocitos.

Métodos – Se aplicaron hipoclorito tópico al 0,05% y agua del grifo a ambos lados del tórax de cuatro perros sanos. El efecto antiinflamatorio sobre los queratinocitos caninos se determinó mediante reacción en cadena de la polimerasa en tiempo real; Se evaluó la integridad de la barrera cutánea analizando los cambios en lípidos del estrato córneo en cultivos epidérmicos estratificados caninos.

Resultados – La viabilidad celular de los queratinocitos primarios tratados con agua y hipoclorito diluido a 0,005 y 0,01%, se redujo el porcentaje en un 10%. La exposición de los queratinocitos primarios al hipoclorito diluido al 0,005% redujo significativamente la inducción de los genes inflamatorios quemoquina ligando-2 (CCL2; $P = 0,015$) y quemoquina tímica regulada por activación (TARC / CCL17, $P = 0,032$). No hubo cambios en las fracciones de ceramida lipídica y de no ceramida en cultivos epidérmicos estratificados mantenidos durante 17 días con hipoclorito al 0,05%. El hipoclorito tópico al 0,05% y el agua del grifo fueron bien tolerados sin signos de irritación de la piel. Aunque se observó una marcada reducción de los recuentos bacterianos a los 20 minutos de la aplicación de la lejía diluida en comparación con el control de agua del grifo, esto fue sólo marginalmente significativo ($P = 0,06$).

Conclusiones e importancia clínica – Los resultados indican que una solución de lejía diluida tópica, a 0,05 o 0,005% de concentraciones de hipoclorito, es un antiséptico bien tolerado que también exhibe propiedades antiinflamatorias.

Zusammenfassung

Hintergrund – Verdünntes Natriumchlorid stellt ein billiges und weithin verfügbares Oberflächenantiseptikum dar. Es gibt dafür allerdings keine Toleranz- und Wirksamkeitsdaten in der Veterinärdermatologie.

Ziele – Eine Bestimmung der *in vivo* antibakteriellen Wirksamkeit und Toleranz der oberflächlichen Anwendung von verdünnter Chlorbleiche und ein Erfassen seiner *in vitro* Wirksamkeit auf die Lipide der Hautbarriere und seiner entzündungshemmenden Eigenschaften auf Keratinozyten.

Methoden – Topisches Hyochlorid bei einer Konzentration von 0,05% mit Leitungswasser wurde bei vier gesunden Hunden an beiden Seiten des Thorax aufgetragen. Der entzündungshemmende Effekt auf die Keratinozyten des Hundes wurde mittels Real-Time Polymerase Kettenreaktion bestimmt; die Integrität der Hautbarriere wurde durch eine Evaluierung der Lipidveränderungen des Stratum corneum in caninen geschichteten epidermalen Konstrukten erfasst.

Ergebnisse – Die Zellviabilität der primären Keratinozyten, die mit Wasser und verdünntem Hypochlorid bei einer Verdünnung von 0,005 und 0,01% behandelt wurden, reduzierte den Prozentsatz der lebensfähigen Zellen um 10%. Die Exponierung von primären Keratinozyten zu 0,005%igem verdünnten Hypochlorid reduzierte die Induktion des Entzündungsgens Chemokin Ligand-2 (CCL2; $P = 0,015$) sowie Thymus und

Activation-regulated Chemokin (TARC/CCL17, *P* = 0,032). Es bestanden keine Änderungen bei den Hautlipidceramiden und in den Nicht-Ceramid Fraktionen in den caninen geschichteten epidermalen Konstrukten, die 17 Tage lang mit 0,05% Hypochlorid kultiviert wurden. Topisches 0,05%iges Hypochlorid mit Leitungswasser wurde ohne Ausbildung von Hautirritationen gut vertragen. Obwohl im Vergleich zur Kontrolle, die aus Leitungswasser bestand eine deutliche Reduzierung der Bakterienzahl innerhalb von 20 Minuten nachdem die verdünnte Chlorbleiche aufgetragen worden war, eintrat, war dieses Ergebnis nur marginal signifikant (*P* = 0,06).

Schlussfolgerungen und klinische Bedeutung – Diese Ergebnisse weisen darauf hin, dass oberflächliche verdünnte Chlorbleichlösungen entweder bei einer Hypochloridkonzentration von 0,05% oder 0,005% eine gut tolerierte antiseptische Lösung darstellen, die auch entzündungshemmende Wirkung zeigen.

要約

背景 – 希釈次亜塩素酸ナトリウムは安価で広く入手可能な局所消毒剤であるが、獣医皮膚科領域において、許容性および有効性のデータはない。

目的 – 局所希釈漂白剤のin vivoにおける抗菌効果および許容性を評価すること、およびin vitroにおけるケラチノサイトに対する皮膚バリア脂質に対する効果および抗炎症効果を評価すること。

方法 – 4頭の健常犬の各胸側に局所次亜塩素酸塩0.05%あるいは水道水を塗布した。イヌケラチノサイトに対する抗炎症効果は、リアルタイムポリメラーゼ連鎖反応を用いて測定した。皮膚バリアの完全性は犬の層状化表皮構造における角質層の脂質変化によって評価した。

結果 – 水および0.005%あるいは0.01%で希釈した次亜塩素酸塩で処理した初代ケラチノサイトの細胞生存率は10%減少した。初代ケラチノサイトを0.005%希釈次亜塩素酸塩に曝露させると、炎症遺伝子のケモカインリガンド-2(CCL2; P = 0.015)および胸腺および活性化制御ケモカイン(TARC / CCL17; P = 0.032)の誘導が有意に減少した。0.05%次亜塩素酸塩で17日間培養した層状化表皮構造において、皮膚脂質セラミドおよび非セラミドに変化は認められなかった。0.05%局所次亜塩素酸塩および水道水に対する皮膚刺激は観察されず、良好な耐容性を示した。希釈漂白剤の塗布により、20分以内に細菌数の顕著な減少が見られたが、これは水道水コントロールと比較してわずかに有意であるのみであった(P = 0.06)。

結論および臨床的な重要性 – 本結果は、0.05または0.005%のいずれの希釈濃度においても、局所希釈漂白剤が良好な許容性を示し、抗炎症性も持つことを示唆している。

摘要

背景 — 稀释的次氯酸钠是一种廉价且被广泛使用的外部抗菌剂。但是,在兽医皮肤病学上,未见有关其耐受性和疗效的数据报道。

目的 — 外部使用稀释漂白剂,判定其抗菌效果和活体耐受性,同时,评估体外试验中对角质细胞上的皮肤屏障脂质和抗炎特性的影响。

方法 — 0.05%的外部次氯酸盐和自来水应用于四只健康犬的胸廓两侧。通过实时PCR检测对犬角质细胞的抗炎作用,通过评估犬表皮层状结构中角质层的脂质变化,来监测皮肤屏障的完整性。

结果 — 用自来水和浓度为0.005%和0.01%的稀释次氯酸盐处理原代角质细胞的细胞活性,活菌百分比降低了10%。原代角质细胞经0.005%稀释次氯酸盐作用后,能明显降低炎性基因趋化因子配体-2(CCL2; P = 0.015)和胸腺活化调节因子(TARC/CCL17, P = 0.032)的诱导。经0.05%次氯酸盐培养17天后,层状表皮结构中的皮肤脂质神经酰胺和非神经酰胺片段没有变化。外用0.05%次氯酸盐和自来水都具有很好的耐受性,无皮肤刺激症状。与自来水对照组相比,虽然使用稀释的漂白剂20分钟后,能观察到细菌数量的明显减少,但是两者差异只是近乎显著(P = 0.06)。

结论和临床意义 — 结果表明外部使用稀释的漂白溶液,不论0.05%或0.005%的次氯酸盐浓度,均具有很好的抗菌耐受性,同时还表现有抗炎特性

Resumo

Contexto – O hipoclorito de sódio diluído é um antisséptico tópico amplamente disponível e de baixo custo. Entretanto, dados a respeito da sua tolerabilidade e eficácia não estão disponíveis na dermatologia veterinária.

Objetivos – Determinar o efeito antibacteriano e a tolerabilidade da aplicação de água sanitária diluída e avaliar o efeito *in vitro* nos lipídeos da barreira cutânea e as propriedades anti-inflamatórias nos queratinócitos.

Métodos – Hipoclorito a 0,05% e água de torneira, por via tópica, foram aplicados nos dois lados do tórax de quatro cães saudáveis. O efeito anti-inflamatório nos queratinócitos caninos foi determinado por PCR-*real time;* a integridade da barreira cutânea foi analisada pela avaliação das mudanças nos lipídeos do estrato córneo na estrutura epidérmica estratificada.

Resultados – A viabilidade celular dos queratinócitos primários tratados com água e hipoclorito a 0,005 e 0,01%, foi reduzida em 10%. A exposição dos queratinócitos primários ao hipoclorito diluído a 0,005% reduziu significativamente a indução de genes inflamatórios ligadores de quimiocina-2 (CCL2; *P* = 0.015) e TARC/CCL17 (*P* = 0.032). Não houve modificações nas frações de ceramídeos e não-ceramídeos na estrutura epidérmica estratificada cultivada por 17 dias com hipoclorito a 0,05%. Tanto o hipoclorito a 0.05% quanto a água de torneira foram bem tolerados por via tópica, sem sinais de irritação cutânea. Apesar da

redução importante no número de bactérias ter sido observada em 20 minutos após a aplicação de água sanitária diluída, quando comparado à água de torneira, esta diferença foi marginalmente significativa apenas.

Conclusões e importância clínica – Os resultados indicam que a água sanitária diluída por via tópica, tanto na concentração de 0,05 quanto 0,005%, é um antisséptico bem tolerado que apresenta propriedades anti-inflamatórias.

