Journal of Equine Veterinary Science 35 (2015) 161–164

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Short Communication

Commercial and Homemade Extremely Dilute Hypochlorous Acid Solutions Are Bactericidal Against *Staphylococcus aureus* and *Escherichia coli* In Vitro

David W. Ramey [a,*], Hailu Kinde [b]

[a] *Ramey Equine, PO Box 16883, Encino, CA 91416*
[b] *CAHFS San Bernardino Branch, School of Veterinary Medicine, University of California Davis, San Bernardino, CA*

ARTICLE INFO

Article history:
Received 19 August 2014
Received in revised form 26 November 2014
Accepted 3 December 2014
Available online 10 December 2014

Keywords:
Hypochlorous acid
Wound
Wound care
Disinfectant
Bacteriology
Cytotoxicity

ABSTRACT

A commercially available extremely low concentration of hypochlorous acid solution (HClO, 0.011%) sold over the counter for wound care in horses completely inhibits growth of *Staphylococcus aureus* and *Escherichia coli* in vitro when in solution. A homemade solution of equivalent concentration of hypochlorous acid (HClO, 0.012%) equally inhibits the two organisms tested, at a much lower cost. Such solutions may have wide potential applications in equine medicine.

1. Introduction

Chlorine is a potent, rapidly acting, short-term disinfectant. However, because chlorine is a gas, it has little practical use. Instead, many compounds that release chlorine find wide application, including hypochlorite and organic and inorganic chloramines. Sodium hypochlorite (NaOCl) is one of the most frequently used chloro-releasing agents used in the field of chemical disinfection; hypochlorous acid (HClO) is the main active ingredient of most household bleaches and cleansers. HClO has a broad antimicrobial spectrum covering bacteria, mycobacteria, bacterial spores, viruses, algae, and protozoa. In human medicine, hypochlorite solutions diluted in water or saline have been used for vaginal, bladder, and urethral irrigations, control of athlete's foot and as infection prophylaxis in the management of burns [1].

Many over-the-counter products are available for use by horse owners for the treatment of wounds in horses. In the last few years, an over-the-counter hydrochlorous acid preparation has been made available (Vetericyn VF Liquid, Innovacyn, Inc, Rialto, CA). Similar products are marketed as a medical device in the human market, but there has been no approval granted for the veterinary product, by the US Food and Drug Administration (FDA), as there are no current requirements by the FDA for animal device clearance [2].

Healing of wounds is thought to be enhanced by decreasing the bacterial burden; however, the need for antimicrobial treatments in wound management is not consistently supported by experimental evidence. Nevertheless, countless topical agents have been used in an effort to prevent wound infection and decrease surface contamination. Unfortunately, the majority of such preparations may be locally toxic and have limited to no proven effectiveness in enhancing wound healing [3].

The first objective of this study was to evaluate the antibacterial effect of a low concentration of hypochlorous

* Corresponding author at: David W. Ramey, PO Box 16883, Encino, CA 91416.
E-mail address: ponydoc@pacbell.net (D.W. Ramey).

0737-0806/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2014.12.004

acid solution (HClO, 0.011%) against two common bacteria, *Escherichia coli* and *Staphylococcus aureus*, in vitro. The second objective of the study was to compare the commercial product to that of a homemade solution (HClO, 0.012%).

2. Materials and Methods

One bottle of the commercially available aqueous preparation was purchased at a retail tack and pet supply store. The preparation consists of electrolyzed water (H_2O, 99.816%), hypochlorous acid (HClO, 0.011%), sodium chloride (NaCl, 0.023%), and sodium phosphate (NaH_2PO/NaH_2PO_4, 0.015%). The pH of the buffered solution was 6.9.

In an effort to duplicate the concentration of HClO in the commercial preparation, the amounts of necessary ingredients (distilled water and bleach) were calculated. A homemade solution of equivalent concentration of chloride ion was thus made by adding 1.5 mL of "concentrated" bleach solution (8.25% Nalco) to 1 L of distilled water, resulting in a concentration of 99.988% H_2O and 0.012% HClO. Because of concerns about possible instability of the homemade bleach and/or water preparation, the homemade preparation was held in a plastic bottle for 1 week before testing. The pH of the unbuffered solution was 8.9, measured after 1 week of storage.

Tests on the commercial and homemade solutions were conducted at the California Animal Health and Food Safety Laboratory, San Bernardino, CA, as follows:

2.1. Trial 1

Pure 18- to 24-hour-old *E. coli* and *S. aureus* cultures growing on a sheep blood agar plates with well-isolated colonies were used to prepare a bacterial suspension. The turbidity of the bacterial suspension was adjusted to a 0.5 McFarland standard; approximately 10^8 colony forming units per milliliter (CFU/mL). One microliters of each bacterial suspension was mixed thoroughly with 1 mL of commercial solution. Similarly, 1 mL of each bacterial suspension was mixed with 1 mL of water (control solution). The mixtures were then allowed to sit for 1 hour at room temperature and then streaked on to blood agar plates. The plates were then incubated in 3%–7% CO_2 for 20–24 hours, and results were recorded as growth present or absent.

2.2. Trial 2

Pure 18- to 24-hour-old *E. coli* and *S. aureus* cultures growing on a sheep blood agar plates with well-isolated colonies were used to prepare six tubes of bacterial suspension. The turbidity of the bacterial suspension was adjusted to a 0.5 McFarland standard; approximately 10^8 CFU/mL. The tubes were centrifuged at 5,000 rpm for 10 minutes, and the supernatant was decanted. Five hundred microliters and 1,000 μL of the commercial solution were added to the tubes containing pellets of *E. coli* and *S. aureus*. Similarly, 500 μL of water (control solution) was added to the bacterial pellets in each of the remaining two tubes. The tubes were then rigorously mixed and allowed to sit for 1 hour at room temperature. After 1-hour incubation, the bacterial suspensions were streaked on to blood agar plates. The plates were then incubated in 3%–7% CO_2 for 20–24 hours, and results were recorded as growth present or absent.

2.3. Trial 3

Trial 3 was the same as trial 2 except homemade solution was used instead of the commercial antibacterial solution.

3. Results

In trial 1 (commercial solution), no growth of either bacteria was seen in test solutions containing the commercial HClO product after 24 and 48 hours of incubation. Bacterial growth was seen in all control samples.

In trial 2 (commercial solution), no growth of *E. coli* was seen at either 500 or 1,000 μL. No growth of *S. aureus* was seen at 1,000 μL, but growth was present at 500 μL. Bacterial growth was present on all control samples.

In trial 3 (homemade solution), no growth of either bacteria was seen at either 500 or 1,000 μL. Bacterial growth was present in all control samples.

4. Discussion

Hypochlorous acid has been recognized as a microbicide since at least 1915 [4]. Dakin's solution has been recognized as a wound disinfectant for nearly a century and has been shown to kill pathogenic microorganisms with minimal cytotoxicity [5]; however, its concentration, 0.5% HClO [6], is much higher than that of the tested solutions. HClO is a potent oxidant, and the bactericidal action appears to be primarily related to the destruction of cellular electron transport chains and the adenine nucleotide pool and abolition of adenosine triphoshate production [7]. Immune cells such as neutrophils, macrophages, and eosinophils produce reactive oxygen species when confronted with infectious agents, so in a sense, there is biological precedent for its application [8].

It has not been established that HClO solutions enhance wound healing; however, the possible cytotoxicity of HClO solutions has been investigated on human dermal fibroblasts in vitro [9]. At concentrations of 0.1% and upward, the compound has a profound cytotoxic effect. At concentrations of ≥0.01% dose-dependent mitochondrial dysfunction is observed, and cell survival increases with decreasing concentrations of the chemical. Dakin's solution at concentrations of 0.5%, 0.125%, and 10-fold serial dilutions from 0.25% to 0.00025% have been evaluated, with most concentrations being shown to be detrimental to macrophage viability and function [10]. The concentration of hypochlorous acid in the tested solutions was 0.011%, a concentration at which cell toxicity of between 20% and 75% was seen in human fibroblasts. At a concentration of 0.01% HClO, there is almost total depletion of cellular adenosine triphoshate in the fibroblasts; however, this appears to be at least partially reversible due at least in part to the relatively short-term action of HClO [11]. (Interestingly, hypochlorite concentrations ranging from 0.005% to