Avenova™ with Neutrox™ (pure 0.01% HOCl) compared with OTC product (0.02% HOCl)

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Key Facts

- Avenova™ with Neutrox™ is the first and only product in ophthalmology containing **pure** hypochlorous acid (Neutrox)
- Pure hypochlorous acid is only stable in NovaBay's proprietary amber glass.
- Avenova with Neutrox has a 3 year shelf life
- Lab studies show Avenova to be non-cytotoxic to cells
- Alternative manufacturing processes for hypochlorous acid produce hypochlorous acid with hypochlorite impurities
- Avenova with Neutrox is by prescription only and bears significant safety and efficacy data

Summary

Avenova with Neutrox is the only non-detergent based, prescription eyelid and lash hygiene product containing pure hypochlorous acid. The manufacturing process for Neutrox utilizes a patented technology for the production of pure hypochlorous acid. Avenova with Neutrox is stable for 3 years in amber glass. Other hypochlorous acid products are either manufactured by Dakin's process or by electrolysis, both of which produce significant amounts of sodium hypochlorite impurities. A hypochlorous acid product introduced during World War I was called Dakin's solution. A more recent hypochlorous acid product with a concentration of 0.02% at pH 6.5-7 in a plastic bottle, has less chemical stability, has up to 27% sodium hypochlorite impurities, and a shelf life 2 of 18 months. A common household product that contains sodium hypochlorite is bleach, i.e. Clorox.

Introduction

Antibiotic therapy currently underpins all of modern medicine. However, resistance to antibiotics is emerging at an increasingly rapid rate with the introduction of each new drug, demonstrating that we can no longer outpace bacterial evolution and resistance. With our current resources, the World Health Organization (WHO) estimates that a post-antibiotic era, with few effective antibiotics available, could be apocalyptic[1].

In the "pre-antibiotic era", the active free chlorine species including chlorine (Cl2), hypochlorous acid (HOCl) and sodium hypochlorite (Na^+OCl^-) proved to be invaluable as a deodorizer, disinfectant and necessity for providing clean drinking water. Hypochlorous acid was shown to be the most valuable of these chlorine species. In the "antibiotic era", research was focused predominantly on the development of numerous antibiotics, with little effort in investigating other types of non-antibiotic antimicrobials. In this era of antibiotic resistance, NovaBay's attention has focused on providing a stable, non-injurious form of hypochlorous acid that can be directly introduced onto and into the human body through open wounds and skin.

An overview of chemical reactions and formation of hypochlorous acid

Hypochlorous acid is formed when chlorine is added to water. During the process of water disinfection, the hydrolysis of chlorine is nearly complete and occurs in only a few seconds.[2]

$$Cl_2 + H_2O \longleftrightarrow HOCl + H^+ + Cl^-$$

Given this extremely rapid reaction, the major disinfecting property and oxidizing capacity of chlorine is actually in hypochlorous acid, the hydrolysis product.[2]

Hypochlorous acid also undergoes instantaneous dissociation in water to form the hypochlorite ion, a reaction that is reversible.

$$HOCl \longleftrightarrow H^+ + OCl^-$$

Hypochlorites, including calcium hypochlorite and sodium hypochlorite, form a similar equilibrium in water, and ultimately produce hypochlorous acid as the primary antimicrobial. For example, calcium hypochlorite initially dissolves in water to form the hypochlorite ion.

$$Ca(OCl)_2 \longleftrightarrow Ca^{2+} + 2OCl^-$$

The hypochlorite ion subsequently associates with hydrogen ion in water to form hypochlorous acid.

$$H^+ + OCl^- \longleftrightarrow HOCl$$

The production of pure hypochlorous acid, available in com-

mercial pharmaceutical form, has been very challenging, as the pH must be maintained at 3.5 to 6 in order for hypochlorous acid to remain in this form3 (**Figure 1**). Over-acidification of hypochlorous acid leads to formation of mostly chlorine in NaCl aqueous solution (**Figure 2**). In alkaline condition, hypochlorite becomes the dominating chlorine species.

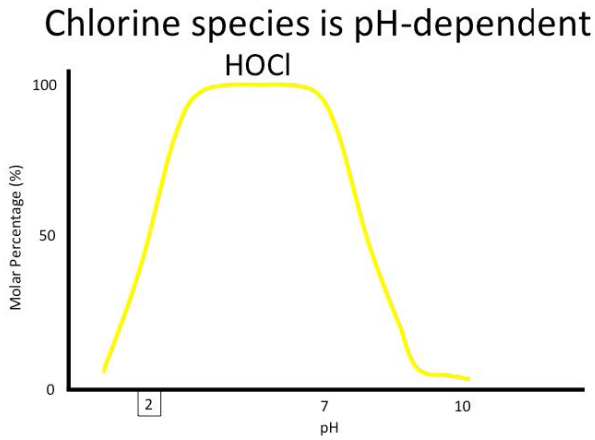

Figure 1. Percentage of hypochlorous acid present at different pH levels.

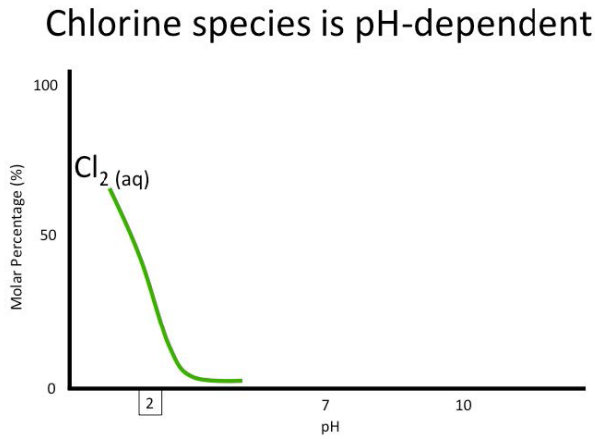

Figure 2. Percentage of chlorine present in 0.9% NaCl solution at different pH levels.

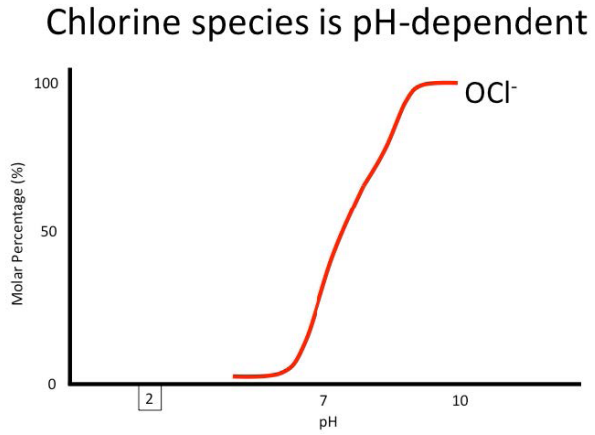

Figure 3. Percentage of hypochlorite present at different pH levels.

(**Figure 3**). Each of these different species has different properties and antimicrobial capabilities.

Pure Hypochlorous Acid Produced by White Blood Cells

Hypochlorous acid is an essential component of the microbial killing capacity of phagocytes, enabling our bodies to defend against bacteria, viruses and fungi.[4]

The respiratory burst, or oxidative burst, is a rapid release of reactive oxygen species and is the critical reaction occurring in phagocytes that allows degradation of bacteria and other internalized material as a part of the innate immune system (**Figure 4**).[4]

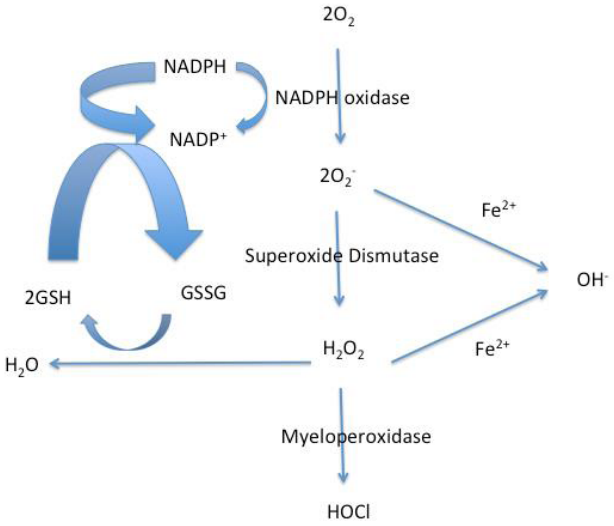

Figure 4. Respiratory burst to create rapid release of reactive oxygen species and hypochlorous acid.

Rates of oxygen uptake increase when phagocytes are exposed to certain stimuli, and they start to produce large amounts of superoxide (O_2^-) and hydrogen peroxide (H_2O_2). This reaction is catalyzed by NADPH oxidase. The O_2^- then reacts with H^+ to produce oxygen and H_2O_2. This reaction is catalyzed by superoxide dismutase.

Myeloperoxidase uses H_2O_2 to catalyze the oxidation of Cl^- to hypochlorous acid, the microbiocidal agent in the respiratory burst.

Why Pure Hypochlorous Acid Matters

NovaBay has developed Avenova, a novel daily eyelid and lash hygiene product, based upon a patented, proprietary manufacturing process of pure hypochlorous acid (**HOCl; see Figure 5**). HOCl is the most active anti-bacterial, anti-fungal and virucidal compound produced by neutrophils. HOCl is a charge neutral, small, inorganic compound that has the ability to rapidly inactive bacteria and fungi[3], bacterial toxins[5], and viruses[6]. Due to its small size and lack of ionic charge, HOCl can penetrate into bacterial biofilm and spores. In addition, HOCl exhibits anti-inflammatory activity by neutralizing inflammatory mediators in

the body[7] and those exuded from pathogens[5].

A number of commercial products, such as Dakin's solution, contain HOCl. However, most HOCl-based products also contain sodium hypochlorite (**Na^+ $^-$OCl; Figure 5**), which is also the primary ingredient in Clorox bleach. Because hypochlorite is a charged compound and therefore cannot easily penetrate into bacteria, sodium hypochlorite is less effective at killing bacteria than HOCl[8].

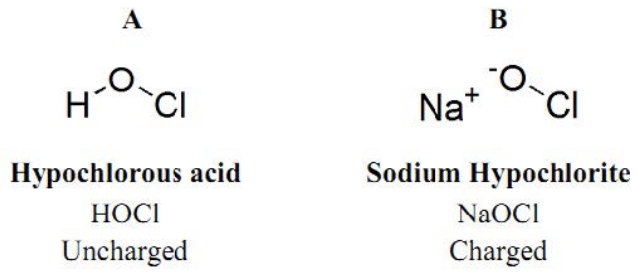

Figure 5. The chemical structures of HOCl (A) and sodium hypochlorite (B)

NovaBay has developed a pure form of HOCl into a product that is made via a proprietary manufacturing process.[9] Avenova is a member of NovaBay's Neutrox family of FDA-cleared products currently marketed: Neutrophase™ and CelleRx™. Avenova is free of hypochlorite impurities. In contrast, other manufacturing processes for making HOCl yield significant amounts of sodium hypochlorite.

Cytotoxicity:

It is important to compare the cytotoxicity of Avenova with Neutrox with a 0.02% hypochlorous product which contains significant sodium hypochlorite impurities.

The cytotoxicity testing was conducted using L929 (ATCC® CCL-1™) mouse fibroblast cells by the method detailed in Rani et al.[8] (please see Appendix for the complete method). Cytotoxicity is defined as less than 50% of viable cell count compared to untreated cells.

The cytotoxicity testing results showed that, when Avenova was assayed, the viable cell count was 73.77% ± 7.32% as compared to the Untreated Control. On the other hand, when the 0.02% hypochlorous acid product was assayed, the viable cell count was 2.44% ± 0.24% as compared to the Untreated Control.

Therefore in this cytotoxicity assay, Avenova was non-cytotoxic, whereas the 0.02% hypochlorous acid product was cytotoxic (**Figure 6**).

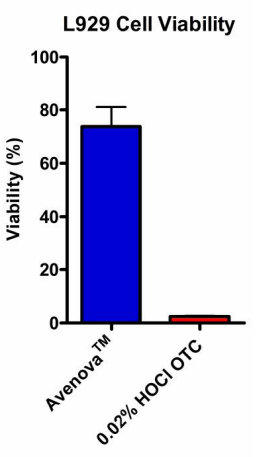

Figure 6.

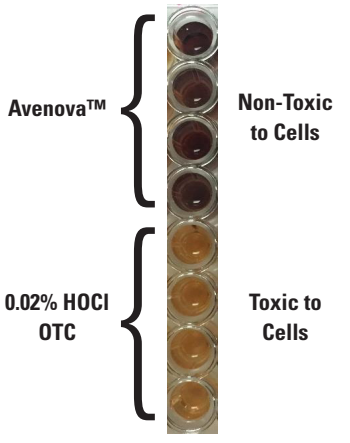

Figure 7.

Conclusion

Avenova with Neutrox (pure hypochlorous acid) is manufactured by a proprietary process and packaged in NovaBay's unique, proprietary amber glass bottles with a shelf-life of 3 years. Avenova with Neutrox, available only by prescription, has been used safely by an estimated 40,000 patients. The intended use of Avenova is clearly stated in the product Indication for Use (IFU) in the package as well as some of its performance attributes. In direct comparative testing, Avenova proved to be non-cytotoxic while the 0.02% hypochlorous acid product was clearly cytotoxic.

Package Insert

Avenova with Neutrox uses the same "Indications for Use" as NovaBay's award winning wound cleanser NeutroPhase which recently was selected as an official wound cleanser of the National Necrotizing Fasciitis Foundation.

Daily Lid & Lash Hygiene
(Rx Only)

INDICATIONS FOR USE:
Avenova is intended for use under the supervision of healthcare professionals for cleansing and removal of foreign material, including: microorganisms and debris from wounds; cleaning minor cuts, minor burns, superficial abrasions, and minor irritations of the skin; as well as moistening absorbent wound dressings. It is also intended for moistening and debriding acute and chronic dermal lesions, such as: Stage HV pressure ulcers, stasis ulcers, leg ulcers, diabetic foot ulcers, post-surgical wounds, first and second degree burns, as well as grafted and donor sites.

DIRECTIONS FOR USE:
1. Wash hands prior to application.
2. Remove any make-up or lotions around your eyes.
3. Apply 2 sprays of Avenova to a 100% cotton round pad.
4. May use a magnifying mirror for better visualization.
5. Close one eye and using a horizontal motion, gently wipe the base of all the upper lid lashes at least 3 times.
6. With the eye open, look up and gently wipe the base of the lower lashes with a horizontal motion at least 3 times.

7. With a 100% new cotton round pad, repeat on the other eye.
8. No rinsing is necessary.
9. Use twice daily or as recommended by your Doctor.

INSTRUCTIONS FOR USE OF SPRAY PUMP:
1. Remove original cap from 40 mL bottle of Avenova.
2. Insert pump sprayer into Avenova bottle and screw sprayer down securely.
3. Hold upright to spray Avenova onto 100% cotton round pad.
4. Replace protective cap on the spray pump.

Use Avenova contents within thirty days of opening or discard/recycle.

CONTACT TIME:
Apply Avenova as directed by your Doctor.

HANDLING:
Non-toxic, non-sensitizing, and non-irritating to the skin and eyes. Harmless to common work surfaces.

ORGANISMS TESTED IN SOLUTION:

Organism (ATCC number)	Time to Kill	% Reduction
Acinetobacter baumannii 19606	60 seconds	>99.99%
Aspergillus brasiliensis 16404	60 seconds	>99.99%
Bacteroides fragilis 25285*	60 seconds	>99.999%
Candida albicans 10231	60 seconds	>99.99%
Clostridium perfingens 13124*	60 seconds	>99.99%
Corynebacterium amycolatum 49368	60 seconds	>99.99%
Enterobacter aerogenes 51697	60 seconds	>99.999%
Vancomycin-resistant *Enterococcus faecium* (VRE) 51559	60 seconds	>99.99%
Haemophilus influenzae 49144	60 seconds	>99.999%
Klebsiella pneumoniae 10031*	60 seconds	>99.999%
Moraxella catarrhalis 8176	60 seconds	>99.9%
Proteus mirabilis 14153*	60 seconds	>99.999%
Pseudomonas aeruginosa 27853	60 seconds	>99.9999%
Serratia marcescens 14756	60 seconds	>99.999%
Methicillin-resistant *Staphylococcus aureus* (MRSA) 33591*	60 seconds	>99.999%
Staphylococcus aureus 29213*	60 seconds	>99.999%
Staphylococcus epidermidis 12228	60 seconds	>99.999%
Staphylococcus haemolyticus 29970	60 seconds	>99.99%
Staphylococcus hominis 27844	60 seconds	>99.99%
Staphylococcus saprophyticus 35552	60 seconds	>99.99%
Streptococcus pyogenes 49399*	60 seconds	>99.99%
Vibrio vulnificus 27562*	60 seconds	>99.999%

No special handling precautions required.

INGREDIENTS:
Neutrox (pure hypochlorous acid) 0.01% (as a preservative) in normal saline.

PRESERVATIVE EFFECTIVENESS:
Avenova passes USP <51>, which demonstrates in-solution eradication of P. aeruginosa, E.coli, S. aureus, C. albicans, and A. niger. Avenova also demonstrates rapid, in-solutioninactivation of MRSA, VRE, MDRA, and other dangerous pathogens (listed below).Reductions in microbial growth in solution have not been shown to correlate withreductions in infections in patients, as clinical studies to evaluate reductions in infection-shave not been performed.

STORAGE:
No special storage conditions are required for the contents. Caution - do not freeze - glass bottle may break if frozen. Non-flammable.

DISPOSAL:
Non-toxic and safe to the environment. No special disposal considerations required.
Container may be recycled.

WARNING:
For external use only. Not for injection. If you experience any redness or irritation, please consult with your Doctor. In case of known allergies to chlorine, please exercise caution and consult with your physician prior to use.

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Appendix

Methodology for conducting Cytotoxcicity Assay:

A cell-based cytotoxicity assay was used to compare the relative cytotoxicity of Avenova™ (NovaBay Pharmaceuticals, Emeryville, CA) and 0.02% hypochlorous acid. *In vitro* cytotoxicity was tested against L929 (ATCC® CCL-1TM) mouse fibroblast cells (American Type Culture Collection, Manassas, VA) by the method detailed in Rani et al.8with some modifications. The L929 cell line was maintained in Minimum Essential Medium Alpha (α-MEM) containing 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine, and 100 IU/mL penicillin with 100 µg/mL streptomycin. Cells were grown and maintained at 37°C with 5% CO_2. 200µL of L929 cells in α-MEM was seeded into a 96-well plate at a density of 10^4 cells/well. The cells were grown for 24 hours at 37°C with 5% CO_2, in order to reach confluency. 120µL of media was removed from eachwell, leaving behind media to represent the neutralizing environment of the lid and-lashes. 80µL of each test article was applied in quadruplicate, for a total volume of 200µL in each well. Wells without treatment were used as a control and wells that contained nocells were used to later determine background absorbance. The treated plates wereincubated for 30 minutes at 37°C with 5% CO2. After incubation, solutions wereaspirated out of the wells and 200 µL of fresh α-MEM was added to each well. Plateswere incubated for 24 hours at 37°C with 5% CO2. Cell viability was determined withCellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI).The cell viability assay contains a tetrazolium compound that is bioreduced by viablecells to produce a dark formazan dye. A darker color corresponds to greater cell viability(Figure 6). The optical density (OD) at 490 nm was measured using a SpectraMax M5Microplate Reader. Cytotoxicity is defined as less than 50% of viable cell countcompared to untreated cells.

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Avenova™

Daily lid and lash hygiene.