A safe and effective wound irrigation solution for sensitive tissues

Background
A prerequisite for successful wound healing is the thorough cleaning and decontamination of the wound. This can be achieved by use of wound irrigation solution, which reduces the microbial load of the wound by the mechanical effect of rinsing and can prevent wound infection when regularly applied. Here we selected a solution which is based on HOCl. It is well known that HOCl is part of the human body’s immune defense system and is involved in destroying pathogens. Aim of this study was to analyze a wound irrigation solution (test substance 1) preserved with a mixture of low concentration hypochlorous acid (50ppm HOCl) and sodium hypochlorite (NaOCl), with respect to its safety and antimicrobial efficacy. In addition it will be compared to safety profile of a high concentration NaOCl rinsing solution (test substance 2) regarding its cytotoxic profile.

Approach
Cytotoxicity data were assessed by cultivation of L929 cells with different sample concentrations for 24 hours in accordance to EN 10993-5. The irritation potential was examined using the HET-CAM test according to Spielmann et al. 1992. The efficacy of antimicrobial preservation of the wound irrigation solution was tested in accordance to EP 5.01.03; EN 13272; EN 13624, or EN 13704 phase 2. The antibacterial activity against E.coli and S.aureus was tested after 30 min of exposure at 37°C in accordance to EN 1040 in the presence of 10% FCS. Chlorate levels were determined via LS-MS/MS.

All experiments analyzing test substance #1* (50ppm HOCl/50ppm NaOCl) or test substance #2** (800ppm NaOCl) were performed in independent, accredited laboratories.

Results

I CYTOTOXICITY, TOLERABILITY AND SAFETY

a) Cytotoxicity
The concentration of a test substance 1 preserved with 500ppm HOCl/50ppm NaOCl caused neither toxicological nor biological damages to subconfluent monolayer of mouse fibroblasts (L929). In contrast, the test substance 2, preserved with a high concentration of 800ppm NaOCl led to severe cytotoxic reactions.

b) Irritation
The Henn’s Egg Test on the Chorionallantoic Membrane (HET-CAM) is considered representative for the physiology of the human eye. The HOCl/NaOCl solution is tested with 100% product concentration reached an irritation score <10 in the HET-CAM assay and is classified as not, or only very slightly irritant.

d) Chlorates
Chlorates may be formed in products containing NaOCl, especially under unfavorable storage conditions. Analyses of chlorates revealed a low content of 4.3 mg/L for the HOCl/NaOCl solution, demonstrating its high stability and safety; and a 30-fold higher content of 120 mg/L for the NaOCl solution.

II ANTIMICROBIAL EFFICACY OF THE PRESERVATIVE

HOCI rapidly reduces the microbial load of gram-positive and gram-negative bacteria, including resistant bacteria, fungi and spores in solution as well as in gauzes and dressings previously moistened with HOCI/NaOCl solutions. All bacteria were reduced by at least 5 log levels after 15-60 sec which corresponds to >99% reduction of the initial microbial load. The tests were performed in the presence of an additional organic load of 0.3 g/L albumin. Candida albicans and spore forming bacteria were reduced by >4 log levels after 15 sec or 5 min respectively.

Even after 1 h of incubation the same log levels of microbial reduction could be obtained, demonstrating the sustainability of efficacy of the antimicrobial effect. Moreover, stability of preservation was confirmed, as even 3 weeks after first opening of the bottle and storage at room temperature, this antimicrobial effect was maintained.

III BIOMICROBILITY INDEX

The biocompatibility index (BI) is defined as the ratio of the mean values of IC50 on L929 cells and of the concentration producing 3 log10 reduction in microbial CFU. A BI >1 represents a solution with a positive benefit/risk ratio whereas, whereas a BI <1 indicates an antimicrobial agent with a relatively high risk side effects.

IV ADVANTAGES OF WOUND CARE PRODUCTS CONTAINING HOCl/NaOCl

HOCI/NaOCl in wound irrigation solutions rapidly reduces the microbial load of wound dressings and does not lead to the development of resistances of clinical relevant pathogens. As the solution is highly tolerated and does not provoke sensitization reactions, it can remain in the wound and does not need to be rinsed out. Hence, HOCl/NaOCl containing solution is the superior active agent to be used for rinsing of cavities with a lack of drainage potential, for peritoneal rinsing, as well as for wounds with the risk of central nervous system exposure. In addition, it can be used to rinse wounds with other exposed sensitive tissues, such as joints or cartilage, or for the eye or mucosa.

Conclusion
• Hypochlorous acid (HOCI) ensures a safe preservation and makes the HOCl/NaOCl containing solution an effective wound irrigation solution. Proliferation of microorganisms in the analyzed irrigation solution or in dressings moistened with the solution can be safely prevented. In addition, unpleasant wound odors are eliminated rapidly.
• The tested HOCl/NaOCl solution is a safe and stable solution and is the only solution in this test setting with a BI >10. Due to its high tolerability it can be used for cleaning, moistening and rinsing of acute or chronic wounds even when sensitive tissues, such as joints, cartilage or CNS structures are exposed. It is also suitable for irrigation of the peritoneum, eye or mucosa.
• However, the results clearly show, that solutions with higher concentrations of hypochlorous acid or sodium hypochlorite show a severe cytotoxicity. Therefore, wound irrigation solutions should be evaluated by their cytotoxic characteristics prior to use for sensitive tissues.

*Test substance #1 = Granudacyn®  ** Test substance #2 = Lavanox®