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Comparative Bacteriology Analysis:

Particulate vs. Ionic Silver

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Purpose

Claims have been made by the manufacturer of Mesosilver that suggest "ionic" silver potency is compromised by Hydrochloric Acid (HCl) in the stomach, and that only the particulate [species], elemental silver (i.e. the primary content in Mesosilver) will survive and therefore be effective in inhibiting microorganisms. Whether or not HCl in the stomach is an issue is well beyond the scope of this paper. Herein we are going to deal with only one issue: the antimicrobial effect of 'particulate' (elemental) vs. (free) 'ionic' silver.

The hypothesis of Natural-Immunogenics, Corp. is that, contrary to the claims above, it is the silver ion [species] that is primarily responsible for silver's antimicrobial efficacy. Natural-Immunogenics, Corp.'s products (Argentyn 23 and Sovereign Silver) are composed in excess of 95% silver ions.

The purpose of this study, then, is to determine the antibacterial efficacy of both species, ionic vs particulate. This was to be achieved by comparing the two products, Argentyn 23 and Mesosilver, after the free ion content in both products was reduced or eliminated equally to the extent that only the particulate content in Mesosilver remained. This of course would reduce the ionic content of Argentyn 23 by the same amount.

This was accomplished by first exposing both products to the same amount of HCl. Identical bacterial concentrations and dilutions of two strains of Staphylococcus aureus (S-1 and S-2) were then used to test each product. This testing was accomplished by exposing healthy strains of the bacteria (in dilution series) to the two products after adding 10µl of HCl solution (in various concentrations).

Materials and Methods

Summary:

The antibacterial activity of both Argentyn 23 and Mesosilver were compared by treating healthy cultures of the bacterial strains, separately, with each of the two products (to which was added 10µl of HCl solution, in various concentrations, to make 1 ml samples of each).

Source, cultivation, and preparation of bacterial samples:

1. The YT media was autoclaved and poured into sterile plates and allowed to dry.
2. Two strains of Staph received from the New York Hospital of Queens.
 - B14192 - wild type/ normal strain (S-1)
 - B14310 - antibiotic resistant MRSA (S-2)
3. The bacteria was grown on YT media for 16 to 24 hours before being harvested.

4. A 3 mm confluent inoculum of S-1 was resuspended in 1,250µl of sterile, 17 to 18 MegOhm (M?) purified lab water.
 - Then a standard 10:1 dilution series was performed on each strain, using sterile purified lab water
 - 1/10, 1/100, 1/1,000, 1/10,000, and 1/100,000 dilutions of each stock bacterial suspension were prepared.
5. The bacterial strain, S-2, was prepared identically, as was strain S-1.

Preparation of Test Media (YT):

1. Each 95mm sterile polystyrene culture plate (Petri dish) was filled with 5mm of YT media (which consists of 0.5% NaCl, 0.6% yeast extract, 0.8% tryptone and 2% agar).

Preparation of the silver products:

1. Argentyn 23 was diluted to 20 PPM (so as to have an equal concentration of silver) using sterile, 18 MegOhm (M?) purified lab water.
 - Mesosilver was determined (separately, by atomic absorption) to have a concentration of 20 PPM.
2. 9 parts of Argentyn 23 (final concentration 18 PPM) and one part of HCl (33, or 44, or 77 PPM, finally diluted to 3, 4, and 7 PPM, respectively) were added to each other and mixed.
3. Mesosilver and HCl were added to each other and mixed, as above.

Treatment of Bacterial Strains with Silver Products

1. 10 µl. of the silver product/ HCl mixture (9µl. each of Silver product and 1 µl. of HCl) were added to 90µl. of each of the sets of dilutions (1/100 through 100,000) of the two bacterial strain, above.
2. The mixture was agitated and allowed to react until spotted onto the YT plate.
 - One 10µl sample of each mixture of bacterial dilution/Silver/HCl of Strain S-1 was spotted onto the YT plates.
 - One 10µl sample of each mixture of bacterial dilution with Silver/HCl of Strain S-2 was spotted onto YT plates.
 - The exposure times were limited to 4 or 8 minutes, respectively, for each set of treatments.
3. This protocol was performed identically for Argentyn 23 and Mesosilver.
4. Negative control plate (positive growth) - No silver/HCl being added to S-1 or S-2 cultures. Sterile, purified water replaced the volume (10µl) of Silver/HCl.
5. Positive control plate - Treatment of bacterial strains with respective silver products without HCl.
6. Plates then placed in a 35°C to 37°C incubator overnight (16 to 24 hours).

Results

Qualitative results can easily be seen on each plate.

The negative control for S-1 grew out 4.5 spots, represented by (++++ ½), as did the negative control for S-2. These samples did not contain the silver or silver/ HCl mix. A (-) represents no Staph growth and (1/2) represents some Staph growth. The efficacy of the silver/HCl in the various ratios can be compared by the number of (+) vs. (-) spots observed.

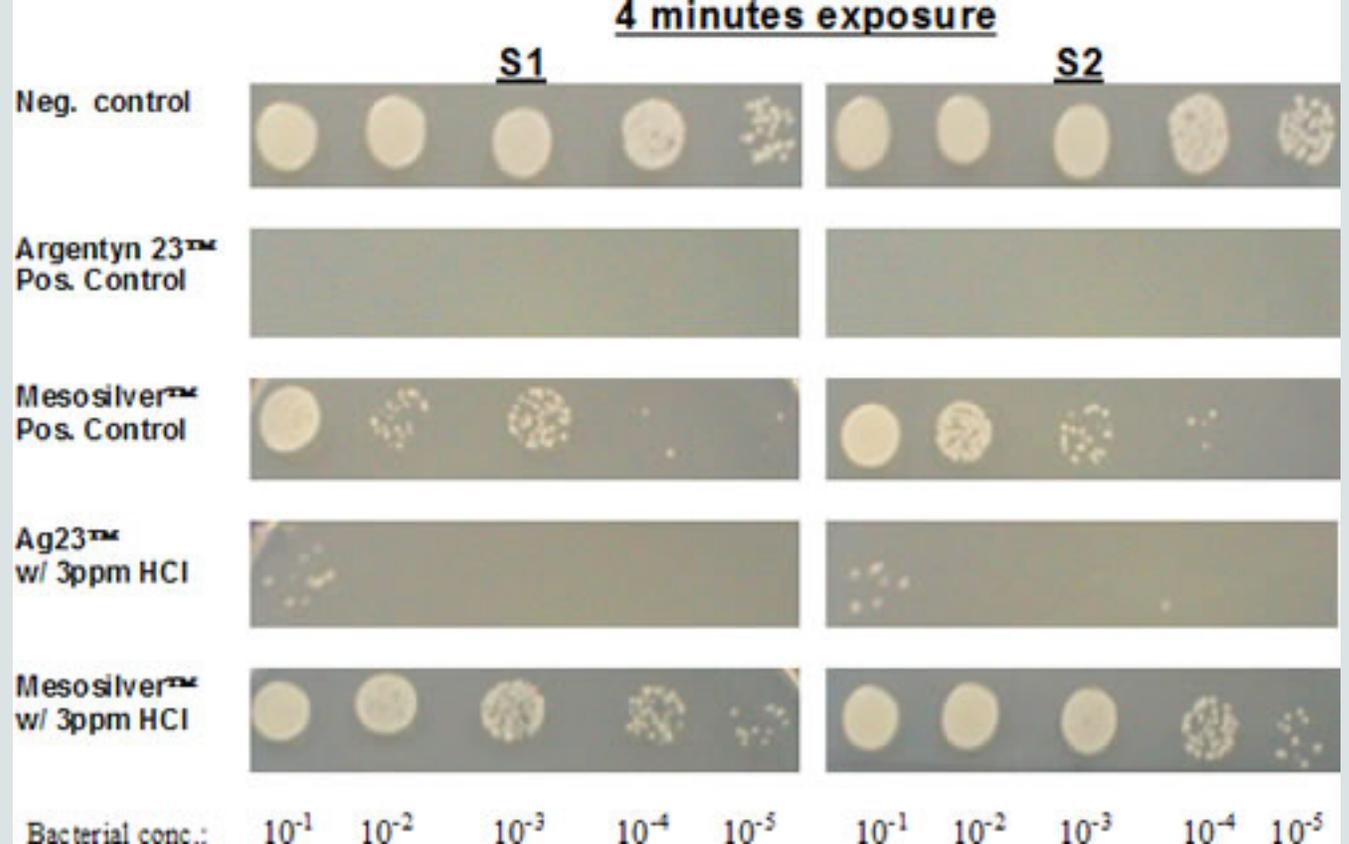
The positive control (silver and NO HCl) for S-1 and S-2. This should have shown the greatest degree of kill/inhibition (since there was no HCl to degrade/inactivate the silver).

A + designation is given for complete or near-complete spot-growth. A 1/2 is given for mottled/speckled appearing growth. A - is given for only a few spots of growth. A - is given for NO growth. The right-most + or - character represents a 1/100,000 dilution of the stock bacterial suspension; the left-most + or - represents a 1/10 dilution. The more - characters from the right, the more potent the antibacterial activity. The negative control (representing NO silver/HCl treatment should show 4 + and one 1/2 characters, demonstrating the viability of the untreated bacterial employed.

Inhibition results

	S-1 4min	S-2 4min	S-1 8min	S-2 8min
Negativecontrol	++++ 1/2	++++ 1/2	++++ 1/2	++++ 1/2
Argentyn23, 0 HCl	-----	-----	-----	-----
Ag23 w/ 3ppm HCl	+/- -----	+/- -----	-----	1/2 -----
Ag23 w/ 4ppm HCl	+ -----	+ -----	+ -----	+ -----
Ag23 w/ 7ppm HCl	+ -----	+ -----	+ -----	+ -----
Mesosilver, 0 HCl	+1/2 1/2 +/- -	++1/2 +/- -	+ 1/2 1/2 - -	+ 1/2 1/2 - -
Meso w/ 3ppm HCl	+++1/2 +/-	+++1/2 +/-	++ 1/2 1/2 -	++++ 1/2
Meso w/ 4ppm HCl	++++ 1/2	++++ 1/2	+++ 1/2 -	++++ 1/2
Meso w/ 7ppm HCl	++++ 1/2	++++ 1/2	++++ 1/2	++++ 1/2

The photographs of the results for the 4 minute exposure of the Silver/HCl (at 3 PPM) are shown below. The observations of the 4 PPM and 7 PPM and for both 4 and 8 minute exposures of Silver/HCl mixtures were virtually identical to those for the illustrated 3 PPM HCl (shown below).



Discussion

The concentrations of HCl added to each of the two products were such that the concentrations of HCl in the final volumes would be 3ppm, 4ppm, and 7ppm. The range of concentration was determined in an earlier experiment. These concentrations were selected because, if the HCl were to react with the silver, the conversion of Ag⁺ to AgCl would not make HCl the limiting reagent. The amount of remaining active silver would be the true limiting factor.

The following conclusions can be drawn from the data:

1. It is evident from the results that the antimicrobial efficacy of both products decreases as the concentration of HCl increases .
2. When comparing the plates where no HCl was added, it can be seen that Argentyn 23™ has far greater antibacterial efficacy than Mesosilver™ . Mesosilver's™ antibacterial efficacy is almost negated by adding just 4ppm of HCl, whereas Argentyn23™ 's antibacterial efficacy is only slightly reduced but still strong, even with the addition of 7 PPM HCl.
3. Adding HCl to the silver products, causes the chloride ion to bind the silver ion forming Silver chloride (AgCl). Since Argentyn23™ is primarily an ionic product there is still a sufficient number of Ag⁺ ions left to kill the bacteria.
4. Mesosilver™ on the other hand is primarily particulate in nature, and has significantly little (compared to Argentyn 23™) silver ion content. Once the silver ions in Mesosilver™ react with the chloride ions, they become almost completely inactivated. We see an almost complete loss of antimicrobial activity when Mesosilver™ is exposed to even modest quantities of HCl.
5. This experiment serves as proof that **it is the active, available silver ions** – not the particulate silver – in which the antibacterial property of silver resides.

