



Anti-inflammatory Activity of Ionized Silver (IM/Q610-419-01A)

Starlight Laboratories

Ionized Silver

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Product

The product under testing was delivered directly to Synexa Life Sciences (Pty) Ltd as a ready-made solution and this was indicated as de-ionized water containing 18.012 ppm (18.012 mg/l) ionized silver (Ag). In parallel, a bottle containing only the de-ionized water was made available as a control for all experiments.

Methodology

The ionized Ag preparation was evaluated for its effect on monocytes at three different concentrations which included 4.503 ppm (25%), 2.252 ppm (12.5%), and 1.126 ppm (6.25%). Due to the nature of the assay and the volumes needed we were restricted to a highest final concentration of 4.503 ppm. At higher concentration interference with cell activity occurred due to the aqueous nature of the sample and the control sample.

The activation of monocytes by a stimulus or antigen brings about an inflammatory response in the human body. This inflammatory response is characterised by the release of self-perpetuating soluble factors which, if left uncontrolled, will cause tissue damage. The purpose of the inflammatory response is to dilute, neutralize or remove the threatening agent and initiate the process of recovery or repair. On the other hand, an inflammatory response can also be harmful to the human body for instance in cases of sport injuries, chronic inflammatory conditions such as Rheumatoid arthritis, etc. Another example where an inflammatory response would be unfavourable is in the case of an HIV infection: the release of the cytokines by the activated monocytes brings about enhanced viral replication. The inflammation caused by an infection causes the recruitment of white cells to the site of infection, which in turn make more cells available for infection.

An *in vitro* whole blood assay was set up where whole blood from a healthy volunteer was incubated in the presence of the different concentrations ionized Ag and stimulated with 6.66 ng/ml lipopolysaccharide (LPS). LPS is a very strong inducer of inflammatory responses and more specific a potent activator of monocytes. The same volume of control water, as the ionized Ag water used in this experiment, was run in parallel with each dilution to compare and exclude any possible effects the water alone would have. The results of these control samples were also used to calculate the difference between the Ag containing samples and the control samples. In order to evaluate the possible anti-inflammatory effect of ionized Ag, the release of two of the major pro-inflammatory cytokines, IL-6 and TNF- α , from all the samples were measured by means of enzyme-linked

immunosorbent assay (ELISA). Any anti-inflammatory effect will be depicted by a decrease in the levels of these two cytokines.

Each result in the following table is expressed as a percentage of its corresponding control using the following formula:

$$\text{Factor decrease (\%)} = \frac{\text{Stimulated Control}}{\text{Sample}}$$

Results

The following table shows the anti-inflammatory capabilities of ionized Ag solution through the down-regulation of cytokines IL-6 and TNF- α :

Concentration Ionized Ag	Factor decrease in IL-6	Factor decrease in TNF- α
4.503 ppm	5.8	4.9
2.252 ppm	2.5	2.4
1.126 ppm	3.5	3.0

Conclusion

The results obtained clearly show the anti-inflammatory action of ionized Ag *in vitro*. At a final concentration of 4.503 ppm (25%) ionized Ag was able to inhibit the production of IL-6 and TNF- α around 5 times compared to the stimulated control. This anti-inflammatory activity was still demonstrated at a final Ag concentration of 1.126 ppm (6.25%) and inhibited the release of the cytokines by a factor of 3.

These results indicate a potential clinical use of the product to control tissue damage in chronic inflammatory conditions. Obviously, we cannot conclude whether the oral intake of the product will demonstrate the same anti-inflammatory activity because the *in vitro* results cannot be extrapolated to

in vivo situations: metabolic transformation of the product, oral uptake, etc. However, it remains an interesting product to consider for topical applications.

A handwritten signature in black ink, appearing to be "Patrick Bouic".

Patrick Bouic
Chief Technical Officer

A handwritten signature in black ink, appearing to be "Wessel Kriek".

Wessel Kriek
BioActivity Screening Manager