Journal of Antimicrobial Chemotherapy (2004) **54**, 546–548
DOI: 10.1093/jac/dkh349
Advance Access publication 8 July 2004

JAC

Anomaly and correlation of killing in the therapeutic properties of silver (I) chelation with glutamic and tartaric acids

Kareen I. Batarseh*

Alpha-OmegaBiologicals, 8610 Larkview Lane, Fairfax Station, VA 22039, USA

Received 9 May 2004; returned 29 May 2004; revised 31 May 2004; accepted 3 June 2004

***Objectives*: To investigate whether silver chelates or silver ions are more effective as therapeutic agents, and to examine their mode of action so that safer and stable compounds that have a broad spectrum of therapeutic activities can be developed.**

***Methods*: Efficacy was investigated against *Pseudomonas aeruginosa* (ATCC 15442) by determining MIC via a broth macrodilution procedure using NCCLS methods for antibiotic susceptibility testing.**

***Results*: It was found that the responsible agent for silver therapeutic properties is the silver chelates rather than silver ions, contradicting previous findings, and the efficacy profiles mimic that of free silver ions present in solution.**

***Conclusions*: Silver therapeutic activities seem to be more effective as complexes—an intracellular package—rather than free silver ions, demonstrating that the effect of silver is linked to cells' DNA unwinding, and not respiratory or membrane functionality as was traditionally recognized.**

Keywords: oligodynamics, silver, antimicrobials, chemotherapeutics, DNA

Introduction

The antimicrobial activity of small amounts of metal, known as oligodynamic action, has been known for a long time and is the basis for the development of many therapeutic agents, especially the use of silver in burn patients.[1] Some metals are also employed in the design of chemotherapeutic compounds. Examples of such metals are gold, copper, iridium and rhodium.[2] The most prominent and promising family of metal-based cytotoxic agents is those referred to as 'oxaliplatin', also known as L-OHP, which are considered third generation platinum complexes.[3]

Recently, ionic silver substances have been resurging again in popularity. What makes silver unique in comparison with other antibiotics is the fact that it has no toxicity and carcinogenic activities. Also, ubiquitous metallothioneins have the property of binding silver and other metals that may enter the body and detoxify them.[4]

The exact mechanism by which silver ions perform such functions is not known. It is accepted that there are three mechanisms for silver deactivation: firstly, catalytic oxidation through the absorption of nascent oxygen which will readily react with thiol groups (–SH) forming R–S–S–R (R is an organic moiety) bonds; secondly, reaction with bacterial cell membranes by the attachment of silver ions to surface radicals; and thirdly, preferential binding of silver atoms with cells' DNA, preventing DNA from unwinding, an essential step for cellular replication.

Researchers have elucidated the significance of the availability of silver as ions in the treatment of infections, and found out that this is completely independent of the total amount of silver chelates.[5–7] Since my interest lies in the potential use of silver (I) as a therapeutic agent, the main impetus of the present work is to investigate whether silver chelates or silver ions are more effective in combating diseases. This in turn will help delineate the mechanism of action, and hence the production of the best preparation and the design of the most effective delivering method.

Materials and methods

Reagents

Sterile, double-distilled-deionized water was used for all microbial and chemical assays. Analytical-grade reagents obtained from Sigma–Aldrich were used for all the experiments.

*E-mail: fractals00@hotmail.com/fractals00@aobio.com

Table 1. Relative weights of glutamic acid/tartaric acid (mg/mg) system at different values of R^a

	$R = 0.25$		$R = 1.0$		$R = 3.0$	
GA/TA	GA	TA	GA	TA	GA	TA
1:1	4.30	4.39	17.2	17.5	51.6	52.6
1:2	2.87	5.86	11.5	23.4	34.4	70.2
1:3	2.15	6.58	8.6	26.3	25.8	78.9
2:1	5.73	2.92	22.9	11.7	68.8	35.1
3:1	6.45	2.19	25.8	8.77	77.4	26.3

[a] R denotes the ratio of number of moles of GA and TA with respect to the number of moles of silver.

Silver chelates

An aliquot of silver ion solution of 1.1×10^5 ppm was prepared at room temperature by dissolving 4.0 g of silver nitrate in 20.45 mL of doubled-distilled-deionized water and 2.55 mL of 85% H_3PO_4. By using a micropipette, 230 μL of this prepared solution was placed in a microtube where designated amounts of glutamic acid (GA) and tartaric acid (TA) were added according to Table 1, and mixed thoroughly. These values represent the number of moles of each organic acid with respect to free silver ion present in solution. The ratio of number of moles of both organic acids used with respect to the number of moles of silver, denoted by R, is given in Table 1. Thus, if $R = 1$, this represents an equimolar ratio of the corresponding organic acids with respect to silver present in solution.

The prepared solutions were then added to 50 mL of doubled-distilled-deionized water and mixed continuously. This resulted in an initial total (free and chelated) silver concentration of 506 ppm.

Bacteriological methods

In this study, the activity of silver was determined using *Pseudomonas aeruginosa* (ATCC 15442), a tenacious species that is difficult to treat due to lower permeability of its cell wall and cell membrane, and efflux pumps that cause enhanced levels of efflux of antimicrobials. Pure cultures of *P. aeruginosa* were incubated in Mueller–Hinton broth (composed of beef infusion 300.0 g/L, casein acid hydrolysate 17.5 g/L, and starch 1.5 g/L) for 18 h at 37°C. Cell suspension concentration was adjusted to yield a bacterial concentration of approximately 1.0×10^6 cells/mL with the addition of nutrient broth.

The bactericidal activity was investigated by determining MIC. MIC testing of antimicrobials was conducted via a broth macrodilution procedure using standard NCCLS methods. The biological experiments were conducted by serial dilution of 10 tubes with Mueller–Hinton broth. The tubes were then incubated at 37 ± 2°C for 24 h, and were observed for the presence and level of visible growth. The test culture was also inoculated with the reagents without the presence of silver to examine if there was any interaction between culture medium and test reagents. This turned out as expected, validating the observations of the actual experiment.

For comparative purposes, a free silver ion solution of 506 ppm was also prepared without glutamic acid and/or tartaric acid. The same procedure was followed as for the above samples.

Silver ion assay (Ag^+)

Standard atomic absorption spectroscopy methods were employed to determine free ionic silver. This was done after ultra-filtration of each sample since the complex has silver, which would mask the results. All the experiments were done in triplicate, and the results averaged and reported.

Results and discussion

For all cases examined, MIC results have revealed that as silver ion concentration increases, significantly lesser efficacies were observed, demonstrating the fact that silver as a therapeutic agent could be administered as a silver complex. Specifically, it was found that the chelated silver efficacy is almost one order of magnitude, and 32 times larger than the efficacy of free silver for the worst and the best cases studied, respectively.

The salient features and the general trend of efficacy and free silver ion profiles were found to be similar at a given R. For instance, it was found that for $R = 0.25$, both profiles were peak-shaped with maxima at 0.5 (data not shown). This shows that the same phenomenon that governs silver is finger printed in the efficacy results, demonstrating that these two variables are strongly dependent on each other, which is to be expected, if only the chelates are responsible for the observed biocidal activities. Therefore, maximum efficacies correspond to minimum free ionic silver (Ag^+) concentrations, contradicting previous findings reported in the literature.[5–7]

As mentioned earlier, there are three suggested mechanisms for the biocidal activities of silver: 1) catalytic oxidation of silver ions with nascent oxygen; 2) reaction with bacterial cell membranes by the attachment of silver ions to surface radicals; and 3) preferential binding of silver atoms with cells' DNA, preventing DNA from unwinding. The first two mechanisms necessitate silver to be as ions for the reaction of the positively charged silver with the negatively charged oxygen, thiol group, and membrane enzymes to take place. Hence, if these two mechanisms are responsible for the biocidal ability of silver, one should see more kill with increasing silver ion concentration, which is not what the present data reveal. The effect of silver atoms on unwinding of cellular DNA, thereby inhibiting replication does seem more plausible. This is because silver is transported intracellularly as a package—a protected complex—and not as a free ion, preventing silver from binding with a host of substances present in the cell membrane or the intracellular space of microorganisms such as thiol groups, enzymes or any other negatively-charged moiety, precluding its biocidal function. What substantiates this conclusion further is the fact that experiments conducted on aerobic bacteria have shown that silver is also quite effective at killing these types of bacteria.[8]

It is possible that the first two mechanisms work synergically with the third, unwinding of cellular DNA. Nevertheless, their combined contribution to killing may not be as effective as unwinding of DNA alone. This can be substantiated by the fact that experiments on *P. aeruginosa* with silver sulfadiazine have revealed that silver was present up to 12% in the DNA fraction, 3% in the RNA fraction, less than 0.5% in the lipid fraction, and the remainder in its proteins and polysaccharides.[9] It is known that when silver bonds with DNA, the resulting complex is not unwound. However, in treating the complex with chlorides,

bromides and cyanides, which remove the silver, regeneration of the native DNA takes place and its function is fully restored.[9]

A good candidate for the above findings, besides its application as antibiotics, is in the design of chemotherapeutic agents. Knowing the fact that silver has a much safer tolerance record than most heavy metals, it might be possible to design chemotherapeutic agents that employ the present silver complexes. The many adverse side effects of the current cancer systemic therapeutic methods such as radiation, immunotherapy, chemotherapy, and antiangiogenic drugs make silver an excellent candidate for alternative therapy. It might also be possible to use the organic moieties described here for the chelation of silver or any other heavy metal, especially since these organic moieties have small molecular weights, from which smaller chemotherapeutic molecules could be developed. Such molecules are useful in the treatment of neoplasia, especially those associated with breast, head and neck, pancreatic, stomach, ovarian, cervical, lung, and prostate since within the intra- or inner-region of solid tumours the network of blood capillaries is too small for large molecular weight agents to be delivered.[10] Thus, further research is warranted.

Acknowledgements

I would like to thank Dr Oumeish Y. Oumeish, MD, FAAD, FACP, FRCP (Glasgow) for his critical review of the manuscript prior to submission, and for his many constructive suggestions.

References

1. Klasen, H. J. (2000). A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. *Burns* **26**, 131–8.

2. Haiduc, I. & Silvestru, C. (1989). Rhodium, iridium, copper and gold antitumor organometallic compounds. *In Vivo* **3**, 285–93.

3. Pil, P. & Lippard, S. (1997). Cisplatin and related drugs. In *Encyclopedia of Cancer* (Bertino, J. R., Ed.), pp. 392–410. Academic Press, San Diego, CA, USA.

4. Stillman, M. J., Presta, A., Gui, Z. *et al.* (1994). Spectroscopic studies of copper, silver, and gold metallothioneins. *Metal-Based Drugs* **1**, 375–94.

5. Grier, N. (1983). Silver and its compounds. In *Disinfection, Sterilization, and Preservation* (Block, S. S., Ed.), pp. 375–89. Lea and Febiger, Philadelphia, PA, USA.

6. Ratte, H. (1999). Bioaccumulation and toxicity of silver compounds: a review. *Environmental Toxicology and Chemistry* **18**, 89–108.

7. Dibrov, P., Dzioba, J., Gosnik, K. K. *et al.* (2002). Chemiosmotic mechanism of antimicrobial activity of Ag^{+} in *Vibrio cholerae*. *Antimicrobial Agents and Chemotherapy* **46**, 2668–70.

8. Dudley, J. M. (1997). The chemical and physical basis of the therapeutic value of colloidal forms of silver. [Online.] http://www.silverfacts.netfirms.com/pages/dudley.html (2 March 2004, date last accessed).

9. Modak, S. M. & Fox, C. L. (1973). Binding of silver sulfadiazine to the cellular components of *Pseudomonas aeruginosa*. *Biochemical Pharmacology* **22**, 2391–8.

10. Jain, R. K. (in press). Vascular and Interstitial Biology of Tumors. In *Clinical Oncology*, 3rd edn (Abeleff, M., Armtage, J., Kastan, M. *et al.*, Eds), Elsevier, Philadelphia, PA, USA.