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Silver complex of salicylic acid and its hydrogel-cream in wound healing chemotherapy

Maria-Eleni K. Stathopoulou[a], Christina N. Banti[a,*], Nikolaos Kourkoumelis[b,*], Antonios G. Hatzidimitriou[c], Angelos G. Kalampounias[d,1], Sotiris K. Hadjikakou[a,*]

[a] Inorganic Chemistry Laboratory, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece
[b] Medical Physics Laboratory, Medical School, University of Ioannina, Ioannina, Greece
[c] Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece
[d] Physical Chemistry Laboratory, Department of Chemistry, University of Ioannina, Greece

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ABSTRACT

The known metallotherapeutic $[Ag(salH)]_2$ (**AGSAL-1**) of salicylic acid ($salH_2$), was used for the development of new efficient silver based material for wounds healing. **AGSAL-1** was characterized by spectroscopic techniques and X-ray crystallography. The wound healing epithelialization of **AGSAL-1** was investigated by the means of scratch assay against immortalized human keratinocytes (HaCaT) cells. The anti-inflammatory activity of **AGSAL-1** was evaluated by monitoring the catalytic peroxidation of linoleic acid to hydroperoxylinoleic acid by the enzyme lipoxygenase (LOX). The antibacterial activity of **AGSAL-1** was evaluated against bacterial species which colonize wounds, such as: *Pseudomonas aeruginosa* (*PAO1*), *Staphylococcus epidermidis* and *Staphylococcus aureus*, by the means of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and their Inhibition Zone (IZ). Moreover, the influence of **AGSAL-1** against the formation of biofilm of *PAO1* and *St. aureus* was also evaluated by the mean of Biofilm Elimination Concentration (BEC). A hydrogel material **CMC@AGSAL-1**, based on the dispersion of **AGSAL-1** in to carboxymethyl cellulose (CMC) was tested for its antimicrobial activity. Molecular Docking was performed, to explore the molecular interaction of **AGSAL-1** with (i) the transcriptional regulator of *PAO1*, LasR. (ii) the mevalonate pathway for the biosynthesis of isoprenoids which is essential for gram-positive bacteria *St. epidermidis* and *St. aureus*. The toxicity of **AGSAL-1** was examined against the HaCaT cells. Its genotoxicity was evaluated using *Allium cepa* model, *in vivo*. No genotoxicity was detected, indicating that **AGSAL-1** is a candidate towards the development on a new efficient medication of the silver based metallodrugs.

1. Introduction

The epithelium is a crucial barrier to infection, and its integrity requires efficient wound healing. There are two distinct stages of wound repair: (i) inflammation and (ii) new tissue formation. In the first stage of the wound repair, which lasts about 48 h after injury, is dominated by abundance of bacteria in wound. In the second stage, the tissue is formed while the epithelial cells are migrated [1]. Bacterial cells and secretomes from *Pseudomonas aeruginosa* or *Staphylococcus aureus* strains *etc*., inhibit the epithelial cell migration *in vitro* and *ex vivo* [2]. Wounds, on the other hand, are more susceptible to bacterial infections mainly by Gram positive or negative bacteria, as result of sepsis [3]. Especially, *Staphylococcus aureus*, a Gram-positive bacteria, is the first bacterium that colonizes the wound [3]. Gram negative bacteria, as *Pseudomonas aeruginosa* and *Escherichia coli*, are found in the wound after 24–48 h [3]. Thus, the development of new materials which activate and promote cells epithelialization with antimicrobial and anti-inflammatory properties is of a great interest.

Silver ions exhibit antimicrobial, antiseptic and anti-inflammatory properties and low human toxicity [4–8]. Metallic silver, silver salts (silver nitrate or silver sulfadiazine) and silver(I) complexes, are used in wounds treatment due to their antibacterial activities [9]. The silver ions react with proteins, change the structure of bacterial membrane and inhibit bacterial replication, through binding to bacterial DNA [10]. One of the greatest disadvantage in the usage of silver(I) nitrate salts in wound healing is their high solubility in human serum under physiological conditions which is accompanied by dissociation. This leads in their rapid inactivation through the formation of insoluble

* Corresponding authors.
E-mail addresses: cbanti@cc.uoi.gr (C.N. Banti), nkourkou@uoi.gr (N. Kourkoumelis), shadjika@uoi.gr (S.K. Hadjikakou).
[1] Institute of Chemical Engineering Sciences/FORTH, P.O. Box 1414, GR-26504, Patras, Greece.

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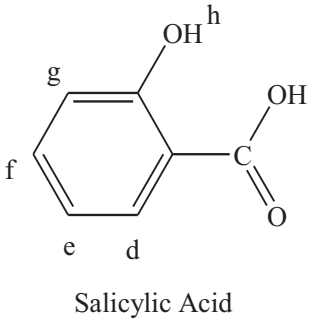


Salicylic Acid

Scheme 1. The ligand used.

complexes by chloride within few hours [11]. Therefore, there is an interest for the development of new silver containing metallotherapeutics, stable in human serum.

Salicylic acid, on the other hand, has been used on burn wounds, as an ingredient of creams, such as Aserbine. Aserbine possess high bioavailability and anti-inflammatory activity on the burn wounds, due to the salicylic acid. However, the antibacterial activity of the cream is limited [12]. Recently, the antibacterial, antifungal and anticancer properties of the metallodrug $[Ag(salH)]_2$ ($salH_2$ = salicylic acid) have tested against the bacteria methicillin-resistant *St. aureus*, *E. coli* and the fungal *Candida albicans and* against Cal-27, Hep-G2 and A-498 cancer cells [13–14]. Several silver(I) compounds with NSAIDs have been synthesized and evaluated for their antimicrobial activity [15]. Thus, complexes of salicylic acid, ibuprofen and nimesulide exhibit antibacterial activity against Gram positive or negative bacteria, which is significantly higher than that of the parent drugs [15].

In the course of our studies on the development of new metallotherapeutics [16–27], the already known metallotherapeutic $[Ag(salH)]_2$ (**AGSAL-1**) ($salH_2$ = salicylic acid (Scheme 1)) [13–14,16–17], was used for the development of new antimicrobial, anti-inflammatory and wound healing chemotherapeutic. Compound **AGSAL-1** was characterized by m.p., FT-IR spectroscopy, ^{1}H NMR, UV–Vis, High Resolution Mass spectrometry (HRMS) and single crystal X-ray crystallography. The potential activity of **AGSAL-1** in wound healing epithelialisation was investigated by the means of scratch assay against immortalized human keratinocytes (HaCaT) cells. The anti-inflammatory activity of **AGSAL-1** was evaluated by its inhibitory activity on the enzyme lipoxygenase (LOX). The antimicrobial activity was examined by means of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and their Inhibition Zone (IZ) against the bacteria of burn wounds (*PAO1*, *St. epidermidis*, *St. aureus*). The influence of **AGSAL-1** against the formation of biofilm was also evaluated by the mean of Biofilm Elimination Concentration (BEC). A hydrogel material **CMC@AGSAL-1**, based on the dispersion of **AGSAL-1** into carboxymethyl cellulose (**CMC**) was prepared and it was tested for its antimicrobial activity by the means of IZ. The cytotoxicity of **AGSAL-1** was examined against HaCaT cells. The genotoxicity of the metallodrug was evaluated by *Allium cepa*, *in vivo*.

2. Results and discussion

2.1. General aspects

AGSAL-1 was synthesized through the reaction of $AgNO_3$ with salicylic acid (1:1) (Scheme 2) in 5 ml of DMF or DMSO with an excess of trimethylamine (0.5 ml). Crystals of **AGSAL-1** have been grown by slow evaporation of the solution. The formula of **AGSAL-1** was initially determined by spectroscopic methods and its structure was confirmed here by single crystal X-ray diffraction analysis. Both the synthesis and the crystal structure of **AGSAL-1** are known [16–17,28–29]. In order to ensure the purity and the formula of the medication, its molecular structure was refined again here from new diffractions dataset. **AGSAL-1** is soluble in the following solvents: Et_2O, n-Hexane, Toluene, $CHCl_3$, CH_2Cl_2, Acetone, EtOH, MeOH, DMF, MeCN, DMSO and H_2O.

2.2. Solid state studies

2.2.1. Crystal and molecular structure of $[Ag(salH)]_2$

The crystal structure of **AGSAL-1** was here re-determined and its crystallographic parameters are: space group: $P2_1/c$; a = 7.3959(5), b = 8.8223(6), c = 10.6836(7) Å, β = 107.5835(18)°, R = 0.0249. These parameters are identical with those reported earlier (space group: $P2_1/c$; a = 7.3973(2), b = 8.6899(2), c = 10.5388(3) Å, β = 107.2560(10)° [16], space group: $P2_1/c$, a = 7.405(1), b = 8.826(2), c = 10.683(2) Å, β = 107.48(4)° [28], and space group: $P2_1/c$; a = 7.4100(10), b = 8.8350(10), c = 10.687(2) Å; β = 107.630(10)° [17]). The molecular structure of **AGSAL-1** is shown in Fig. 1.

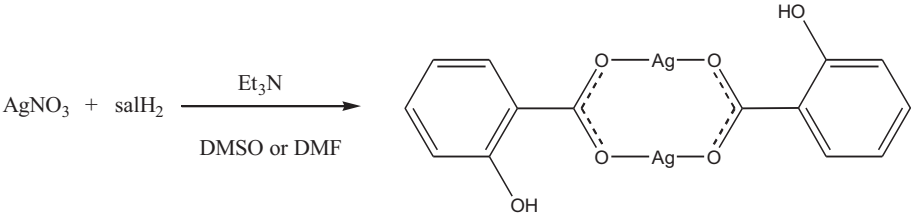
2.2.2. Vibrational spectroscopy

The purity of the sample of **AGSAL-**1 is examined with FT-IR spectrum. The FT-IR spectrum of **AGSAL-1** shows vibrational bands at 1556 cm^{-1} and 1334 cm^{-1} which are attributed to ν_{as} and ν_s of the COO− of the ligand, respectively. The corresponding bands for the free ligand, salicylic acid, are appeared at 1659 cm^{-1} and at 1325 cm^{-1}. The $\Delta\nu[\nu_{as}(COO\text{-})\text{-}\nu_s(COO\text{-})]$ value of **AGSAL-1** is higher (225 cm^{-1}) than the corresponding one of the sodium salt of $salH_2$ ($\Delta\nu$ = 205 cm^{-1}) indicating that the −COO− group bridges to silver(I) ion [30] (Fig. S1). Since the interpretation of the FT-IR spectrum is based on the crystal structure (see above X-ray analysis) the bulk of the sample contains **AGSAL-1**.

The purity of the sample of **AGSAL-1** is further examined by comparing the FT Raman spectra of the single crystal with the corresponding one of the bulk of the sample (Fig. 2). These spectra are identical confirming the purity of the sample.

2.2.3. Powder X-ray diffraction analysis

The purity of the sample is confirmed by XRPD analysis. The XRPD pattern of the **AGSAL-1** (Fig. S2) is of single phase without any detectable impurity and narrow peaks indicate the good crystallinity of the material. The simulated XRPD pattern using single crystal XRD data, which is overlaying the experimental one, adequately resembles the experimental pattern.



Scheme 2. Synthesis reaction.