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The use of ^{111}Ag as a tool for studying biological distribution of silver-based antimicrobials

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Abstract

Recently, there has been an emergence of significant interest in silver-based antimicrobials. Our goal was to develop a radioactive tracer for investigating the biological fate of such compounds. Purified ^{111}Ag was incorporated into the methylated caffeine analogue, IC1 to yield the silver carbene complex designated as [^{111}Ag]SCC1 and investigated in biodistribution studies.

Different antimicrobials have been developed over the years to treat bacterial lung infections. With increasing prevalence of pathogenic bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), multi-drug resistant *Acinetobacter baumannii* (MRAB) and *Pseudomonas aeruginosa* gaining resistance to conventional antimicrobial agents such as penicillin, vancomycin, ampicillin, ceftazidime and kanamycin,^{1, 2} there is a challenge to continuously develop new effective antimicrobials. The use of silver as an antimicrobial agent can be dated as far back as the early 1800s until the birth of penicillin and other antibiotics.³ Due to the emergence of resistant microbial strains, an interest in the use of silver in the management of wound infection and as a topical antimicrobial has renewed recently.^{4, 5} Several studies have been published on the use of silver as an antimicrobial for in vivo therapy, of particular interest is the use of stable N-heterocyclic carbene metal complexes as silver delivery agents.^{6–9} Silver has long been used as a broad-spectrum antibacterial at very low concentrations,¹⁰ with very little evidence of bacterial resistance.^{11, 12} Although, the bactericidal mechanism of silver is not completely understood, it has been suggested that the interaction of Ag^+ with thiol compounds or the DNA helical chains in bacteria cells could be the cause of the observed cell membrane damage or bacterial growth inhibition.^{13, 14} Since cationic silver shows very minimal toxicity and side effects (rare cosmetic side effect known as argyria),^{12, 15} new silver based compounds have been

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synthesized as potential antimicrobials for pulmonary infections related to cystic fibrosis.^{16, 17}

Previously, silver was coupled to derivatives of caffeine-like xanthine compounds as shown in Scheme 1 to obtain silver N-heterocyclic carbene complexes (SCCs). These compounds have been shown to have a broad-spectrum antimicrobial activity, even against bacteria resistant to conventional antimicrobials.^{18–20} The cytotoxicity, acute reaction and efficacy of the nebulized silver carbene complex designated as SCC1 has been investigated in mice models.¹⁸ In murine infection models, complete eradication of bacteria in the lungs was observed in more than 50% of the mice. These results demonstrate the potential of caffeine carbene derivatives as carriers of silver in lung infections therapies. Little, however, is known about the dose deposited in the lungs of these treated animals via the aerosol route. This information is important for the translation of these compounds to clinical studies and thus, prompted the following studies.

Our study investigates the dose delivered into the lungs via aerosol and the tissue distribution of a radiolabeled carbene complex in mice models. ¹¹¹Ag was chosen as the radiotracer due to its favorable half-life, low beta and gamma energy and ease of production. ¹¹¹Ag has a $t_{1/2}$ = 7.47 days, decays 92% by β^- emission (1.037 MeV) and has characteristic γ rays at energies of 245 keV (1.3%) and 342 keV (6.7%) useful for detection and monitoring by gamma spectroscopy.²¹ ¹¹¹Ag can be produced from neutron irradiation of Pd targets and subsequent β^- decay of ¹¹¹Pd to ¹¹¹Ag: (¹¹⁰Pd(n, γ) ¹¹¹Pd ($t_{1/2}$ = 23.4 m) $\rightarrow$ ¹¹¹Ag) then purified using ion-exchange chromatography monitored via gamma spectroscopy. In the crude irradiated sample, the presence of Pd is followed using ¹⁰⁹Pd ($t_{1/2}$ = 13.7 h, γ = 88 keV, 100% β^- emission to excited energy levels or the ground state of ¹⁰⁹Ag) which is co-produced during the irradiation of natural palladium (¹⁰⁸Pd, 26.46% natural abundance). A representative gamma spectrum of the irradiated Pd target (¹⁰⁹Pd, 88 keV) and purified ¹¹¹Ag (97, 245, 342 keV) is shown in Figure S1. As shown in Table 1, a number of nuclear reactions are possible during the neutron bombardment of natural palladium. One of these reactions produce the metastable Ag isotope, ^{110m}Ag via neutron bombardment and subsequent β^- decay of ¹⁰⁸Pd to ¹⁰⁹Ag and thereafter ^{110m}Ag: (¹⁰⁸Pd(n, γ) ¹⁰⁹Pd ($t_{1/2}$ = 13.7 h) $\rightarrow$ ¹⁰⁹Ag; ¹⁰⁹Ag(n, γ) ^{110m}Ag (657 keV, and 884 keV, $t_{1/2}$ = 249.8 days)). ^{110m}Ag was observed along with ¹¹¹Ag, thus can also be used to monitor the elution of silver from the ion-exchange resin.

Anion exchange chromatography (AG1-X8 resin) was used to separate the palladium ions from the silver ions using a reversed modified method described by Aardaneh *et al.*²² In nitric acid solutions (HNO₃ 1M), Pd²⁺ forms anionic complexes such as [Pd(H₂O)(NO₃)₃]⁻ and [Pd(NO₃)₄]²⁻ which are retained strongly on the AG1-X8 resin,²³ while Ag⁺ forms neutral AgNO₃ species which adsorb weakly to the resin. Different acid concentrations and column lengths were investigated to determine the optimal elution conditions. Figure S2 shows a superior elution profile of ¹¹¹Ag from AG1-X8 anion exchange resin packed in a long, thin column (0.7 x 20 cm) and eluted with 3M nitric acid as compared with 2M nitric acid or 3M nitric acid in a shorter column (1.0 x 10cm). The average recovery of pure ¹¹¹Ag was calculated as (92.9 $\pm$ 23.7)% by comparing the activity measured before and after purification via gamma spectrometry. ICP-MS measurement of the eluted fractions demonstrated a final concentration of <25 ppb of Pd in all solutions used for subsequent radiolabeling which indicates that >99.9% of the palladium was removed by our purification process.

The isolated ¹¹¹Ag recovered was used in *in vivo* studies either incorporated into a carbene or used as the nitrate or acetate salt. Synthesis of the carbene compound was accomplished as previously described by Kascatan-Nebioglu *et al.*¹⁶ ¹¹¹Ag was successfully incorporated

into the xanthinium salt, IC1 with a radiochemical yield of $(25.5 \pm 2.5)\%$. The product was reconstituted in 0.24% acetic acid at a final pH of 7.0 for *ex vivo* biodistribution studies. The amount of radioactivity in each organ as a percent dose per gram administered per animal is shown below in Figure 1. Although, single and multiple aerosol dosing experiments were performed separately, no significant difference was observed in the biodistribution of the normal mice used. This observation could be attributed to the lack of entrapment of [^{111}Ag]SCC1 in any organ except the lungs where it was continuously cleared and excreted daily with no cumulative dose observed after 3 days. Based on these findings, we concluded that one dose administration for 5 m was sufficient, resulting in a total dose received per mouse of $(1.07 \pm 0.12)\%$ of the aerosolized dose. The average dose taken up in the lungs (% ID/g) of the mice was estimated to be $(21.5 \pm 1.2)\%$ of the total dose received per mouse, thus $(0.22 \pm 0.01)\%$ of the aerosolized dose. The presence of the radiotracer in the stomach and the intestine is indicative of activity that may have been coughed up from the airways and subsequently swallowed. Since, coughing is one of the natural ways in which the body gets rid of foreign particles dislodged in the respiratory airways,^{24, 25} it is reasonable to conclude that any activity in the stomach or intestine is associated with the coughing and swallowing process. Also, as shown in Figure S3, the multi-dosing animal chamber is airtight such that no activity reaches other body parts of the mice except the nose.

Conclusions

Silver-111 has been purified from neutron irradiated palladium targets as a carrier-free radioisotope and successfully incorporated into the methylated caffeine carrier, IC1 to yield [^{111}Ag]SCC1. [^{111}Ag]SCC1 was delivered into healthy mice via nebulization to determine the average deliverable dose. The ^{111}Ag based compounds cleared mostly through feces with good accumulation in the lungs 24 h after nebulized dose delivery. Pre-clinical studies using ^{111}Ag incorporated in SCC1 carbene as an antimicrobial compound show high drug dosing via nebulization and good lung retention. The average %ID/g in the lungs was observed to be 10–50 times greater than most of the other organs—liver, spleen, kidneys and bone. It is expected that the transportation of silver based therapeutics into and through the lungs of healthy animals would be different from that of infected mice with thick mucus and biofilm. Thus, it would be important to determine the biodistribution of the inhaled silver therapeutics in the lungs of infected mice or in polymeric artificial mucus. The results obtained indicate ^{111}Ag is a useful radiotracer for investigating the biodistribution of silver therapeutics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Biodistribution of nebulized [^{111}Ag]SCC1 in normal mice. Two dose delivery approaches; 1 aerosol dose for 5 m and 5 doses administered within 3 days were investigated. No significant difference was observed in the lungs or any organ due to the fast and continuous clearance of the radiotracer. Excretion data (urine and feces) is only given for the 1 dose cohort.

Scheme 1.
Synthesis of SCC1 from caffeine

Table 1

Nuclear reactions and products produced during neutron bombardment of natural palladium

Reaction Target	Percent Abundance	Product (half-life)	Subsequent decay product
$^{102}\text{Pd}(n, \gamma)$	1.02	^{103}Pd (16.99 d)	^{103}Ag (1.1 h)
$^{106}\text{Pd}(n, \gamma)$	27.33	^{107}Pd (6.5×10^6 yr)	^{107}Ag (stable)
		$^{107\text{m}}\text{Pd}$ (20.9 s)	^{107}Pd
$^{108}\text{Pd}(n, \gamma)$	26.46	^{109}Pd (13.5 h)	^{109}Ag (stable)
$^{110}\text{Pd}(n, \gamma)$	11.72	^{111}Pd (23.4 m)	^{111}Ag (7.47 d)
$^{109}\text{Ag}(n, \gamma)$	[48.1] *	$^{110\text{m}}\text{Ag}$ (249.8 d)	

* Stable isotope of Ag with natural abundance of 48.1%. Neutron capture leads to production of $^{110\text{m}}\text{Ag}$, a long lived radiosilver contaminant.