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Investigating the pharmacokinetics and biological distribution of silver-loaded polyphosphoester-based nanoparticles using ^{111}Ag as a radiotracer

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Abstract

Purified ^{111}Ag was used as a radiotracer to investigate silver loading and release, pharmacokinetics and biodistribution of polyphosphoester-based degradable shell crosslinked knedel-like (SCK) nanoparticles as a comparison to the previously reported small molecule, N-heterocyclic silver carbene complex analogue (SCC1) for the delivery of therapeutic silver ions in mouse models. Biodistribution studies were conducted by aerosol administration of ^{111}Ag acetate, [^{111}Ag]SCC1 and [^{111}Ag]SCK doses directly into the lungs of C57BL/6 mice. Nebulization of the ^{111}Ag antimicrobials resulted in an average uptake of $1.07 \pm 0.12\%$ of the total aerosolized dose given per mouse. The average dose taken into the lungs of mice was estimated to be $2.6 \pm 0.3\%$ of the dose inhaled per mouse for [^{111}Ag]SCC1 and twice as much dose was observed for the [^{111}Ag]SCKs ($5.0 \pm 0.3\%$ and $5.9 \pm 0.8\%$ for [^{111}Ag]aSCK and [^{111}Ag]zSCK, respectively) at 1 h post administration (p.a.). [^{111}Ag]SCKs also exhibited higher dose retention in the lungs; 62 – 68% for [^{111}Ag]SCKs and 43% for [^{111}Ag]SCC1 of the initial 1 h dose was observed in the lungs at 24 h post administration (p.a.). This study demonstrates the utility of ^{111}Ag as a useful tool for monitoring the pharmacokinetics of silver loaded antimicrobials *in vivo*.

Abstract

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

Purified ^{111}Ag was used as a radiotracer to investigate silver loading and release, pharmacokinetics and biodistribution of polyphosphoester-based degradable shell crosslinked knedel-like (SCK) nanoparticles as an alternative to the small molecule, N-heterocyclic silver carbene complex (SCC1) for aerosol delivery of therapeutic silver in mouse models. Twice as much dose was observed in the lungs for [^{111}Ag]SCKs ($5.0 \pm 0.3\%$ and $5.9 \pm 0.8\%$ for [^{111}Ag]aSCK and [^{111}Ag]zSCK respectively) compared with $2.6 \pm 0.3\%$ for [^{111}Ag]SCC1 at 1 h post inhalation.

Keywords

Radiotracer; Aerosols; Pharmacokinetics; Biodistribution; Silver antimicrobials; Nanoparticles; Autoradiography; Nose-only inhalation

INTRODUCTION

Silver has long been recognized as a metal with antimicrobial and medicinal properties, often used to treat a variety of topical wounds, cuts and minor burns.^[1, 2] The emergence of pathogenic bacteria that have exhibited multi-drug resistance has sparked an increased interest in the study of the antimicrobial properties of silver. Studies of species such as *Staphylococcus aureus*, have shown the potential of silver as an antimicrobial, as well as the inability of the bacteria to demonstrate resistance even after repeated exposure to silver ions.^[3] Common commercial applications include the incorporation of silver into sulfadiazine to make the antimicrobial cream, Silvazine, for topical treatment of burns and the coating of catheters to prevent the growth of bacteria.^[4] Given the low toxicity of silver in humans,^[5] new silver based compounds have been synthesized as potential antimicrobials for pulmonary infections related to cystic fibrosis.^[6, 7] The use of caffeine derived, stable N-heterocyclic carbene metal complexes as silver ion carriers has been studied *in vitro* and *in vivo* to demonstrate that these silver carbene complexes (SCC) have broad-spectrum antimicrobial activity, even against bacteria resistant to conventional antimicrobials. In addition, SCC1 has been demonstrated to show no acute toxicity to the airway epithelial cells and mice treated with SCC1 appeared to have normal lung histology with no sign of inflammation.^[8, 9] Silver, as an antimicrobial agent, is often required at very low concentrations at the site of infection.^[10] To investigate the sustained release of silver, silver incorporated into larger molecules is currently under consideration for *in vivo* applications.^[11, 12]

Studies have shown silver nanoparticles to be effective antimicrobials against antibiotic resistant bacteria. These silver nanoparticles, aggregates of reduced silver ions which can be oxidized *in vivo* to Ag (I) ions for bactericidal activity, exhibit similar bacterial inhibitory mechanisms as seen with N-heterocyclic carbene silver antimicrobial complexes.^[13] Other studies have demonstrated that silver nanoparticles can accelerate the wound healing process with less scarring than silver sulfadiazine, due to their ability to reduce local and systemic inflammatory responses during wound healing.^[14] Nanoparticles are attractive as they can be tailored with a specific charge, size, hydrophobicity, and targeting agent for biocompatibility with the cell or tissue of interest.^[15, 16]

Delivery of nebulized drugs directly into the lungs is an attractive model for treating pulmonary related infections and diseases. Direct lung delivery for pulmonary infections allow topical application of the drug to the disease site, potentially leading to higher drug retention and efficacy in the lungs while minimizing the systemic side effects.^[17, 18] Small molecules have been administered via direct inhalation and were shown to diffuse and clear quickly from the lungs,^[19] therefore requiring repeated dosing for effective therapy. For example, SCCs were administered as aerosolized silver antimicrobials in mouse models allowing for localized delivery to the lungs, but required twice daily dosing to demonstrate significant efficacy.^[8, 12] In our previous work, aerosolized, non-degradable shell crosslinked knedel-like (SCK) nanoparticles were employed to deliver silver ions and SCCs in a depot fashion, to treat *P. aeruginosa* infected mice. These silver-loaded SCKs were delivered once daily, yet showed equivalent efficacy at a 16-fold lower dose compared with the small compounds by themselves.^[12] Here, the next generation polyphosphoester-based degradable SCKs were used as biocompatible, biodegradable vehicles with superior loading and dosing capabilities, which would allow for improved sustained release of the therapeutic silver ions in the lungs.^[20] In this work, we evaluated the pharmacokinetics (PK) and biodistribution (bioD) of silver loaded polyphosphoester-based degradable and hydrocarbon-based non-degradable SCK nanoparticles and compared our findings to our previous work with a silver N-heterocyclic carbene complex (SCC1), using ¹¹¹Ag as a radiotracer.

MATERIALS AND METHODS

Chemicals and Materials

Palladium (Pd) wire was irradiated at the Missouri University Research Reactor (MURR) as described previously.^[21] Silver acetate and diethyl ether were purchased from Sigma-Aldrich (Milwaukee, WI). Other solvents were obtained from Fisher (Pittsburgh, PA) or Burdick and Jackson (Morristown, NJ). AG1-X8 resin was purchased from Biorad (Hercules, CA). The Plexiglass inhalation box was built by the machine shop at Washington University in St. Louis. The nebulizer unit was obtained from Aeroneb Lab through Kent Scientific Corp (Torrington, Connecticut). Amicon ultra centrifugal filter units (30 and 100 kDa MWCO) were obtained from EMD Millipore (St. Charles, MO) and aqueous solution of formaldehyde (16%) was purchased from Electron Microscopy Sciences (Hatfield, PA). Flex chromatography columns were purchased from Thomas Scientific (Swedesboro, NJ). Materials for autoradiography; slides and adhesive tape were purchased from Leica Biosystems (Richmond, IL) while the Cryo-M-Bed embedding compound was obtained

from A-M systems (Sequim, WA). Radioactive samples were counted using a 1480 automatic gamma counter (PerkinElmer, Downers Grove, IL). All chemicals and solvents were used as purchased without any further purification unless otherwise noted.

Dissolution of irradiated Palladium wire

Dissolution of the irradiated Pd target was accomplished with some modifications as previously described.^[21, 22] Briefly, irradiated Palladium wire (4 – 6 mg Pd, ¹¹¹Ag ranging from 44.4 to 118.4 MBq) was dissolved in 2 mL of a conc. HCl: conc. HNO₃ (1:1) mixture by gentle heating. To prevent the formation of insoluble oxide precipitates,^[23] the resultant brownish red solution was heated gently to near dryness and reconstituted in 3 M HNO₃ twice and heated to near dryness each time to expel traces of HCl. After allowing the resulting volume (0.5 – 0.65 mL) to cool to room temperature, an additional 2 mL of 3 M HNO₃ was added and the final volume measured, typically 2.5 – 2.65 mL. A 10 µL aliquot was removed and diluted in 1 mL 3 M HNO₃ for gamma spectroscopy analysis on a high purity germanium (HPGe) detector (Canberra).

Separation of ¹¹¹Ag from Palladium isotopes

Isolation of ¹¹¹Ag from the dissolved palladium wire target was achieved with the anion exchange AG1-X8 resin using a method described by Aardaneh *et al.*^[24] Briefly, as described in the modified method,^[21] 4.7 g of AG1-X8 resin was made into a slurry with 3 M HNO₃ and loaded into a Flex column (0.7 × 20 cm) and conditioned further with 15 mL of 3 M HNO₃. Typically, 2.5 - 2.7 mL of dissolved Pd wire- ¹¹¹Ag target in 3 M HNO₃ was loaded onto the resin and eluted with 3 M HNO₃ in 1 mL fractions. The eluted fractions were analyzed by gamma spectroscopy for the presence of ¹¹¹Ag while excluding fractions containing any traces of co-produced ¹⁰⁹Pd. Fractions containing purified ¹¹¹Ag were combined and dried down under air with gentle heating in a glass vial. Samples were also analyzed by ICP-MS after several months of decay.

Preparation of Degradable Anionic SCKs (aSCKs)

Briefly, polyphosphoester-based anionic amphiphilic diblock copolymer was synthesized from poly(2-ethylbutyl phospholane)-*b*-poly(butynyl phospholane) by thiol-yne reaction with 3-mercaptopropanoic acid. The aSCKs were constructed by the direct dissolution of so formed anionic amphiphilic diblock copolymer in water and followed by covalent crosslinking throughout the shell region^[16]. D_{av} (TEM) = 16 ± 3 nm, D_h (DLS, number) = 16 ± 4 nm; D_h (DLS, volume) = 19 ± 6 nm; D_h (DLS, intensity) = 25 ± 8 nm.

Preparation of Degradable Zwitterionic SCKs (zSCKs)

Briefly, polyphosphoester-based zwitterionic amphiphilic diblock copolymer was synthesized from poly(2-ethylbutyl phospholane)-*b*-poly(butynyl phospholane) by thiol-yne reaction with cysteine hydrochloride monohydrate. The zSCKs were constructed by direct dissolution of the formed zwitterionic amphiphilic diblock copolymer in water and followed by covalent crosslinking throughout the shell region. D_{av} (TEM) = 21 ± 5 nm, D_h (DLS, number) = 23 ± 6 nm; D_h (DLS, volume) = 31 ± 12 nm; D_h (DLS, intensity) = 55 ± 22 nm.

Preparation of [^{111}Ag] Acetate and [^{111}Ag]SCC1

^{111}Ag acetate was prepared by adding 1 mL of 0.24% acetic acid into a vial containing dried ^{111}Ag . Synthesis of the carbene compound known as imidazolium complex 1, IC1 (a precursor for SCC1), was accomplished as previously described by Kascatan-Nebioglu *et al.*,^[6] and the synthesis of [^{111}Ag]SCC1 was performed as previously published.^[21] Briefly, the carbene compound, IC1 (32 mg, 0.095 mmol) was dissolved in 1 mL of methanol before adding into a vial containing dried ^{111}Ag . This mixture was stirred for 10 min to resuspend the ^{111}Ag . Silver acetate (29 mg, 0.174 mmol) and 300 μL methanol was added into the ^{111}Ag -IC1 solution producing a dirty yellow mixture. After 2 h of stirring at room temperature, the residue was centrifuged at $2500\times g$ for 5 min. The product was precipitated from the supernatant with 35 mL of ice-cold diethyl ether, collected by centrifugation, and dried under air with gentle heating. Chemical yield was estimated from a non-radioactive reaction (same amount of IC1 and silver acetate as in the radioactive synthesis) performed in parallel with the radioactive reaction.

^{111}Ag Loading of Degradable Nanoparticles, aSCK and zSCK

Two different types of polyphosphoester-based degradable particles were investigated; zwitterionic Shell Cross-linked Knedel-like nanoparticles (zSCK) and anionic Shell Cross-linked Knedel-like nanoparticles (aSCK). These two differ only in the portion of the hydrophilic shell that coordinates with the Ag (I) species, Figure 1. Stock solutions of zSCK or aSCK in water (1 mL, 2.5 mg/mL) were mixed with ^{111}Ag nitrate for 10 min to re-solubilize the dried ^{111}Ag nitrate. Silver nitrate (10 μL , 25 mg/mL) was then added and the mixture stirred for 2 h at room temperature in the dark. The product was purified with an Amicon ultra centrifugal filter unit (100 kDa MWCO) and continuous washing with 3 to 4 mL of water to remove free silver and weakly bound silver. The amount of radioactivity in the flow-through was determined to monitor the breakthrough of low molecular weight ^{111}Ag species. After multiple washes, when minimal (less than 74 kBq) or no activity was observed in the flow-through washes, the [^{111}Ag]aSCK and [^{111}Ag]zSCK samples were further concentrated for mice studies.

In Vivo Studies with ^{111}Ag Antimicrobial Therapeutics

Healthy female C57BL/6 mice (28 – 30 days old, 14 – 16 g) were used for animal studies. All animal experiments described were performed according to animal use protocols approved by the Washington University Institutional Animal Care and Use Committee (IACUC). The isolated ^{111}Ag was incorporated into a carbene small molecule or SCK nanoparticles for use in *in vivo* biodistribution studies or administered as the nitrate or acetate salt. Administration of the ^{111}Ag labeled compounds in mice was achieved by aerosol delivery as described below.

Aerosol Delivery

Aerosol doses were delivered to the lungs of mice via direct nebulization of the silver carbene complex, [^{111}Ag]SCC1 or ^{111}Ag nanoparticles in a multi-dosing animal chamber. The multi-dosing chamber consisted of a square Plexiglas box with inner dimensions of 20.32 L $\times$ 20.32 W $\times$ 11.43 H cm with a nebulizer mounted in the center of the lid and

equipped on opposite sides with 4 Plexiglas tubes. The nebulizer, obtained from Aerogen, produces aerosol particles with a volume median diameter of 2.5 - 4.0 μm at a low velocity (0.1 mL/min). Animals were individually placed into the Plexiglas tubes for nose-only delivery. The dose, 200 μL (0.59 – 1.48 MBq)/ 4 mice was administered over a 5 min period, although aerosol production was observed to end at the 2 min mark. The extended time allows for the non-inhaled saturated aerosol droplets in the box to settle, avoiding air and personnel contamination. Each set of mice in the study was dosed once per time point before sacrificing for biodistribution.

Dose Determination and Biodistribution Studies

Dose was delivered to (4 mice per group) for the four different ^{111}Ag labeled compounds at different time points and one mouse was randomly selected per group and immediately sacrificed to be used as the reference dose inhaled per mouse. The amount of radioactivity in mice treated with different ^{111}Ag compounds ($n = 3$) was measured to investigate the dependence of particle type, size or charge on the total amount of radioactivity inhaled. The radioactivity in each sacrificed mouse was measured on a gamma-counter. The radioactivity in the bulk dose (200 μL total nebulized dose/ group) was also measured and used to estimate the fraction of the bulk dose inhaled per mouse. For biodistribution studies, the radioactivity measured in one sacrificed mouse per administration was used as the standard “whole body” dose for each mouse in the group. The remaining mice ($n = 3$ per group) were sacrificed at 1, 4, and 24 h after receiving aerosolized doses. Mice to be sacrificed at 4 and 24 h were kept in metabolism cages until time of sacrifice to collect feces and urine to assess excretion. The lungs and other normal organs of interest and feces and urine were collected, weighed, and measured for radioactivity content using a gamma counter. The data was background and decay corrected and expressed as the percent of inhaled dose per weight, gram (%ID/g) of each organ as calculated by normalization to the standard whole body dose. In two separate experiments, the aerosol dose was administered to 4 mice for 2 min (200 μL) and for 5 min (600 μL , 200 μL dose diluted with water). The mice were immediately sacrificed after the aerosol inhalation, the lungs and other normal organs including the head (no brain) and carcass were collected and counted on the gamma counter. The data was processed as previously described. These two experiments were performed to investigate the effect of aerosol exposure duration and the volume of bulk dose applied on the percent inhaled dose per mouse as well as the biodistribution pattern.

Autoradiography

Lungs were harvested from each mouse, weighed and immediately fixed in 4% neutral buffer formalin and then counted for radioactivity. Lungs were embedded in Cryo-M-Bed embedding compound and frozen at -30°C on a Vibratome cold snapTM. The frozen lungs were coronally cut (sliced along their vertical planes) into 40- μm slices on a Vibratome 8850 whole body cryo-microtome set at -19°C . Sixteen consecutive frozen sections were transferred unto adhesive glass slides with adhesive tape and scanned directly by electronic autoradiography (InstantImager from Packard Instrument Company) for 2 - 4 h.

Intravenous Injection

Aerosol dose administration was compared with intravenous injection (i.v.) dose administration using [^{111}Ag]SCC1. Healthy female C57BL/6 mice of the same age and size as the ones used for the inhalation study were warmed gently under heat lamp prior to receiving 15.9 – 14.4 MBq of [^{111}Ag]SCC1. This dose was prepared in a more diluted acetic acid final concentration, 0.012% v/v for tail vein administration to achieve *in vivo* tolerance in the mice. Mice (n = 3) per time point were sacrificed at 1 h and 24 h post administration. The lungs and other normal organs of interest were collected, weighed, and measured for radioactivity content using a gamma counter. The data was background and decay corrected and expressed as the percent of injected dose per weight, gram (%ID*/g) of each organ as calculated by normalization to the total activity injected into the animals. Statistical Analysis. Statistical calculations were made using GraphPad Prism. One-way analysis of variance at 95% confidence level ($p < 0.05$) was considered to be statistically significant.

RESULTS AND DISCUSSION

In this study, our objective was to investigate the silver loading capacity, biodistribution and pharmacokinetics of antimicrobial nanoparticles administered *via* direct nebulization into the lungs of mice. ^{111}Ag was chosen as a silver radiotracer due to its favorable half-life, low beta and gamma energy and ease of production. ^{111}Ag 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, 25] The average recovery of purified ^{111}Ag was $92.9 \pm 23.7\%$ from the palladium target as assessed 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 indicated that >99.9% of the palladium was removed by our purification process. The yield and physical state of the recovered ^{111}Ag was useful for radiochemical and biological experiments. ^{111}Ag was successfully incorporated into the xanthinium salt (carbene molecule), IC1 with a radiochemical yield of $25.5 \pm 2.5\%$ while the chemical yield obtained from the cold (non-radioactive) reaction performed in parallel was 70%. The product, an off-white or white powder was reconstituted in 0.24% v/v acetic acid at a final pH of 7.0 for biodistribution studies. The degradable nanoparticles having zwitterionic or anionic functional groups within their shells (zSCK and aSCK, respectively) showed excellent ^{111}Ag nitrate or acetate loading properties 72% and 92%, respectively, with good preparation reproducibility and high retention of the ^{111}Ag ions. The ^{111}Ag -loaded degradable SCKs could also be concentrated into smaller volumes thus, increasing dosing capabilities. The high binding affinities of silver cations with the thioether groups within the shell domain of aSCK and both the thioether groups and amino groups within the shell domain of zSCK, Figure 1, allow for the high silver loading and also provide for longer solution-state stability when challenged against saline solutions.

Aerosol delivery was chosen as the preferred method to administer the radiolabeled doses into the lungs due to its relevance to clinical application and non-invasiveness.^[19] Nebulizing the radiotracer ensures that the dose is delivered directly into the target organ,

the lungs, while minimizing dose uptake in other organs. The percent dose accumulation per mouse was very consistent from one set of nebulization delivery to another (1 mouse selected from each set of 3 different dosings); Table 1. The average dose inhaled per mouse was determined to be $1.07 \pm 0.12\%$ ($n=3$) of the total aerosolized bulk dose after 5 min nebulization. The excellent reproducibility in the percent dose per mouse supported our method of choosing a random mouse from each administered group as the standard dose in our biodistribution studies. No statistically significant difference in the dose accumulated was found for single or multiple aerosol administrations in the lungs of the normal mice (25.6 ± 4.6 and $23.9 \pm 9.3\%$ of total Inhaled Dose per gram (% ID/g) of the lungs, respectively).^[21] The biodistribution showed that there was a continuous clearance and excretion of the accumulated [^{111}Ag]SCC1 compound. Minimal dose accumulation was observed in the clearance organs – liver, kidney and spleen- and no statistical difference was observed for the single dose or multiple dose cohorts. Excretion of the cleared dose was observed mostly in the feces with the whole intestines showing the second highest dose after the lungs; 5.2 ± 0.8 and $2.0 \pm 0.6\%$ ID/g (g – weight of organ) for the single and multiple dose cohorts, respectively.

The dosing capabilities and pharmacokinetics were investigated with the ^{111}Ag labeled compounds after single aerosol dose administration. The amount of radioactivity in each organ as a percent inhaled dose per gram administered per animal at 1, 4 and 24 h post administration (p.a.) for ^{111}Ag acetate, [^{111}Ag]SCC1 and ^{111}Ag loaded degradable SCKs is shown in Figure 2. The highest radioactivity concentrations were found in the lungs, digestive system (stomach, small intestine and large intestine) and feces for the ^{111}Ag labeled compounds. [^{111}Ag]SCC1 showed the fastest clearance and thus, the lowest dose in the lungs, $21.6 \pm 0.8\%$ ID/g, $16.6 \pm 2.7\%$ ID/g and $9.3 \pm 0.7\%$ ID/g at 1, 4 and 24 h p.a., respectively or $2.6 \pm 0.3\%$, $2.1 \pm 0.2\%$ and $1.0 \pm 0.2\%$ of the total dose inhaled by the mouse, Figure 2. These values are equivalent to $0.027 \pm 0.003\%$, $0.021 \pm 0.002\%$ and $0.010 \pm 0.002\%$ of the total nebulized dose at 1, 4 and 24 h p.a. respectively.

The amount of radioactivity residing in the lungs of animals that received [^{111}Ag]aSCK and [^{111}Ag]zSCK was approximately twice as much at all time points as shown in Figure 3a. Although ^{111}Ag acetate exhibited the highest lung dose accumulation, there was no significant difference between the time points with a rather large data scatter, which suggests precipitation of the Ag ions by biological chloride ions.^[26, 27] Significant clearance of activity from the lungs after 24 h p.a. was observed for [^{111}Ag]SCC1, [^{111}Ag]aSCK and [^{111}Ag]zSCK. For all ^{111}Ag compounds, there was very minimal activity (10-50 times lower than the lung dose) observed in other organs (liver, kidney, blood, bone, etc.) with the highest dose observed in the liver (2.4% ID/g). The relatively high skin dose was attributed to handling contamination during dose administration. Significant amount of radioactivity was observed in the stomach for all ^{111}Ag labeled compounds, which was observed to clear through the intestines (increasing radioactivity dose observed in the large intestine with time) and subsequently excreted in the feces.

The degradable SCK nanoparticles have potential for hydrolytic breakdown for ultimate clearance *in vivo*. They also showed better silver loading capacity, thus more silver ions could be administered per volume/ weight of nanoparticle. Although [^{111}Ag]SCC1 exhibited

a similar biodistribution trend as the [^{111}Ag]SCK nanoparticles, the SCKs had twice as much dose of $^{111}\text{Ag}^+$ residing in the lungs at 1 h, Figure 3a. Also, the rate of clearance of [^{111}Ag]SCC1 was significantly faster than the SCKs which showed no significant difference in accumulation at 1 and 4 h. Higher dose retention at 24 h p.a. was observed in the lungs of mice that received [^{111}Ag]SCKs with 62 – 68% of the initial 1 h dose, whereas 43% of [^{111}Ag]SCC1 was observed in the lungs at this time point. In two separate experiments, the aerosol dose was administered to 4 mice for 2 min (200 μL) and for 5 min (600 μL). There was no significant difference in the dose retained in the lungs or the biodistribution pattern of the mice that received the 2 min or 5 min aerosol administration, Figure 3b. Immediate accumulation of the aerosol particles was observed in the gastrointestinal tract as early as 2 min post inhalation. This could be important for other drug administration through inhalation, thus close attention to potential toxicity or immune response arising from the gut must be considered. The autoradiographs of the lung sections showed homogeneous uptake of the ^{111}Ag labeled compounds with dose accumulation more prominent at the core of the lung sections, Figure 4. The intensity of the autoradiographs was in good agreement with the amount of dose accumulated (% ID/g) in the lungs per administered ^{111}Ag compound.

Further biodistribution studies were performed to compare aerosol dose delivery with intravenous (tail vein) injection. As expected, higher dose uptake was observed in the lungs of mice that inhaled the radiolabeled doses when compared to the mice that received i.v. injections of [^{111}Ag]SCC1, Figure 5. Mice that received tail vein injections show significant accumulation of dose in the clearance organs especially in the liver. There was no significant difference in the dose retained in the liver of the i.v. mice at 1 h and 24 h, while high amount of activity was not observed in the gastrointestinal tract (large intestine) until 24 h post injection. The activity in the gastrointestinal tract is likely due to clearance. The results obtained from the tail vein injections suggests that the aerosol delivery provides a better dose delivery route for lung infection drugs while limiting systemic drug exposure. The superior loading and dosing capabilities of degradable SCKs as evident in the biodistribution show that these nanoparticles are ideal carriers, which provide sustained release of the encapsulated silver ions into the lungs with the additional possibility of nanoparticle cellular uptake. Moreover, neutral or negatively-charged small-sized molecules administered for lung infections have been shown to clear rapidly from the lungs,^[19] thus there is a need for larger, biocompatible and biodegradable vehicles with improved pharmacokinetics. Studies from collaborators and corroborated in our lab have suggested that the degradable SCK nanoparticles allow for a slow release of the therapeutic Ag^+ in *in vivo* studies. Shah and co-workers have published *in vivo* data showing SCKs loaded with Ag^+ and or SCC1 required lower amounts of Ag^+ (up to 16-fold less) and a lower number of administered doses to achieve the same therapeutic outcome as SCC1 only.^[12] In another study, SCC10, a derivative of SCC1, encapsulated into L-tyrosine polyphosphate (LTP) nanoparticles also exhibited improved drug dosing, sustained release and better mice survival outcomes when compared with the non-capsulated SCC10.^[11]

Autoradiography showed that the ^{111}Ag loaded carbene and SCK nanoparticles diffused immediately throughout the lungs after administration with dose accumulation evident in the core of the lung sections. This shows that the dose was delivered beyond the trachea and

per administration with less need for repeated dosages in therapeutic applications. Mucociliary clearance was the dominant mechanism, as observed by the high radioactivity counts in the feces as compared to the excreted urine, as well as an absence of radioactivity in the blood. Biodistribution of ^{111}Ag radiotracers in mouse infection models are currently under investigation. Although this study represents an important demonstration of the utility of ^{111}Ag as a useful tool for monitoring the pharmacokinetics of silver loaded antimicrobials *in vivo*, combination with a second label to track the fate of the nanoparticles themselves is needed to fully understand the mechanism and kinetics by which the ^{111}Ag species are released from the nanoparticles to undergo PK and bioD as a small molecule *vs.* trafficking of the ^{111}Ag while still loaded within the nanoparticle carrier.

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

TABLE 1Dose inhaled per mouse after 5 min administration of nebulized ^{111}Ag labeled compound

^{111}Ag Species	Mouse Dose (CPM)	Nebulizer Bulk Dose (CPM)	Percent Inhaled Dose/ Mouse ^a
[^{111}Ag]SCC1	21516	2113550	1.02
[^{111}Ag]aSCK	53842	5433667	0.99
[^{111}Ag]zSCK	47249	3897300	1.21

^a Average accumulated dose was calculated from the three compounds to be $1.07 \pm 0.12\%$ inhaled dose per mouse.