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Original article

Influence of chitosan coating on the oral bioavailability of gold nanoparticles in rats

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ABSTRACT

Gold nanoparticles are one of the most extensively investigated metallic nanoparticles for several applications. It is less toxic than other metallic nanolattices. The exceptional electrical and thermal conductivity of gold make it possible to be administered as non-invasive radiofrequency irradiation therapy that produces sufficient heat to kill tumor cells. Nanoparticles are generally administered intravenously instead of orally due to negligible oral absorption and cellular uptake. This study evaluated the oral bioavailability of gold nanoparticles coated with chitosan (C-AuNPs), a natural mucoadhesive polymer. We employed traditional method of evaluating bioavailability that involve estimation of maximum concentrations and area under the curve of 3 nm chitosan coated gold nanoparticles (C-AuNPs) in the rat plasma following intravenous and oral administrations (0.8 mg and 8 mg/kg body weight respectively). The oral bioavailability of C-AuNPs was found to be 2.46% (approximately 25 folds higher than polyethylene glycol (PEG) coated gold nanoparticles, reported earlier). These findings suggest that chitosan coating could be better than PEG coating for the enhancement of oral bioavailability of nanoparticles.

1. Introduction

Nanotechnology has emerged as an attractive research technology with potential adaptability in several areas including agriculture, biomedical engineering, cosmetology, disease diagnosis, drug therapy and energy. Nanotechnology has been used in several industries to develop materials with substantially improved properties, functionalities and utilities. The pharmaceutical, biotechnology, biomedical engineering and material sciences industries have developed nanotechnology-based materials, such as nanocrystals (Seabra et al., 2018), carbon nanotubes (Liu et al., 2009), quantum dots (Hassan et al., 2018), metallic nanoparticles (Lohse and Murphy, 2012) and polymeric nanoparticles (Kumari et al., 2010) for use as diagnostics, therapeutics or drug delivery carriers. Among these nanomaterials, nanoparticles have been studied extensively as therapeutics or as nano-therapeutic carriers for small or large drug molecules, including genetic materials and biologics such as DNA (Kumari and Kondapi, 2018), RNA (McCall et al., 2017), proteins (Lee and Cho, 2017) and enzymes (Mokhtari et al., 2017).

Nanoparticles are nano-sized objects of any shape where all three dimensions occupy 1–100 nm (Auffan et al., 2009). This ultrafine size imparts unique physicochemical properties that are not exhibited by bulk material. Nanoparticle preparations are generally administered intravenously. However, rapid clearance by the reticuloendothelial system decreases their circulatory

Abbreviations: AUCcall, area under curve calculated till last quantifiable time point; AUCINF, area under curve extrapolated till infinite time; AUCiv, Area Under the Plasma Concentration-Time Curve of intravenous administration; AUCpo, Area Under the Plasma Concentration-Time Curve of oral administration; C-AuNPs, gold nanoparticles coated with chitosan; CL, clearance; Cmax, maximum concentration of gold nanoparticles in blood; F_AUCINF, bioavailability calculated from Area Under the Plasma Concentration-Time Profile from Time 0 to Infinity; T ½, biological half-life (time required to eliminate half amount of drug from body); Vd, volume of distribution.

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half-life, and the formulated drug is often eliminated before reaching its target. Oral administration of nanoparticles is also problematic due to negligible oral absorption and cellular uptake that are likely caused by the absorption of the nanoparticles through passive transport. Absorption and uptake are significantly influenced by nanoparticle size, with smaller nanoparticles exhibiting better oral absorption than bigger ones (Florence et al., 1995; Geiser et al., 2005; Hillyer and Albrecht, 2001).

Metallic nanoparticles have been investigated extensively due to easy fabrication, relatively small sizes with small polydispersity index when compared with polymeric nanoparticles. The very tiny metallic particles with the corresponding very large effective surface area are highly reactive but potentially toxic as well (Kirchner et al., 2005; Lanone and Boczkowski, 2006). However, gold nanoparticles have been found to be biocompatible and comparatively less toxic (Connor et al., 2005). Gold nanoparticles are extensively investigated as nanotherapeutics, diagnostics and imaging probes (Huang et al., 2006; Patra et al., 2007), intracellular drug delivery carriers (Gibson et al., 2007), intracellular gene regulators (Rosi et al., 2006) and tumor-targeting therapeutics (Malugin and Ghandehari, 2010). Gold nanoparticles are also excellent conductors of electrical and thermal energy, making it possible to administer non-invasive radiofrequency irradiation with sufficient heat to kill tumor cells (Huang et al., 2006; Kong et al., 2008).

Gold nanoparticles can be fabricated easily in very small size such as 1 nm with a very small polydispersity index employing simple and rapid preparation method that is commercially viable. In addition, gold nanoparticles possess characteristics that make them suitable for pharmacokinetic evaluation after oral administration, such as high electron density, low background concentration and poor solubility in biological matrices (Hinkley et al., 2015).

We have previously investigated the oral bioavailability of gold nanoparticles in rats, wherein, gold nanoparticles of approximately 5 nm were coated with 1, 2 and 5 kDa of polyethylene glycol (PEG) (Alalaiwe et al., 2017). PEG coating was supposed to enhance the oral bioavailability of the nanoparticles as it known to minimize the immune system recognition and elimination, thereby increasing circulatory half-life (Alalaiwe et al., 2017). Furthermore, PEG coating has also been reported to minimize the agglomeration of nanoparticles (Hinkley et al., 2015). Bioavailability of low molecular weight PEG coated gold nanoparticles (1 kDa of PEG) was approximately 3–4 fold higher than those coated with 2 and 5 kDa of PEG. However, it was still too low to produce any clinically significant effect. Therefore, we proposed coating of gold nanoparticles with chitosan, a polysaccharide-based muco-adhesive polymer with absorption enhancement capabilities, to enhance their oral bioavailability.

Chitosan is a natural nontoxic biodegradable macromolecule consisted of glucosamine and N-acetyl-glucosamine (Hirano, 1996; Hejazi and Amiji, 2003; Singla and Chawla, 2001). It has been used to formulate particulate systems, such as liposomes and nanoparticles, wherein drug candidates are encapsulated in the polymer membrane. Chitosan's positively charged surface tends to bind to negatively charged cell membranes. It binds with occludin, redistributes F-actin, disrupts the plasma membrane, and decreases the trans-epithelial electrical resistance (TEER) of cells thereby increasing transcellular and paracellular diffusion of the enclosed drugs (Artursson et al., 1994; Dodane et al., 1999; Schipper et al., 1996; Schipper et al., 1997; Thanou et al., 2001). Chitosan's muco-adhesive properties that enhance the diffusion or absorption of the enclosed drugs by prolonging the gastric residence time (Behrens et al., 2002; Kockisch et al., 2003). Due to low toxicity, biodegradability, pH responsiveness and muco-adhesion, chitosan coated systems have been used as effective oral delivery carriers for numerous drugs (Akhtar et al., 2012; Mohammed et al., 2017), proteins and peptides (Ansari et al., 2016; Kawashima et al., 2000; Makhlof et al., 2011; Takeuchi et al., 2005; Zhang et al., 2010) and non-viral genes, DNA and nucleic acid vaccines (Chew et al., 2003; Mao et al., 2001).

To the best of our knowledge, chitosan coating of AuNPs to improve oral bioavailability has not been reported. Some chitosan derivatives have been used to coat AuNPs or gold nanorods for imaging, probing, or anticancer applications (Chen et al., 2013; Duan et al., 2014; Sun et al., 2014). Chen et al., (2013) reported chitosan-coated gold nanoparticles as a sensitive probe for the recognition of heparin. Doxorubicin loaded and thiolated-chitosan coated Gold nanorods were evaluated in vitro for their combined chemical and photothermal effects on cancer cell lines (Duan et al., 2014). In another report, glycol chitosan coated gold nanoparticles (GC-AuNPs) were evaluated for tumor targeting using computed tomography. The biodistribution of the GC-AuNPs was compared with heparin-coated nanoparticles (Sun et al., 2014).

The aim of this study was to investigate the influence of chitosan coating on the pharmacokinetics and bioavailability of nanoparticles after administering coated or uncoated nanoparticles in the rats. We employed the classical method that involved comparisons of blood concentrations of gold (Cmax) and area under the curve (AUC) after oral & intravenous administrations.

2. Methodology

2.1. Preparation and coating of gold nanoparticles

Gold nanoparticles of approximately 3 nm were fabricated by the method reported elsewhere (Alalaiwe et al., 2017). The nanoparticles, which formed rapidly upon addition of the sodium borohydride, were then allowed to stand overnight prior to coating. Chitosan (Aldrich) was dissolved in deionized water until saturated. The prepared gold nanoparticles were incubated with 1 mL of the saturated chitosan solution under vigorous stirring. The coated nanoparticles were acidified using 0.1 M HCl (Fisher ACS Grade) until the suspension of chitosan-coated nanoparticles (C-AuNPs) was stable.

2.2. Nanoparticle characterization

Nanoparticles were characterized for size, shape and zeta potential similarly as described elsewhere (Alalaiwe et al., 2017). Particle size (hydrodynamic radius) and size-distribution were investigated by Nanotrac with DLS technique (Microtrac, Inc., Montgomeryville, PA), whereas zeta potential was evaluated by using ZetaPlus, Brookhaven. The size and shape of AuNPs were further confirmed by transmission electron microscope (Hitachi H7000).

2.3. In vivo study

In vivo pharmacokinetic bioavailability of coated and uncoated gold nanoparticles were investigated in Male Wistar rats as per the approved protocol by the University of Florida Institutional Animal Care and Use Committee. Rats were treated caringly as per the guidance provided in the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, 1985). All the animals between 180 and 220 g, were divided in two groups of five each and caged individually in the standard environment (18–26 °C, and 30–70% humidity), with free access to water and standard diet. Animals in group one received 8 mg/kg body weight of coated nanoparticles through oral gavage after 12 h of fasting which continued two hours post dose. Animals in group two received 0.8 mg/kg body weight of coated gold nanoparticles via the jugular

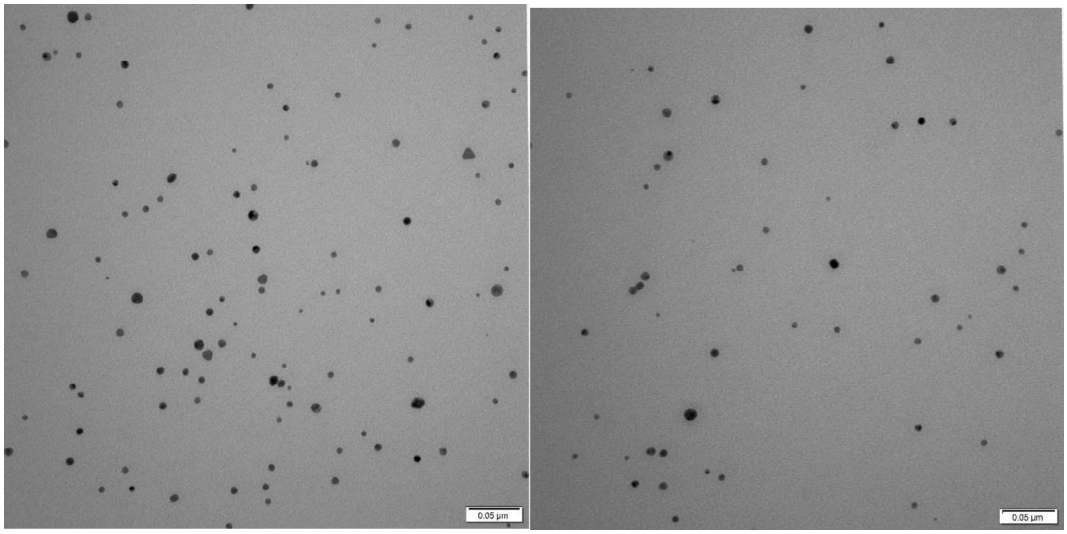

Fig. 1. Particle Characterization: Transmission electron micrograph (TEM) of chitosan coated 5 nm particles. One-hundred particles were measured to determine the average primary size to be 3 ± 1.2 nm.

cannula. Blood samples were extracted at 0, 0.5, 1, 2, 4, 8, 12, 24 and 48 h post dose.

2.4. Determination of gold concentrations in blood

The concentration of gold nanoparticles in blood was determined by Inductive coupled plasma mass spectrometry (Thermo Electron X Series II ICP-MS, Thermo Scientific, West Palm Beach, FL) Briefly, 0.2 mL blood was processed with nitric acid, Hydrogen peroxide and aqua regia, followed by filtration, 0.22 mm PTFE and determination using indium as internal standard (Alalaiwe et al., 2017).

2.5. Estimation of bioavailability of gold nanoparticles

Bioavailability of gold nanoparticles were estimated using pharmacokinetic parameters such as maximum blood concentration (Cmax) and area under the curve (AUC) of blood concentration versus time profiles. Non-compartmental analysis of concentration versus time data was done by using Phoenix (Pharsight, Mountain View, CA). Oral bioavailability of gold nanoparticles (F) was determined by using the formula given below

$$F = AUCpo \times Dose\,iv / AUC\,iv \times Dose\,po$$

2.6. Statistical evaluation

Statistical evaluation of concentration data were carried out on Prism (GraphPad Software Inc., La Jolla, CA). The concentrations of gold in the blood are presented as a mean ± SD. One-way ANOVA and Tukey's test were used to determine the differences that were considered as statistically significant (p value ≤ 0.05).

3. Results

3.1. Size, shape and morphology of gold nanoparticle

The chitosan coated gold nanoparticles were found to have an average size of 3 ± 1.2 nm. Zeta potentials for the C-AuNPs in water were indistinguishable from the neutral measurement: 1.3 ± 0.9 mV. Size and shape of gold nanoparticles were confirmed by TEM (Fig. 1).

3.2. Oral bioavailability of gold nanoparticles

As the animals in this study received either oral or intravenous doses of the nanoparticles, the comparative oral bioavailability was

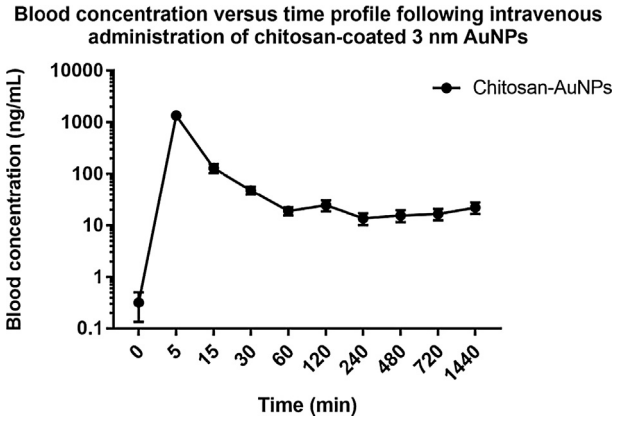

Fig. 2. The concentration vs time profile of IV data of 5 nm AuNPs of chitosan coated. Gold concentration was measured using ICP-MS. Data are presented as average ± SD.

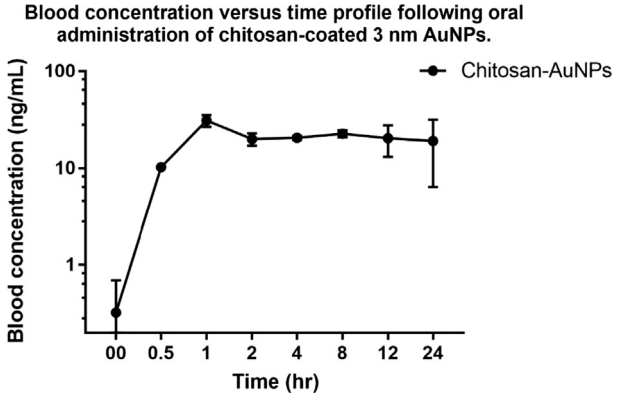

Fig. 3. The concentration vs time profile of ORAL data of 5 nm AuNPs of chitosan coated. Gold concentration was measured using ICP-MS. Data are presented as average ± SD.

Table 1
Pharmacokinetic parameters of gold nanoparticles.

Route	N	T 1/2 (h)	C max (ng/ml)	AUCcall (h*ng/ml)	AUCINF (h*ng/ml)	Vd (ml)	CL (ml/h)	Tmax (h)
IV (0.8 mg/kg)	N1	9.38	1170.6	698.1	824.8	3282	242.4	
	N2	8.22	1369.6	750.3	840.4	2832.8	237.9	
	N3	10.68	1528.2	976.4	1191.4	2586.8	167.8	
	Mean	**9.43**	**1356.1**	**808.27**	**952.1667**	**2900.5**	**216.03**	
	SD	**1.230**	**179.179**	**147.918**	**207.329**	**352.6**	**41.831**	
ORAL (8 mg/kg)		28.11	6.54	105	237.7	341283.3	8414.66	13
		50.02	5.61	92.1	312.93	461210.4	6391	12
		18.74	4.52	84.91	151.85	356243.7	13170.2	11
	Mean	**32.29**	**5.57**	**94.003**	**234.16**	**386,245**	**9325.2**	**12**
	SD	**16.05**	**1.011**	**10.179**	**80.598**	**65350.8**	**3480.1**	**0.12**
F_AUCINF	**2.46%**							

N- number of animals, T½: half-life; Cmax: Maximum concentration of gold nanoparticles in blood, **AUCcall:** Area under curve calculated till last quantifiable time point; **AUCINF:** Area under curve extrapolated till infinite time; CL: Clearance; Vd: volume of distribution. **F_AUCINF**: bioavailability calculated from Area Under the Plasma Concentration-Time Profile from Time 0 to Infinity.

estimated as the ratio of average AUCpo and AUCiv calculated from gold concentration-time profiles, Figs. 2 and 3, respectively. Results of pharmacokinetic evaluations are presented in the Table 1. The oral bioavailability for the C-AuNPs was estimated as 2.46%.

4. Discussions

Bioavailability assessments of metal-based nanoparticles have mainly utilized nanoparticle measurements in matrices such as urine and feces. However, biliary excretion of metallic nanoparticles are documented, which could lead to inaccurate bioavailability estimations (Hinkley et al., 2015; Li et al., 2016). There are very limited number of studies that utilized comparative AUC/Cmax after oral and intravenous doses for estimating oral bioavailability. Lipka et al. (2010) and Park et al. (2011) estimated oral bioavalibilities of ZnO nanoparticles and citrate-coated silver nanoparticles respectively in the rats.

Hinkley et al., 2015, reported oral bioavailability gold nanoparticles of about 23 nm with or without coating with PEG in mice. However, they did not report pharmacokinetic parameters as they collected samples form blood and tissues at three time points only (6, 12, 24 h post dose). Moreover, they did not compare it with IV administration, rather compared it with the amount they administered. They found significantly low bioavailability of uncoated gold nanoparticles due to substantial agglomeration when compared with coated ones which prevented agglomeration. However, oral bioavailability of coated gold nanoparticles was still very low (1% of administered dose). One of the reasons of low oral bioavalibilities of PEG coated particles has been attributed to the reduction in opsonization due to the presence of PEG thus leading to minimized phagocytosis or cellular uptake (Gref et al., 2000). Furthermore, the extent of cellular uptake is reported to be dependent on the size of the particles, and smaller particles are absorbed better than larger particles (Hillyer and Albrecht, 2001; Win and Feng, 2005).

We have already investigated the effect of PEG coating on the oral bioavailability of gold nanoparticles (Alalaiwe et al., 2017). We found an inverse relationship between the PEG molecular weight used in the coating and the oral bioavailability achieved. However, the highest oral bioavailability achieved with 1 kDa PEG coating was only 0.1%. The present investigation reported the oral bioavailability of Chitosan coated gold nanoparticles (C-AuNPs) by comparing AUCinf after intravenous and oral administration of 0.8 and 8 mg/kg body weight of rats respectively. Chitosan, a mucoadhesive polymer, is known to enhance permeation or absorption thus improving oral bioavailability (Fonte et al., 2011). The chitosan-coating resulted in 2.46% oral bioavailability, approximately 25-fold higher than that achieved with the PEG coatings.

5. Conclusions

The estimation of oral bioavailability of the chitosan coated gold nanoparticles in rats by using conventional method wherein pharmacokinetics parameters such as Cmax, AUC, Vd, CL etc. following oral administration were compared with those of achieved following intravenous administration. Results obtained in this report were promising as compared to our previous reports. However, it is still too low to depict any clinically significant effect for therapies. We plan to have more investigation on the chitosan coating and agglomeration of the gold nanoparticles in near future.

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Conflict of interest

We declare that there was no conflict of interest in this investigation.

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