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In Vivo distribution dynamics of Gold Nanoparticles: A quantitative analysis

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Abstract: This study evaluated the biodistribution of gum arabic-functionalized gold nanoparticles (GA-AuNPs) in Balb/C Nude mice following intravenous administration. Two activity regimens (200 μCi and 600 μCi) were analyzed at two different time points (3 and 24 hours post-injection). The results showed predominant accumulation of GA-AuNPs in the liver, spleen, and gallbladder, suggesting hepatobiliary excretion as the primary clearance route. A reduction in liver uptake after 24 hours indicates potential nanoparticle metabolism or elimination. Although this study focused on intravenous delivery, previous work from our group using BSA-coated AuNPs administered intratumorally demonstrated higher tumor retention and reduced systemic accumulation, reinforcing the importance of administration route and surface coating in defining nanoparticle biodistribution. These findings contribute to the understanding of how delivery strategy influences nanoparticle bioavailability and support the development of safer and more targeted therapeutic platforms.

Keywords: Gold Nanoparticles (AuNPs), Nanobrachytherapy, Gum Arabic (GA), Biodistribution.

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Dinâmica de distribuição *in vivo* de Nanopartículas de Ouro: Uma análise quantitativa

Resumo: Este estudo avaliou a biodistribuição de nanopartículas de ouro funcionalizadas com goma arábica (GA-AuNPs) em camundongos Balb/C Nude após administração intravenosa. Dois regimes de atividade (200 μCi e 600 μCi) foram analisados em dois tempos distintos (3 e 24 horas pós-injeção). Os resultados demonstraram acúmulo predominante das GA-AuNPs no fígado, baço e vesícula biliar, sugerindo a excreção hepatobiliar como principal via de eliminação. A redução da captação hepática após 24 horas indica possível metabolismo ou eliminação das nanopartículas. Embora este estudo tenha se concentrado na via intravenosa, trabalhos anteriores do nosso grupo com AuNPs revestidas com albumina bovina (BSA) administradas por via intratumoral evidenciaram maior retenção tumoral e menor acúmulo sistêmico, ressaltando a importância da via de administração e do tipo de revestimento na distribuição das nanopartículas. Esses achados contribuem para o entendimento da influência da administração na biodisponibilidade dos nanomateriais e para o aprimoramento de sistemas terapêuticos mais seguros e direcionados.

Palavras-chave: Nanoparticulas de Ouro (AuNPs), Nanobraquiterapia, Goma Arábica (GA), Biodistribuição.

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1. INTRODUCTION

Biomedical nanotechnology has advanced significantly in recent decades, offering innovative tools for the diagnosis and treatment of various diseases. Among these tools, gold nanoparticles (AuNPs) have stood out due to their unique physical and chemical properties, making them suitable for a variety of medical applications, including imaging diagnostics, photothermal therapy, and controlled drug delivery [1, 2].

Surface functionalization is a critical factor in modulating the biological interactions and biodistribution of AuNPs. Among various coating agents, gum arabic (GA), a natural biopolymer, has attracted attention due to its biocompatibility, stabilizing properties, and influence on *in vivo* distribution [3, 4].

Khlebtsov and Dykman (2011) demonstrated that natural polymers such as GA and bovine serum albumin (BSA) offer good colloidal stability and low toxicity, with predominant accumulation in the liver and spleen behavior that may be beneficial for targeting the mononuclear phagocyte system. In contrast, synthetic coatings like polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP) promote prolonged systemic circulation and reduced hepatic uptake, which are advantageous for systemic drug delivery strategies [5].

Expanding this discussion, Rosero et al. (2024) conducted a comprehensive review of gold nanoparticle functionalization strategies, emphasizing the versatility of GA as a natural coating with strong potential for drug conjugation, physiological stability, and application in radiolabeled nanoparticle systems [6].

Previous studies indicate that intravenously administered AuNPs tend to accumulate primarily in the liver and spleen, due to macrophage recognition by the mononuclear phagocyte system. However, the excretion pathway particularly hepatobiliary clearance has

emerged as a relevant topic, as it directly impacts the therapeutic safety and elimination of nanoparticles [6–8].

Although intratumoral administration has been shown in previous studies to result in more favorable biodistribution profiles, with reduced accumulation in vital organs and greater tumor retention, this study does not directly evaluate that route. Nonetheless, it draws on such findings for comparative discussion. For instance, our group previously demonstrated that BSA-coated AuNPs delivered intratumorally exhibited enhanced tumor retention and reduced systemic toxicity [9].

In this context, the present study investigates the biodistribution of gum arabic-coated gold nanoparticles (GA-AuNPs) in healthy Balb/C Nude mice using two radioactivity regimens (200 μCi and 600 μCi) and two evaluation time points (3 and 24 hours after intravenous administration). The findings aim to improve our understanding of systemic nanoparticle distribution and support the development of safe and targeted nanomedicine strategies.

2. MATERIALS AND METHODS

2.1. Preparation of Gum Arabic-Functionalized Gold Nanoparticles

Gold nanoparticles (AuNPs) were synthesized following the protocol described by Souza et al. (2022) [11]. To ensure isotopic purity, gold irradiated in the EA-R1 nuclear reactor at IPEN was used. The synthesis was conducted using chloroauric acid ($H^{198}AuCl_4$) in a closed system with air filtration to prevent contamination.

The reagents used in the synthesis included nitric acid, sodium hydroxide, sodium citrate, gum arabic, and ultrapure water. Functionalization of the AuNPs with gum arabic was carried out at 100°C. Initially, an aqueous solution of gum arabic was prepared and added

to the AuNPs under constant stirring. Subsequently, NaOH and sodium citrate were added to complete the formation of the nanoparticles (NPs).

The resulting GA-AuNPs exhibited a spherical morphology with a mean diameter of 19 ± 4 nm and a zeta potential of −28.3 ± 1.7 mV, indicating good colloidal stability. Each animal received 100 μL of the nanoparticle solution, corresponding to an estimated gold content of 10 μg.

2.2. Characterization of Nanoparticles

The GA-functionalized AuNPs were characterized using various techniques to confirm their functionalization and determine their physicochemical properties. UV-Vis absorption spectroscopy was used to analyze the formation and stability of the nanoparticles. Transmission electron microscopy (TEM) was employed to determine the size and morphology of the particles. Zeta potential analysis was conducted to evaluate the colloidal stability of the GA-AuNPs in suspension.

2.3. Selection of Animals and Administration Protocols

Male Balb/C Nude mice, weighing between 20 and 25 grams, were obtained from the animal facility of the Institute for Energy and Nuclear Research (IPEN). The animals were kept under controlled conditions of temperature (22±2°C) and a 12/12-hour light/dark cycle, with free access to water and food. All experimental procedures were approved by the Institutional Animal Care and Use Committee (Protocol Nº 243/19).

Each animal was injected with 100 μL of GA-AuNP solution via the tail vein. The groups were organized according to dosage and post-injection time (200 μCi and 600 μCi evaluated at 3 and 24 hours). After administration, the animals were anesthetized with isoflurane, and a 30 μL blood sample was collected by the retro-orbital method.

2.4. Sample Collection and Analysis

The collected organs (blood, heart, lungs, liver, kidneys, gallbladder, spleen, stomach, small intestine, large intestine, pancreas, bones, muscles, brain, fat, and bladder) were weighed and subsequently stored in labeled tubes and analyzed quantitatively with a gamma counter (Gama-2470 Automatic Gamma Counter by PerkinElmer) to evaluate the biodistribution of the nanoparticles (%ID/g). This protocol was repeated for all animals at the determined times (3 and 24 hours post-administration).

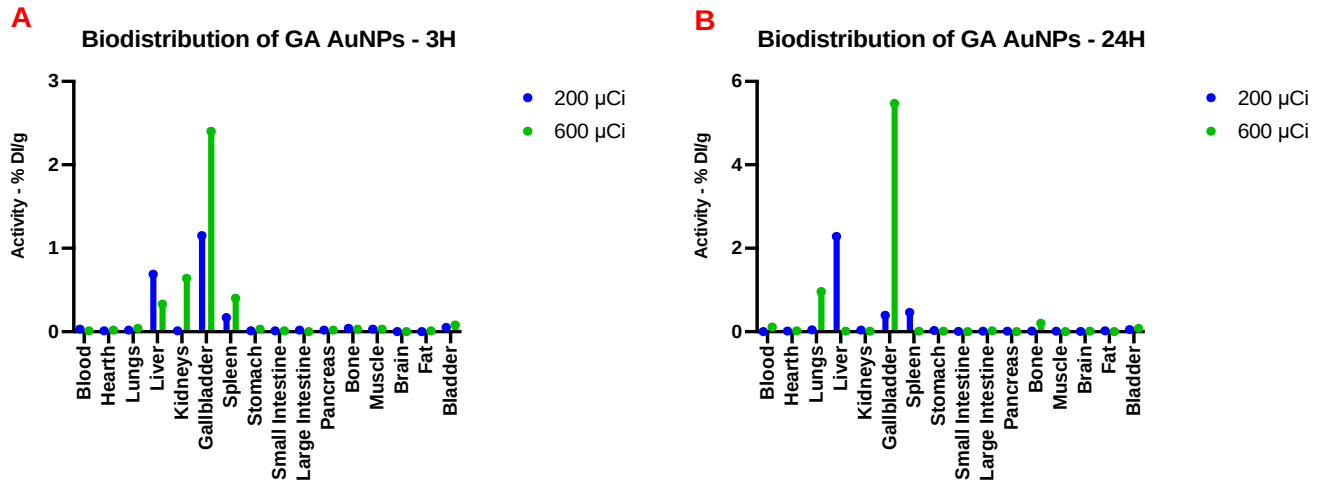
2.5. Statistical Analysis

The data obtained were expressed as mean ± standard deviation (SD). Differences between groups were evaluated using analysis of variance (ANOVA) followed by Tukey's post-hoc test. The level of significance was set at $p < 0.05$. All statistical analyses were performed using GraphPad Prism software version 8.0.

3. RESULTS AND DISCUSSIONS

This study brings important contributions to the understanding of the biodistribution of intravenously administered GA-AuNPs, especially in the context of surfactant-free formulations.

Figure 1: Biodistribution of Gum Arabic-Functionalized Gold Nanoparticles (AuNPs GA) in Balb/C Nude mice. **Panel A** shows biodistribution 3 hours post-intravenous injection, and **Panel B** at 24 hours. Blue bars represent 200 µCi, and green bars represent 600 µCi. Significant uptake is observed in the liver and gallbladder, with time-dependent changes indicating nanoparticle distribution and clearance dynamics.



Source : Authors.

The preferential accumulation in the gallbladder suggests an excretion route for GA-AuNPs or a sequestration mechanism by the organ. This finding aligns with previous studies indicating that nanoparticles can be excreted via the biliary route, especially at higher dosages . Significant liver uptake corroborates existing literature identifying the liver as a primary site for nanoparticle accumulation following intravenous administration, likely due to opsonization and subsequent phagocytosis by the reticuloendothelial system (RES). The variation in biodistribution between doses indicates that the injected quantity of GA-AuNPs directly influences the distribution profile within the body.

To complement the graphical analysis, quantitative biodistribution data are presented in Table 1, expressed as the percentage of the injected dose per gram of tissue (%ID/g). At 3 hours post-injection, liver accumulation reached 0.69 and 0.33 %ID/g for the 200 µCi and 600 µCi doses, respectively. After 24 hours, hepatic uptake increased to 2.285 %ID/g in the 200 µCi group, while the gallbladder showed a remarkably high value of 5.47 %ID/g in the 600 µCi group, suggesting active hepatobiliary excretion. The spleen also exhibited consistent accumulation levels. These quantitative results reinforce the visual patterns observed in the graphs and contribute to a more robust understanding of the in vivo behavior of GA-AuNPs.

Table 1. Biodistribution of gum arabic-coated gold nanoparticles (GA-AuNPs) in Balb/C Nude mice at 3 and 24 hours post-injection. Data are expressed as the percentage of the injected dose per gram of tissue (%ID/g), for two different activity doses (200 µCi and 600 µCi). The results demonstrate time- and dose-dependent variations in organ-specific uptake, with higher liver accumulation at lower doses and pronounced gallbladder retention at higher doses, suggesting hepatobiliary clearance.

Organ	3 hours after injection		24 hours after injection	
	200 µCi	600 µCi	200 µCi	600 µCi
Blood	0,03	0,01	0	0,11
Heart	0,01	0,02	0,015	0,02
Lungs	0,02	0,04	0,045	0,96
Liver	0,69	0,33	2,285	0,01
Kidneys	0,01	0,64	0,04	0,01
Gallbladder	1,15	2,4	0,395	5,47
Spleen	0,17	0,4	0,46	0,01
Stomach	0,01	0,03	0,03	0,01
Small Intestine	0,01	0,01	0,005	0
Large Intestine	0,02	0	0,01	0,02
Pancreas	0,02	0,02	0,01	0
Bone	0,04	0,03	0,015	0,2
Muscle	0,03	0,03	0,01	0
Brain	0	0	0,005	0,01
Fat	0	0,01	0,02	0
Bladder	0,05	0,08	0,05	0,08

Biodistribution results after 24 hours suggest clearance of GA-AuNPs from the liver, with a decrease in detected activity percentage. This could be due to biotransformation or biliary excretion of the nanoparticles. The increased activity percentage in the gallbladder for the 600 µCi samples after 24 hours further supports the biliary excretion hypothesis. These findings are crucial for understanding the fate of gold nanoparticles post-intravenous administration and can aid in designing GA-AuNPs with optimized biodistribution properties for specific clinical applications.

Additionally, the low uptake in the brain and adipose tissue is favorable from a safety perspective, minimizing the risk of adverse effects in non-target organs. This study aligns

with existing literature and enhances our understanding of GA-AuNPs interactions with biological systems, a critical aspect for advancing nanomedicine. Continued exploration of the underlying mechanisms of nanoparticle biodistribution and excretion can lead to significant advancements in targeted therapy and medical imaging.

3.1. Comparative Analysis with Previous Studies

Intratumoral administration of GA-AuNPs, as presented by Barbezan et al. (2024) [9], showed a more favorable biodistribution profile, with less accumulation in vital organs. This delivery method resulted in significantly higher concentrations in tumor cells and reduced uptake by non-target organs, as illustrated in recent results. Direct intratumoral administration ensures that a larger fraction of the administered dose remains at the tumor site, potentially increasing therapeutic efficacy and reducing systemic toxicity. Compared to intravenous administration, as performed in this study, there might be rapid absorption by the reticuloendothelial system, particularly in the liver, as observed in the 3 and 24-hour samples.

The findings align with those reported by Jakic et al. (2024) [12], where a similar biodistribution profile was observed with predominant accumulation in the liver and spleen. Furthermore, Sharon et al. (2024) [13] reported a significant uptake of gold nanoparticles in the liver, which they attributed to the RES, corroborating our results.

3.2. Therapeutic Implications

The data underscore the importance of considering the administration route in designing therapeutic strategies using gold nanoparticles functionalized with gum arabic (GA-AuNPs). Strategies that minimize exposure to non-target organs and maximize tumor delivery are essential for advancing the clinical use of nanoparticles in medicine. Furthermore, the favorable biodistribution observed with GA-AuNPs, characterized by reduced accumulation in vital organs and enhanced targeting of tumor sites, suggests a

significant potential to reduce side effects commonly associated with conventional therapies, such as hepatotoxicity and renal toxicity [14, 15].

These findings highlight the necessity of optimizing administration routes not only for more efficient treatment delivery but also to enhance patient safety and potentially improve quality of life. The comparison with data from Barbezan et al. reinforces the need to refine administration strategies to achieve safer treatments. Future studies should focus on these aspects to further validate the clinical benefits of GA-AuNPs in targeted cancer therapies [9].

4. CONCLUSIONS

The biodistribution study of radioactive gold nanoparticles functionalized with gum arabic (GA-AuNPs) in Balb/C Nude mice revealed key aspects of their *in vivo* behavior. The significant accumulation in the liver, spleen, and gallbladder highlights the involvement of the reticuloendothelial system and hepatobiliary excretion in the clearance of these nanoparticles.

Although intravenous administration is widely used in preclinical studies to assess nanoparticle biodistribution, the data obtained in this study suggest that intratumoral injection may offer a more favorable profile, with higher retention at the tumor site and reduced accumulation in vital organs. This feature may enhance therapeutic efficacy and minimize undesirable side effects.

These findings are consistent with previous reports and reinforce the potential of radioactive GA-AuNPs as promising candidates for nanobrachytherapy applications and oncological nanomedicine. Future research should focus on optimizing dosage, surface functionalization, and administration route to maximize clinical efficacy and safety.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

REFERENCES

[1] DREADEN, E. C.; ALKILANY, A. M.; HUANG, X.; MURPHY, C. J.; EL-SAYED, M. A. The golden age: gold nanoparticles for biomedicine. Chem. Soc. Rev., v 41, n 7, p 2740-2779, 2012.

[2] JAIN, S. ; HIRST, D. G. ; O'SULLIVAN, J. M. Gold nanoparticles as novel agents for cancer therapy. Br. J. Radiol., Belfast UK, v 85, n 1010, p 101-113. 2012.

[3] PATEL, S. ; GOYAL, A. Applications of Natural Polymer Gum Arabic: A Review. Int. J. Food Prop., San Diego - USA, v 18, n 5, p 986–998, 2015.

[4] KHLEBTSOV, N. ; DYKMAN, L. Biodistribution and toxicity of engineered gold nanoparticles: a review of in vitro and in vivo studies. Chem. Soc. Rev., Russia, v 40, n3, p 1647-1671, 2011.

[5] KHLEBTSOV, N.G., DYKMAN, L.A. Biodistribution and toxicity of gold nanoparticles. Nanotechnol., Russia 6, 17–42 (2011).

[6] ROSERO, W. A. A.; BARBEZAN, A. B.; DE SOUZA, C. D.; VELLOSO, J. P. R.; NASCIMENTO, S.; CARVALHO, D. S.; BERNARDES, E. S.; ZEITUNI, C. A.; VIEIRA, D. P.; SPENCER, P. J.; RIBEIRO, M. S.; ROSTELATO, M. E. C. M.

[7] Review of advances in coating and functionalization of gold nanoparticles: From theory to biomedical application. Pharmaceutics, v. 16, n. 2, p. 255, 2024

[8] ALBANESE, A. ; TANG, P. S. ; CHAN, W. C. The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annu Rev Biomed Eng, Ontario – Canada, v 14, p 1-16, 2012.

[9] SURES, K. ; BALASUBRAMANIAN, J. J. ; MANIKANDAN, J. ; ONG, C-N. ; YU, L. E. ; ONG, W-Y. Biodistribution of gold nanoparticles and gene expression changes in the liver and spleen after intravenous administration in rats. Biomaterials, Singapore, v 31, n 8, p 2034-2042, 2010.

[10] BARBEZAN, A. B. ; ROSERO, W. A. A. ; VIEIRA, D. P. ; RIGO, M. E. Z. ; SILVA, G. D. ; RODRIGUES, A. A. ; ALMEIDA, L. F. ; SILVA, F. F. A. ; RIVERA, A. G. ; SILVA, N. G. ; BERNARDES, E. S. ; ZEITUNI, C. A. ; ROSTELATO, M. E. C. M. Radioactive gold nanoparticles coated with BSA: A promising approach for prostate cancer treatment. Nanotheranostics, v 8, n 1, p 112-126, 2024.

[11] JAIN, T. K. ; REDDY, M. K. ; MORALES, M. A. ; LESLIE-PELECKY, D. L. ; LABHASETWAR, V. Biodistribution, clearance, and biocompatibility of iron oxide magnetic nanoparticles in rats. Mol. Phar., Lincoln-Nebraska, v 5, n 2, p 316-327, 2008.

[12] DE SOUZA, C. D. ; BARBEZAN, A. B. ; ROSERO, W. A. A. ; NASCIMENTO, S. ; VERGAÇAS, D. ; CARVALHO, D. S. ; ZEITUNI, C. A. ; BERNARDES, E. S. ; VIEIRA, D. P. ; SPENCER, P. J. ; RIBEIRO, M. S. ; ROSTELATO, M. E. C. M. Synthesis, In Vitro Testing, and Biodistribution of Surfactant-Free Radioactive Nanoparticles for Cancer Treatment. Nanomaterials, v 12, p 187, 2022.

[13] JAKIC, K.; SELC, M.; RAZGA, F.; NEMETHOVA, V.; MAZANEOVA, P. ; HAVEL, F. ; SRAMEK, M. ; ZARSKA, M. ; PROSKA, J. ; MASANOVA, V. ; UHNAKOVA, I. ; MAKOVICKY, P. ; NOVOTOVA, M. ; VYKOUKAL, V. ; BABELOVA, A. Long-Term Accumulation, Biological Effects and Toxicity of BSA-Coated Gold Nanoparticles in the Mouse Liver, Spleen, and Kidneys. Int. J. Nanomed., Bratislava Slovakia, v 19, p. 4103-4120, 2024.

[14] SHARON, Y. ; MOTIEI, M. ; TZROR-AZANKOT, C. ; SADAN, T. ; POPOVTZER, R. ; ROSENBAUM, E. Gold nanoparticles for safe delivery of cisplatin. SPIE, Israel, v.

Nanoscale Imaging, Sensing, and Actuation for Biomedical Applications XXI, n. 128580D, 2024.

[15] MOHAMED, S.A.; ELSHERBINI, A.M.; ALREFAEY, H.R.; ADELRAHMAN, K.; MOUSTAFA, A.; EGODAWATTHA, N.M.; CRAWFORD, K.E.; NESNAS, N.; SABRA, S.A. Gum Arabic: A Commodity with Versatile Formulations and Applications. Nanomat., v. 15, p. 290, 2025

[16] CIFTCI, F.; ÖZARSLAN, A.C.; KANTARCI, İ.C.; YELKENCİ, A.; TAVUKCUOGLU, O.; GHORBANPOUR, M. Advances in Drug Targeting, Drug Delivery, and Nanotechnology Applications: Therapeutic Significance in Cancer Treatment. Pharmaceutics, v. 17, p. 121, 2025.

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