

## Bio-distribution of Gold Nanoparticles in Tumor Mass and Different Organs in Implanted Mice with Mammary Adenocarcinoma AM3 (*in vivo* study)

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### ABSTRACT

Nanoparticles have many properties, especially in treatment of medical field, but the controversy continues about their cytotoxicity. Hence, this research was conducted to estimate the concentration of gold nanoparticles (GNPs) in tumor and other organs such as kidney, liver, and spleen after injecting GNPs by two routes, intratumoral and intraperitoneal to compare between the two methods in mice implanted with mammary adenocarcinoma for 28 days. Atomic Absorption Spectroscopy was used to measure the GNPs concentrations. The results revealed that the GNPs concentrations were significantly ( $P \leq 0.05$ ) increased ( $3.75 \pm 1.75$ ,  $2.42 \pm 0.31$  ppm) in kidney tissue after intratumoral and intraperitoneal administration, respectively, when compared to the other organs (liver and spleen), followed by tumor mass ( $2.66 \pm 0.01$ ,  $1.09 \pm 0.06$  ppm) in tissue. While the concentrations of GNPs in spleen and liver were  $1.40 \pm 0.33$ ,  $0.726 \pm 0.01$ , and  $0.602 \pm 0.03$ ,  $0.517 \pm 0.02$  after intratumoral and intraperitoneal administration, respectively. Also, the experiment showed that the injection by intratumoral method was more efficient than the intraperitoneal method for tumor treatment, and the nanoparticles were cleared by responsible lymphoid organs of body.

**Keywords:** Gold nanoparticles, *In vivo*, Bio-distribution, Intratumoral, Intraperitoneal

### Introduction

The unique properties of nanoparticles encourage the belief that they can be applied in a wide range of fields, from medical applications to environmental sciences, this can lead to increasing the quality of life via early diagnosis and treatment of disease (1). Due to the small size of nanoparticles, it can be greatly used in oncology, such as biomedical engineering for controlling invasive and metastasis behavior of melanoma cells (2). Various types of nanoparticles (NPs) have been used in delivering anticancer drugs to the site of action. This area has become more attractive in recent years due to the optimal size and negligible undesirable side effects caused by the NPs (3) as

well as in enhanced permeability and retention effect caused by leaky tumor vasculatures for better drug accumulation at the tumor sites (4). These benefits are pro-mising candidate of NPs to replace current chemotherapy, in addition, NPs can enhance the intracellular concentration of drug in cancer cells, while avoiding toxicity in normal cells (5), and due to their antioxidant activities, some NPs act against the damaging effects for some drugs (6). GNPs possess optical diagnostic and phototherapeutic applications, such as structure, shape, optics, and surface chemistry; hence, it was used in various types of biomedical application including photothermal therapy, biosensing, drug delivery, and gene therapy (7). The surface plasmon of GNPs enhances strongly light scattering, then, it can be used in molecular-specific diagnostics and detection in cancer (8). Several studies compared between the methods of injection (oral, intravenous (IV), intraperitoneal (IP) and intratumoral (IT)) for limited toxicity level.

Some studies showed that the toxicity of GNPs may be related to administration routes as in the

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experiment of Jo and his team (9), in which they found that oral and intraperitoneal were more toxic than tail vein injection, and the potential toxicity occurred only after long term exposure with high concentration of nanoparticles.

Other experiments revealed that when the GNPs entered blood, the plasma protein bound with surface of nanoparticles, then, protein activation leads to inflammatory responses, or it accumulated in various organs such as liver, spleen, lung, kidney, stomach, and heart, and it can pass to brain via blood-brain barrier, due to the small size of particles (10). On the other hand, some researchers reported through their experiments that GNPs did not cause any toxicity when accumulated in vital organs like liver and kidney following IP injection in mice for 72 h (11). Similar findings by Shukla and his colleagues (12) proved that GNPs are not cytotoxic and non-immunogenic because they can reduce production of ROS and do not elicit secretion of pro-inflammatory cytokines, like TNF- $\alpha$  and IL-1 $\beta$ . In contrast, when Abdelhalim and Jarrar (13) exposed hepatic cells in Wistar-Kyoto rat to GNPs in different size, dose, and duration time, they found that GNPs can cause cytoplasmic degeneration and nuclear destruction by interacting with proteins and enzymes of hepatic tissue and interfering with the antioxidant defense mechanism, then, leading to ROS generation which in turn induce stress, atrophy and necrosis.

Hence, this study was done for investigating the level of cytotoxicity and bio-distribution of GNPs in tumor and vital organs after IT and IP injection.

## Materials and Methods

### Gold Nanoparticles Characterization

Colloidal gold nanoparticles were purchased from Sigma Aldrich Company with the following description: concentration=  $\sim 6.0 \times 10^{12}$  particles/ml, Optical density=1, diameter =10 nm, absorption =510-525 nm wave length, storage temperature = 2-8 °C, shape=spherical, and color dark red wine.

### Laboratory Mice Preparation

Fifteen laboratory mice bearing tumor were divided into three groups as follows: First group- Control positive without treatment (n=5). Second group- Intratumor injection with colloidal GNPs (150  $\mu$ l in concentration of  $4.5 \times 10^{12}$  particles/ml) for 28 days (n=5).

Third group- Intra peritoneal injection with colloidal GNPs (150  $\mu$ l in concentration of  $4.5 \times 10^{12}$  particles/ml) for 28 days (n=5). Five healthy mice were used as negative control (without tumor and treatment). All these groups were sacrificed after 28 days for the estimation of bio-distribution of particles in tumor, liver, spleen, and kidney.

After the treatment period, tumor and organ specimen (liver, spleen and kidney) were sectioned carefully, weighted and prepared for Atomic Absorption Spectroscopy with flame.

Briefly, around 0.1 gm of tissue (n=5) was dried at room temperature and put in glass tube, then, 4 ml of aqua regia (1part of nitric acid combined with 3 parts of hydrochloric acid by volume, in a fume hood) were slowly added to each sample, after that all tubes were put in water bath (80-100 °C) for 4-5 hrs.

Then, the temperature was raised in order to complete digestion of the sample and to safely dissolve the gold nanoparticles (14). GNPs concentration in each sample was determined by Atomic Absorption Spectroscopy with flame.

### Statistical Analysis

Data were subjected to one-way ANOVA using Statistical Analysis System SAS. Least significant difference (LSD) test was used to assess differences between means at  $P \leq 0.05$ .

## Results and Discussion

In the first group (control positive), the tumor size increased with the time, the increase continued till day 28 of the experiment. While in the second group, a significant reduction in tumor size occurred after injecting the GNPs by IT method, which was more than that seen in IP method (third group).

This may be due to the ability of GNPs to eliminate ROS induced under neoplastic condition, thereby, restoring the balanced level of antioxidant defense system; this affirms the therapeutic application of GNPs (15).

Tumor cells regression occurred especially with the long term of treatment (after fifteen to twenty day, data not published) because the cancer cells have a tendency to absorb substantially higher concentrations of GNPs than the surrounding tissue (16). Also, GNPs can aggregate in situ in tumor

after IT injection, this was more than that following IP injection.

Table 1 shows the comparison between the two routes of administration (IT and IP). The table shows that the kidney had higher concentration in both methods of injection ( $3.75 \pm 1.75$  and  $2.42 \pm 0.31$  ppm), respectively, followed by tumor  $2.66 \pm 0.01$  and  $1.09 \pm 0.06$  ppm in IT and IP, respectively. While the concentrations of GNPs in liver were  $0.602 \pm 0.03$  and  $0.517 \pm 0.02$  ppm in IT and IP, respectively, and in spleen were  $1.40 \pm 0.33$  and  $0.726 \pm 0.01$  ppm in IT and IP, respectively.

There was a significant difference ( $P < 0.05$ ) between kidney, spleen, liver and tumor injected by IT route, and highly significant variations ( $P < 0.01$ ) among them were observed in IP route. The accumulation of GNPs in tumor mass and tested organs of mice-bearing tumor post IT treatment was significantly higher (about 2 folds) than that in IP injection. There were significant variations between the two routes in spleen and tumor, but were non-significant in case of liver and kidney.

Gold nanoparticles can easily permeate tumor vasculature and remain in tumor to the enhanced permeability and retention (EPR) effect.

The gap in tumor vasculature are about  $100 \text{ nm}^{-2}$   $\mu\text{m}$  larger than that in normal endothelial lining, thus, GNPs can easily pass through these gaps, and because the tumor loses lymphatic clearance and has a disordered extracellular matrix; therefore, these particles are able to remain in the tumor tissue (17).

Tumor uptake to GNPs is significantly lessened by the opsonization of the NPs with plasma protein and their subsequent phagocytosis by reticuloendothelial system (RES) components (monocytes and macrophages). Hence, parts of GNPs are eventually sequestered in the liver and spleen (18).

The bioaccumulation of GNPs in the liver and spleen may be regulated by the RES, which is part of the immune system involved in the uptake and metabolism of exogenous molecules and particles in these tissues. In addition, the nanoparticles penetrate cell membrane by endocytosis and taken up primarily by Kuepfer cells in the liver and secondarily by macrophages in other places (19). The results of this study referred to more accumulation of GNPs in kidney, this means that GNPs with small size can be metabolized by renal clearance and thus the toxicity can be significantly

decreased (20). When GNPs (especially large sizes) accumulate in kidney, they cannot pass via the glomerular filtration, which has pores measured about 5.5 nm (21).

In contrast, Abdulhalim reported that GNPs up to size 50 nm may be highly cleared via urine and bile (22). Also, the table shows that the bioaccumulation of GNPs in spleen and liver was lower than that in kidney, this explains the major role of these organs in detoxication and clearance (23). In support of this study, the report of Jo and his team (9) proved that GNPs were slowly absorbed into the bloodstream and slightly accumulated only in kidney. Also, the present study revealed that GNPs accumulated in tumor after IT injection were about 2.5 folds higher compared to IP administration, however, not more than 2.5 folds accumulation in any organ indicates that IT injection might improve drug distribution to tumor tissue.

The longer elimination of GNPs from the liver may reflect the deposition of GNPs in tumor tissue after IT injection and redistribution process. However, more accumulation of GNPs in kidney may be attributed to the renal elimination to these particles, while the concentration of GNPs in liver and spleen might reflect the higher saturation concentration of RES to nanomaterial (24, 25). Finally, the accumulation of GNPs in vital organs may be related to concentration of nanoparticles and routes of administration. Nanoparticles have attracted a great deal of attention for targeted drug delivery to tumors. The vast majority of work has focused on intratumor administration to target solid tumors. While some success and clinical impact has been achieved, the overall efficiency of tumor delivery for the field has not markedly increased in recent years. The bio-distribution of nanoparticles through the tumor and different organs depends on nano-particle characteristics such as size, charge, shape, and synthesis methods. We need other data to support several strategies (EPR-based targeting, reducing macrophage uptake, increasing blood circulation time, and active targeting) that are widely touted. It is also briefly emphasized that while nanoparticles are predominantly cleared by the liver, kidney and spleen much remain to be learned about this process.

The route of administration plays an important role in bio-distribution and potential therapeutic effect on cancer cells.

**Table 1.** Bio-distribution of GNPs ppm in tumor masses and various organs (comparison between IT and IP)

Organs	Intra tumor Mean±SE	Intraperitoneal Mean±SE	LSD value	P-value
Liver	0.602 ±0.03 <sup>B a</sup>	0.517 ±0.02 <sup>B a</sup>	0.284 NS	0.451
Kidney	3.75 ±1.75 <sup>A a</sup>	2.42 ±0.31 <sup>A a</sup>	1.41 NS	0.293
Spleen	1.40 ±0.33 <sup>B a</sup>	0.726 ±0.01 <sup>B b</sup>	0.426 *	0.038
Tumor	2.66 ±0.01 <sup>A a</sup>	1.09 ±0.06 <sup>B b</sup>	0.772 *	0.032
LSD value	1.194 *	0.602 **		
P-value	0.0206	0.0036		

\*(P<0.05),\*\* (P<0.01), NS: Non-significant. Different capital letter represents significant differences (P<0.05), (P<0.01) between means of the same column; Different small letter represents significant difference (P<0.05), P<0.01) between means of the same row

### Conflict of Interest

The authors declare that there is no conflict of interest.

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## التوزيع الحيوي لجزيئات الذهب النانوية في الورم والأعضاء المختلفة للفئران المغروسة بسرطان الغدد اللبنية (دراسة داخل الجسم الحي)

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### الخلاصة

تمتلك الجزيئات النانوية عدة خصائص, خصوصاً في المجالات العلاجية الطبية, لكن الجدال مازال مستمر حول سمية هذه الجزيئات. لذلك أنجزت هذه الدراسة لغرض تحديد تركيز جزيئات الذهب النانوية في الورم والأعضاء المختلفة كالكلية والكبد والطحال بعد حقن هذه الجزيئات بطريقتين, داخل الورم وتحت الخلب للمقارنة بين الطريقتين في الفئران المغروسة بسرطان الغدد اللبنية ولمدة 28 يوم. أظهرت النتائج ان الكلية هي العضو الأكثر جمع لهذه الجزيئات من بقية الأعضاء المدروسة وبمعدل  $1.75 \pm 3.75$  و  $0.31 \pm 42.2$  (جزيئة لكل مول) في 0.1 غرام من نسيج الكلية وبطريقتي ادخال الورم وتحت الخلب على التوالي, يتبعها الكتلة الورمية وبمعدل  $0.01 \pm 2.66$  و  $0.06 \pm 1.09$  (جزيئة لكل مول) في 0.1 غرام من نسيج الورم, فيما اظهر الطحال تجمع الجزيئات الذهب النانوي بمعدل  $0.33 \pm 1.40$  و  $0.01 \pm 0.726$  (جزيئة لكل مول) في 0.1 غرام من نسيج الطحال وفي الكبد  $0.03 \pm 0.602$  و  $0.02 \pm 0.517$  (جزيئة لكل مول) في 0.1 غرام من نسيج الكبد وبطريقتي ادخال الورم وتحت الخلب على التوالي. أظهرت الدراسة أيضاً ان طريقة الحقن داخل الورم اكثر كفاءة من طريقة الحقن تحت الخلب للعلاج بالجزيئات النانوية, وان الجزيئات النانوية يتم التخلص منها من قبل الأعضاء للمفاوية المسؤولة عن ذلك.

الكلمات المفتاحية: دقائق الذهب النانوية، داخل الجسم الحي، التوزيع الحيوي، داخل الورم، تحت الخلب