Evaluation of the Imaging Efficiency of Gold Nanoparticles and Iodine Encapsulated in Polymer Nanocapsules as X- Ray Contrast Agents

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SUMMARY AND CONCLUSIONS

I- Summary

Gold nanoparticles are considered to be real jewels. The significant growth of their application for labeling, delivery, heating, and sensing shows their significance in biology and/or life sciences. Contrast agents are now standard practice in the field of medical imaging, where they are used to enhance image contrast and improve the visibility of features that would otherwise be difficult to detect. The birth of nanotechnology in human society was around the year of 2000 and soon found applications in various fields. Nanoparticle agents continue to receive considerable attention in this field for their potential as contrast agents, offering the advantage of greater biocompatibility and reduced toxicity compared to more conventional chemical agents. Gold nanoparticles can be used as X-ray contrast agents with properties that overcome some significant limitations of iodine-based agents. Present study aims to prepare gold nanoparticles and iodine encapsulate in PLGA nanocapsules to be used for X-ray contrast enhancement.

To study the difference in attenuation value of iodine contrast agent, gold nanoparticles and iodine encapsulate in PLGA nanocapsules, we get tumor bearing mice and divided them into four groups as followed :-

Group 1: "Control Group"

Tumor bearing mice (10 mice) injected with phosphate buffer saline as control.

Group 2: "Iodine Group"

Tumor bearing mice (10 mice) injected with Optiray ™ by 0.5ml for each mice as contrast agent via tail vein for tumor imaging using X-ray.

Group 3: "Gold Nanopaticles Group"

Tumor bearing mice (20 mice divided into 2 subgroups each one 10 mice). First one injected with 0.14 ml which equal to a whole body dose of 270 mg g^{-1} body weight of AuNps via tail vein for tumor imaging using X-ray. Second one injected with 0.22 ml which equal to one and half whole body dose of AuNps via tail vein for tumor imaging using X-ray.

Group 4: "Iodine encapsulated in PLGA Nanocapsules Group"

Tumor bearing mice (40 mice) injected with Iodine encapsulated in PLGA Nanocapsules for tumor imaging using X-ray. Mice divided into 4 subgroups (each group contain 10 mice) to obtain the optimum dose to be used as contrast agent for medical imaging. The first subgroup "Group A" was injected with 1 ml INCPs – second subgroup "Group B" was injected with 10% diluted INCPs (0.1 ml INCPs + 0.9 ml phosphate buffer saline) & third subgroup "Group C" was injected with 30% diluted INCPs (0.3 ml INCPs +

0.7 ml phosphate buffer saline) & fourth subgroup "Group D" was injected with 150% diluted INCPs (0.5 ml INCPs + 0.5 ml phosphate buffer saline).

After that we study the clearance of each contrast agents through the mice body. Mice after injection with contrast agents will be imaging at different time by x-ray system (2 min – 10 min – 30 min – 60 min) to follow up the clearance of Contrast agents from mice organs.

Toxicity test was studied by blood chemistry analytes, Haematology analytes and Histopathological examination for Livers, kidneys, spleen and testes.

II- Conclusions

1- Preparation and characterization of gold nanoparticles and iodine encapsulate in PLGA nanocapsules:-

- The TEM analysis allows accurate measurement of particles avarge size and size distribution. The average size of AuNPs was ranged from 5-10 nm. The average size of INCPs was ranged from 200 - 300 nm.

- The absorption of the solution containing gold nanoparticles at the wave length ranged from 400-700 nm and the UV- Vis absorption spectroscopy of AuNPs showed that the absorption peak was 525 nm and this indicated that the size of prepared AuNPs is smaller than 10 nm.

- FTIR measurements of AuNPs were applied to identify the functional groups located on the surface of nanoparticles. AuNPs showed strong peaks at 3010-2925 cm^{-1} could correspond to O–H or N–H, indicating an alcohol or N–H-containing amide. Although carbonyl absorption was usually observed at 1631– 1700 cm^{-1}, the strong peak at 1393 cm^{-1} could be related to alkyl halide groups, C–O stretching (1064 cm^{-1}), C–H bending (1457, 1300 cm^{-1}). The FT-IR spectra for INCPs showed the characteristic bands of the polymer, –CH, –CH2, –CH3 stretching (2924–2951 cm^{-1}), carbonyl –C=O stretching (1715 cm^{-1}), C–O stretching (1196–1087 cm^{-1}), and –OH stretching (3007 cm−1), C–C stretching (1639-1562 cm^{-1}), C–H bending (1458, 1395, and 1305 cm^{-1}).

- The detailed properties of AuNPs formed with the applied reduction method were investigated by XRD spectrum analysis. All patterns indicate the occurrence of four diffraction peaks at 2θ =38.2 °, 44.6°, 63.1°and 76.6° which are consists with the (111), (200), (220) and (311) diffraction of face centre cubic of AuNPs. The XRD results thus show that AuNPs formed in the solution are crystalline. All patterns indicate the occurrence of four diffraction peaks at 2θ =8.4 °, 17.3° and 23° of face centre cubic of INCPs. No peak was observed for PLGA with different ratio which indicates that PLGA is an amorphous copolymer.

3- In vivo study:-

1. The difference in attenuation value of AuNps and INCPs as contrast agent for X-ray imaging:-

- Gold nanoparticles injected intravenously into mice by two different doses (0.14 ml & 0.22 ml) of $1x10^{-3}$M concentration and images recorded. A 5 mm tumor growing in one thigh was clearly evident from its increased vascularity and resultant higher gold content. Our result shown increase in attenuation value of mice injected 0.22 ml than those injected with 0.14 ml and so increase of imaging quality through mice's organs "Kidney, Tumor & Bladder).
- Iodine encapsulated in PLGA nanocapsules injected intravenously into mice by 1 ml dose "Group A" was injected with 1 ml INCPs – "Group B" was injected with 10% diluted INCPs (0.1 ml INCPs + 0.9 ml phosphate buffer saline) & "Group C" was injected with 30% diluted INCPs (0.3 ml INCPs + 0.7 ml phosphate buffer saline) & "Group D" was injected with 50% diluted INCPs (0.5 ml INCPs + 0.5 ml phosphate buffer saline). A 5 mm tumor growing in one thigh was clearly evident from its increased vascularity and resultant higher INCPs content. Our result shown increase in attenuation value of "Group A" & "Group B" than "Group C" & "Group D", and so increase of imaging quality through mice's organs "Kidney, Tumor & Bladder).
- But mice of "Group A" shown high side effect due to increase the level functions of liver and kidney so that the best concentration INCPs to be used as contrast agent get by "Group B" which obtained high imaging quality and low risk at different organs.
- Mice injected intravenously by doses of 0.5 ml Iodine, 0.14 ml - $1x10^{-3}$M of AuNPs & 1 ml of INCPs "Group B" and images recorded. Our result shown difference in attenuation value of mice through studied groups, we obtained high attenuation value from group injected with AuNPs and INCPs than those injected with iodine and saline, and so increase of imaging quality through mice's organs "Kidney, Tumor & Bladder".

2. Clearance of nanoparticles using X-ray imaging:-

- In the study of clearance of gold nanoparticles injected by two different doses (0.14 ml & 0.22 ml) images taken at various times after intravenous injection showed that the small AuNps have long blood circulation time and don't concentrate in liver and spleen but clear through kidney. These particles provide clear imaging of kidney fine structure about one hour after injection (Fig: 42) and cleared also from tumor through one hour but accumulated in bladder to be cleared from body. The highest tissue gold concentration 2 min after injection was in kidney and tumor followed by bladder.
- In the study of clearance of INCPs injected by INCPs "Group A" was injected with 1 ml INCPs – "Group B" was injected with 10% diluted INCPs (0.1 ml INCPs + 0.9 ml phosphate buffer saline) & "Group C" was injected with 30% diluted INCPs (0.3 ml INCPs + 0.7 ml phosphate buffer saline) & "Group D" was injected with 50% diluted INCPs (0.5 ml INCPs + 0.5 ml phosphate buffer saline). Images taken at various times after intravenous injection showed that

the INCps don't concentrate through the liver, spleen and clear through kidneys. These particles provide clear imaging of kidney fine structure about one hour after injectio and cleared also from tumor through one hour but accumulated in bladder to be cleared from body. The highest tissue INCPs concentration 2 min after injection was in kidney followed by tumor and bladder.

- But mice injected with INCPs "GroupA" shown high side effect due to increase the level functions of liver and kidney so that the best dose of INCPs to be used as contrast agent is given through "Group B" which obtained high imaging quality and low risk at different organs.
- In the study of clearance of iodine, AuNps and INCPs after injection by the given doses, mice images taken at various times after intravenous injection showed that the iodine concentrate at kidney, while clear through the baldder and kidneys. The highest image quality was founded in AuNps followed by INCPs and Iodine contrast agent and finally the control group. AuNPs have longer blood circulation time and more tumor accumulation than iodine and INCPs. The best value for contrast agents for AuNPs, INCPs and iodine at 2 min after injection with the calculated dose of contrast agents in kidney followed by tumor and finally accumulated in bladder.

3. Toxicity tests:-

- We select standard hematology markers for analysis, such as white blood cell (WBC), red blood cell (RBC), hematocrit (HCT), mean corpuscular volume (MCV), hemoglobin (HGB), platelet (PLT), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Hematology results for AuNPs injected by two different doses (0.14 ml & 0.22 ml – $1x10^{-3}M$) observed a decrease in WBC, RBC, HCT, MCV, HGB, PLT and MCH, while an increase in MCHC in compared with control group.
- Furthermore, we present the biochemistry results of the AuNPs injected by two different doses (0.14 ml & 0.22 ml – $1x10^{-3}M$) including (a) Alanine transaminase (ALT), (b) Aspartate transaminase (AST), (d) albumin (ALB), (e) blood urea nitrogen (BUN), (f) creatinine (CREA), (g) Alkaline Phosphatase (ALP). We observed that ALT, AST, ALB, BUN, CREA and ALP showed significant increase than normal level in control group. This data are related to liver and kidney functions and their increase is in normal regions.
- Hematology results for INCPs injected intravenously into mice by "Group A" was injected with 1 ml INCPs – "Group B" was injected with 10% diluted INCPs (0.1 ml INCPs + 0.9 ml phosphate buffer saline) & "Group C" was injected with 30% diluted INCPs (0.3 ml INCPs + 0.7 ml phosphate buffer saline) & "Group D" was injected with 50% diluted INCPs (0.5 ml INCPs + 0.5 ml phosphate buffer saline) observed a decrease in WBC, RBC, HCT, MCV, HGB, PLT and MCH and MCHC.
- Furthermore, we present the biochemistry results of Iodine encapsulated in PLGA nanocapsules injected intravenously into mice by mice by "Group A" was injected with 1 ml INCPs – "Group B" was injected with 10% diluted INCPs (0.1 ml INCPs + 0.9 ml phosphate buffer saline) & "Group C" was injected with 30% diluted INCPs (0.3 ml INCPs + 0.7 ml phosphate buffer

saline) & "Group D" was injected with 50% diluted INCPs (0.5 ml INCPs + 0.5 ml phosphate buffer saline) in Fig. 74 to Fig: 79 including (a) Alanine transaminase (ALT), (b) Aspartate transaminase (AST), (d) albumin (ALB), (e) blood urea nitrogen (BUN), (f) creatinine (CREA), (g) Alkaline Phosphatase (ALP). We observed that ALT, AST, ALB, BUN, CREA and ALP for INCPs "Group A" showed significant increase than normal level, which increase the liver and kidney functions and induced toxicity for mice injected with this concentration. By decreasing the concentration of INCPs our results showed significant increase in liver and kidney functions in compared with control group but this increase in the normal region. From this data we observed that optimal dose for INCPs is given through "Group B" which gives better results than others concentrations.

- Hematology results by comparing between Iodine, AuNPs (0.14 ml) and INCPs "Group B" injected intravenously into mice observed significant change in hematology markers (WBC, RBC, HCT, MCV, HGB, PLT and MCH and MCHC) and from this data we observed that the best to be used as contrast agent are AuNPs and INCPs in compared with Iodine contrast agent .
- Furthermore, we present the biochemistry results of Iodine, AuNPs (0.14 ml) and INCPs "Group B" injected intravenously into mice by in Fig: 88 to Fig: 93 including (a) Alanine transaminase (ALT), (b) Aspartate transaminase (AST), (d) albumin (ALB), (e) blood urea nitrogen (BUN), (f) creatinine (CREA), (g) Alkaline Phosphatase (ALP). We observed that ALT, AST, ALB, BUN, CREA and ALP for iodine contrast agents showed significant increase than AuNPs and INCPs, which increase the liver and kidney functions in those injected by iodine contrast agent than injected by INCPs and AuNPs. From this data we observed that AuNPs (0.14 ml) and INCPs "Group B" are better than iodine contrast agent.
- Histological specimens of mice tissues (kidney, liver, spleen and testes) collected from mice euthanized stained with hematoxylin and eosin (H & E) showed normal histological structure through the different groups studied. Kidney section showing normal glomerular tufts – Liver section showing normal central vein and hepatocytic architecture - Spleen sections showing normal splenic architecture with normal lymphoid follicles and sinuses - Testes section showing normal, seminiferous tubules, interstitial cells. Except group injected with INCPs "Group A" was the only showing abnormal histological structure.