Gold Nanoparticles in Liver Cells: The Applications of In Vitro and In Vivo Studies Towards Toxicological Determination

Introduction

Although Paino et al. were able to produce results that which show cytotoxicity related to two different types of capped gold nanoparticles (AuNPs) among hepatocellular carcinoma (HepG2) cells , the evidence of cytotoxicity in these AuNPs does not show the temporal effects of cell viability. Moreover, without showing how cell viability is affected over time, the evidence overlaying that AuNPs are cytotoxic is weakened. In order to show the efficacy of induced cell death by AuNPs, Fraga et al. included the temporal effect of the AuNPs over time periods of 24, 48, and 72 hours and were able to find insignificant results showing cytotoxicity of the AuNPs.

The use of gold nanoparticles can be applied to many fields of study. Specifically, it is appealing to the pharmaceutical industry in that AuNPs are able to target sites of drug delivery. Generally, gold is an inert material having advantageous optical and thermal properties. However, studies of AuNPs are still recent, and much is still unknown on the potential side effects of their use. Areas that are of concern are their toxicological effects on the human body, which may include their cytotoxicity, genotoxicity, and even toxicity among human organs such as hepatotoxicity. While evidence has leaned towards showing that toxicity is due to AuNPs, there has not been significant research that which identifies the source of toxicity. Two types of studies have arisen to address the source of toxicities: in vitro and in vivo. Furthermore, it has been documented extensively that AuNPs are known to accumulate in the human liver and therefore studies have been done specifically on the toxicology of AuNPs in the liver and its related cells such as hepatocellular carcinoma cells (HepG2) and peripheral blood mononuclear cells (PBMC). While there are many evidence-based studies as to why AuNPs may produce toxicity towards the liver and liver cells, toxicity cannot be accurately determined without including temporal effects on the liver cells, in vivo applications to the in vitro studies of biodistribution, the effect of biodistribution due size of the AuNPs, and the method of uptake of the AuNPs. Furthermore, results of in vivo studies must also be followed by further studies showing the long term effects of the AuNPs.

Paino et al. performed an in vitro study with HepG2 cells to show the cytoxicity and genotoxicity on two types of capped AuNPs: sodium citrate AuNPs (Cit-) and polyamidoamine dendrimer AuNPs (PAMAM-). A mixture of positive and negatively charged AuNPs of various sizes ranging from 7 to 20 nm was used. To find the cytotoxicity of the AuNPs, the cell viability of the HepG2 cells using MTT assay were found after a 24 hour incubation period. The percentage of cell viability was determined for different concentrations of both capped AuNPs: 0.1, 0.5, 1.0, 50 μm. For both Cit-AuNP and Pamam-AuNP, it was found that cell viability decreased with increasing concentration. Thus, all concentrations caused cytotoxicity of the AuNPs. From this study, it was found that Cit-AuNP and PAMAM- AuNP were likely to be cytotoxic. However, cell viability was not observed after the 24 hour incubation period. This may have been a missed opportunity to obtain data in which there may have been a decrease in number of viable cells or an insignificant amount of change in the number of viable cells, which would not allow Paino et al to conclude that Cit-AuNPs and Pamam-AuNPs are cytotoxic. While Paino et al. were able to show that cytotoxicity was induced in both Cit-AuNP and Pamam-AuNP, the temporal effect on cell viability of the AuNPs were not observed and creates a conflict of interest of whether the two capped AuNPs are cytotoxic.

On the other hand, Fraga et al. were able to produce a study that monitored coated AuNPs over a specific time period. In order to determine the role of capped AuNPs and cytotoxicity, a 20 nm AuNP was used and coated with either citrate (Cit-) or 11-mercaptoundecanoic acid (11-MUA). Before testing, coated AuNPs were either grouped into different concentrations of 0- 200 μm or exposed to different vehicles. To test for cytotoxicity, Fraga et al. used MTT and LDH assay methods on HepG2 cells and observed them over time periods of 24, 48, and 72 hours. Furthermore, the toxicity of AuNPs due to capping was also tested for in concentrations of 10, 30, 100, and 200 μm. It was found that there were not significant findings of cytotoxicity after exposure to the vehicle AuNPs or capped AuNPs even after 72 hours of AuNP exposure to the HepG2 cell. After analysis of the effect of surface modification, it was found that there were no significant uptake differences among Cit and 11-MUA surface modifications, although apoptosis and necrosis had occurred. However, Fraga et al. failed to reproduce their findings of the AuNPs being non-toxic in their in vivo study. The in vivo study revealed DNA damage to the HepG2 cell and it is questionable how AuNPs were distributed among their liver sample. The amount of AuNPs in their liver sample might significantly tell the cytotoxic effect of the AuNPs. Although this study successfully was able to rule out cytotoxic effects of surface coating of AuNPs in vitro, an in vivo biodistribution of the AuNPs should be carried out to effectively demonstrate AuNPs as being non-toxic.

Moreover, Morais et al. studied the effect of surface coating and biodistribution of coated AuNPs in vivo. Morais et al. obtained AuNPs of different surface coatings and assessed toxicity among liver samples in the rat after injection of AuNPs over time. Citrate, 11-MUA, and 3 pentapeptides (CALNN, CALND, and CALNS) were synthesized as the cappings of AuNPs and had an average of 18.4 ± 4.9 nm in size. To test for biodistribution within the rat post-injection, GFAAS technique was used. As predicted, a high concentration of AuNPs was found in the liver of the rat regardless of capping amongst all other organs of the rat. The peptide-coated AuNPs seem to increase accumulation of them in the liver. This suggests that capping type does directly affect the toxicity. Between all of the capping types, it is seen that Cit-AuNPs were poorly eliminated compared to the others in which adds to toxicity. It is speculated that the relative size of the AuNP might be a source to poor elimination and that the uptake. By using an average size of AuNP, the sizes become variable, in which is a factor to take into account when showing the amount of AuNPs remaining within the liver. Morais et al. failed to explain why the average size of 18.4 nm was chosen or carry out a study on the most beneficial size of AuNP. While this study successfully showed the toxicity of AuNPs due to capping in accumulation of AuNPs, it failed to study the effect of AuNP size on the biodistribution.

In contrast, Cho et al. provided an in vivo study of biodistribution of AuNPs and cited another study, in which recommended the AuNP size that Cho et al. had again synthesized. Cho et al. analyzes the biodistribution of AuNPs injected in mice and their effect on cellular components, in which are involved in uptake of the AuNPs. Cho et al. uses 13 nm-sized PEG-coated AuNP and administered dosages of 0.85mg/kg and 4.26 mg/kg to the mice. Using the inductively coupled plasma technique, concentrations were obtained after 5 min, 30 min, 4 h, 24h, and 7 days. From their findings, it appeared that inflammation of the liver occurred immediately after the nanoparticles post-injection and once again after 7 days. Apoptosis also occurred in the hepatocytes, but even more so in the higher dosage of 4.26 mg/kg . The occurrence of apoptosis from this in vivo study directly shows hepatoxicity and cytotoxicity in mice. However, without further analysis of whether the AuNPs were taken up by the cells of the liver, toxicity results may be easily resolved if the AuNP was not taken up. Without uptake of the AuNP to the cell, inflammation and apoptosis may have occurred indirectly from the presence of AuNP. Although Cho et al. provided a study showing the negative side effects of the introduction of AuNP in an animal vehicle, the study failed to examine the uptake of the AuNPs.

On the other hand, Dragoni et al. provided both an in vitro and in vivo study on uptake and was able to make a fair conclusion on the cytotoxicity of polyvinylpyrrolidone (PVP) coated AuNPs. Dragoni et al. used a 5 nm AuNP. In their in vitro study, precision-cut slices of rats were used to determine the cytotoxicity and determine whether the AuNPs were taken up by the cells. The interaction of AuNPs with the slices was observed. It was seen that over 40 percent of the cells interacted with the AuNPs, concluding that the cells were taken up. Observation after 6 hours also showed that AuNPs entered the cell and were observed in the cell membrane. Furthermore, the in vivo study matched the results of the in vitro study. In the in vivo study, AuNPs were injected into rats and their liver fragments were observed after 12 hours. Traces of AuNPs were found amongst the hepatocytes as well as in endothelial and Kupffer cells. While the uptake of the cells was successful, no toxic effects such as apoptosis were found. However, from the in vitro they had only recovered about 6 percent of the AuNPs in from the precision-cut slices. While this demonstrates successful uptake, it is necessary to provide more research on the long-term effect of AuNPs that were taken up by cells. Thorough study of the toxicity of the AuNPs is critical towards the overall recommendation of using AuNPs for drug delivery. Thus, further studies on long term effects should be taken into account to successfully show the extent of toxicity in AuNPs.

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