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The Potential of Gold Nanoparticle Conjugates to Kill Cancer Cells in Culture

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Abstract

The anticancer potential of gold nanoparticle conjugates was investigated. The gold nanoparticles were conjugated to paclitaxel (Taxol) to determine if the gold nanoparticle conjugates are as effective at killing cancer cells as the Taxol alone. Cytotoxicity of samples against breast, skin and lung cancer cell lines was determined using the MTT viability assay (a colourimetric assay detecting survival in microplates). The cancer cells were not significantly killed by the gold nanoparticles. However, the skin cancer and breast cancer cells were killed by the Taxol alone and the gold nanoparticle-Taxol conjugate with approximately 65% cell killing at the highest dose.

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Keywords: Gold nanoparticles; cell culture; cytotoxicity; paclitaxel; breast cancer; skin cancer

1. Introduction

1.1. Background

Chemotherapy is currently the method of choice for treating most cancers. The problem with most chemotherapy used to treat cancer is the difficulty in delivering a high enough dose of the therapeutic to the cancer cells without causing overwhelming adverse side-effects on normal cells throughout the body of the patient. There are many anti-cancer therapeutics on the market, but by far the great majority have adverse side-effects. One solution to this problem is to deliver the therapeutic directly to the cancer cells so that less of the drug is needed and normal cells are not harmed. This represents a big shift in the focus of current anticancer research. One emerging area for enabling this shift to occur is the use of nanoparticle-based drugs. Work involving anticancer effects and imaging potential has included studies of quantum dots, dendrimers,gold nanoparticles, polymer gels, Fe3O4, and ZnO [1]. For example, several different amides of gold(I) diphosphines prepared from N-heterocyclic sources have been shown to have activity against cancer cell lines *in vitro* [2]. Despite the success of the study, the authors concluded that compounds with greater efficacy (ie. Less toxic to non-cancer cells yet more toxic to cancer cells) still need to be identified [2]. One way to achieve this is to use the gold nanoparticles as a delivery vehicle and use gold nanoparticles that are inherently non-toxic and conjugate the nanoparticles to a toxic agent. The conjugate would then act as a safe delivery vehicle. For example, Joshi et al. [1] found that breast cancer cells were

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sensitive to killing *in vitro* by chloroquine-gold nanoparticle conjugates. It has also been shown that hepatic cancer cells can be significantly killed by gold nanoparticles conjugated to either doxorubicin, cisplatin, or capecitabine and hence showed that conjugated gold nanoparticles enter hepatic cancer cells and are potential anticancer drugs [3].

1.2. Aim of the current study

Gold nanoparticles as a potential packaging system were investigated by our group for the delivery of Paclitaxel (Taxol) to cancer cells using an *in vitro* cell culture model. The aim of the study was to determine the effect of gold nanoparticles, the anticancer therapeutic taxol and a gold-nanoparticle-taxol conjugate on three cancer cell lines (lung, breast and skin origin) using the MTT assay to monitor loss in viable cell numbers following treatment.

2. Methods, Results and Discussion

2.1. Methods

The cell lines tested were three human cell lines derived from cancer tissues. All cell lines were maintained in RPMI supplemented with 10% Foetal Bovine Serum and were grown at 37°C, in a fully humidified atmosphere. The cell lines were Breast cancer T47-D cell line; Skin cancer A431 cell line and Lung cancer A549 cell line.

The samples tested for cytotoxicity were prepared in house, with the exception of the Taxol which was from Sigma Aldrich. The final samples were:

A. Citrate capped gold nanoparticle (11-12 nm in diameter)
B. Thiol capped gold nanoparticle (nanoparticles A, with some citrate removed and replaced with 16-mercaptohexadexanoic acid)
C. Taxol (commercial Paclitaxel)
D. Conjugate: gold nanoparticle with Taxol added

The MTT Assay was used to monitor cytotoxicity. In the MTT assay, viability is indicated by presence of mitochondrial activity via which yellow MTT dye is reduced to purple formazan in the mitochondria of living cells [4-5]. The amount of purple formazan product is directly proportional to the number of viable cells. The absorbance of formazan is quantified by measuring OD at 570nm, with 630 nm as the reference wavelength. Briefly, cells were plated at 10,000 cells/well in 96-well plates, allowed to adhere overnight then treated with nanoparticles, Taxol or conjugate for 24 hours. After washing the wells, the cells were incubated with MTT dye, then any formazan generated by living cells was solubilised in acidified SDS. An interference assay with known numbers of cells/well was conducted to make sure the particles didn't interfere with the MTT metabolism or SDS solubilisation steps.

2.2. Results

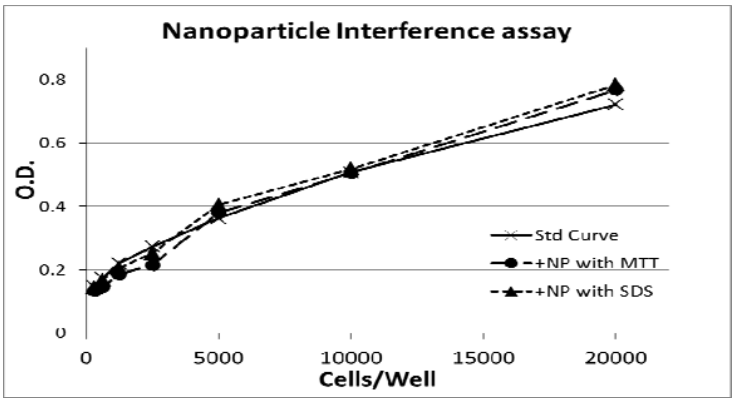

Fig. 1. The Thiolated Gold nanoparticles do not interfere with the MTT assay. A549 cells were plated in 96-well microplates at the concentrations indicated then either the standard curve was run either with nanoparticles added with the MTT dye (+NP with MTT); nanoparticles added with the acidified SDS (+NP wth SDS) or with without any nanoparticles being added (Std Curve).

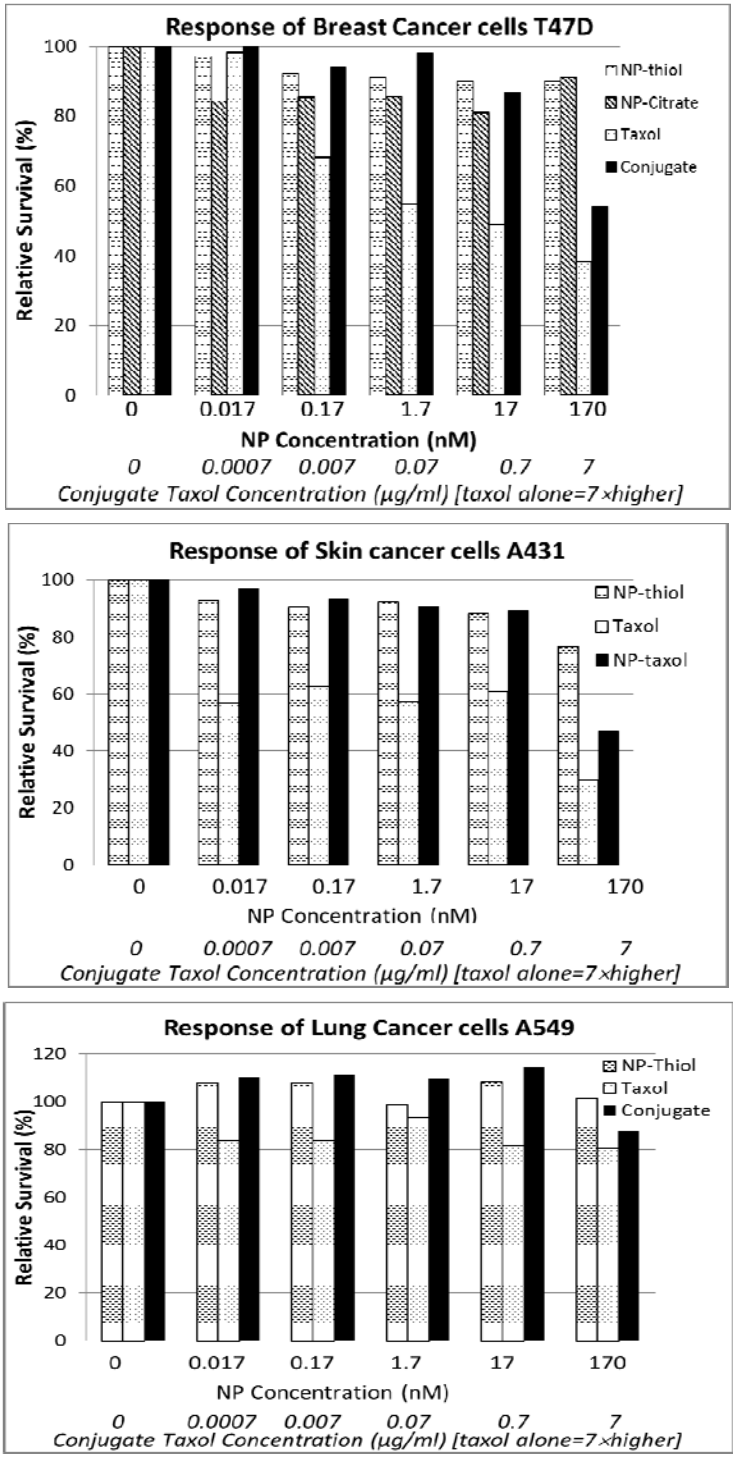

Fig. 2. Cytotoxicity of Thiolated Gold nanoparticles (NP-thiol); citrate capped gold nanoparticles (NP-citrate); Taxol alone or Taxol conjugated with the NP-citrate. Concentration of NP was as indicated, including for the conjugate. Concentration of Taxol alone and as a part of the conjugate is also indicated beneath the x-axis. As indicated above, cell lines were Breast cancer T47-D cell line; Skin cancer A431 cell line and Lung cancer A549 cell line. Cells were treated for 24 hours then assayed with the MTT assay. Relative survival (%) was calculated from numbers of surviving cells/well values relative to the untreated control.

Shown in figure 1 is an example of the results obtained for the interference assays. No significant shift in the standard curve O.D.s was induced by the gold nanoparticles in any interference assays in our laboratory. Therefore it is valid to use the MTT assay to assess the cytotoxicity of these gold nanoparticles. Interestingly, there was some interference with the colourimetric development in the Crystal Violet assay for these particles (data not shown) and so this assay was not used for further investigations

The nanoparticles used by themselves to treat the cells, did not induce any significant cytotoxicity to any of the three cancer cell lines (see fig 2). However, a dose-dependent decrease in survival is observed for the treatment of Breast cancer cells with Taxol and there is also cytoxicity of the Taxol towards the skin cancer cells. At doses of the conjugate, equivalent to non-toxic doses of the nanoparticle, there is killing of the breast and skin cancer cells. This cytotoxicity is observed at a dose of Taxol predicted to be 7 times lower than the original doses of Taxol alone (as indicated on the x-axis labels of fig 2).

2.3. Discussion

The human breast and skin cancer cell lines tested were as expected, sensitive to killing by the Taxol alone. Importantly, they were also killed by the Taxol when delivered as part of the conjugate. There was not a similar effect for lung cancer cell line tested, which appears not to be sensitive to either the Taxol alone or the Taxol conjugate. Wang et al. [6] worked with nanoscale preparation of Paclitaxel (Taxol) and found for a murine breast cancer cell line, that the nanopreparation and Taxol alone induced very similar levels of dose-related cell killing. They observed 50% cell killing at a dose of approximately 18μg/ml [6]. In our current study, we observed approximately 50% cell killing for the human breast and skin cancer cell lines at doses of 5-50μg/ml for Taxol alone and at 0.7-7μg/ml for the conjugated Taxol preparation. These are all of the same order of magnitude, however we need to carry out additional experiments with more doses around the IC_{50} to better estimate the IC_{50} for the human cell lines. Two differences between our current study and that of Wang et al. [6], were that we used human cell lines rather than murine and our conjugate involved gold nanoparticles with the potential for targeted drug delivery.

3. Conclusion

This study has successfully demonstrated a pilot set of experiments in support of the use of gold nanoparticles as drug delivery agents for breast and skin cancer. The major finding is that an equal or lesser dose of Taxol when present as a conjugate kills the human breast and skin cancer cells as effectively as the Taxol used as an individual treatment. Also, by implication, there Taxol is effectively taken up by the cells when present as a conjugate, however this remains to be further investigated by cell imaging. Additional experimental work is being initiated to determine the mechanism of killing.

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