Cancer-selective toxicity of gold nanoparticles: effects of synthesis time and charge

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Version: Version of Record
Cancer-selective toxicity of gold nanoparticles: effects of synthesis time and charge

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Introduction

It is estimated that 50% of people born after 1960 in the UK will develop some form of cancer in their lifetime. Cancer treatments include surgery, radiotherapy and chemotherapy. Each of these methods carries a risk of damaging healthy tissues surrounding the tumour.

Nanotechnology offers novel tools for cancer diagnosis, early detection and treatments. Gold nanoparticles show great promise in targeted cancer therapy due to their unique physicochemical properties. They offer the advantage of interaction with biomolecules both at the cell surface level and inside the cell. This interaction is highly dependent on their ligand composition.

Midatech's technology is based on carbohydrate-coated gold nanoparticles (GNPs). The particles used in this project consist of a gold core with an average diameter of 3 nm to which an organic layer of a thiocacolate derivative and thiol PEG amine are attached via gold-sulphur bonds (Figure 1). The highly water-soluble carbohydrate layer provides colloidal stability in aqueous solutions, while the thiol PEG amine creates positive charges which enable the particle to interact with biomolecules.

The key advantages are:

- Solubility
- Stability
- Releasability
- Excretability
- Scalability

Fig 1: Representation of the chemical structure of positively charged carbohydrate-coated nanoparticles.

Methodology

1. Synthesis of GNP
- GNP were synthesized by the reduction of HAuCl4 with NaBH4 in the presence of disulphide-containing ligands (Figure 2).

2. Clonogenic Assays
- Isolated human skin cancer (HSC-3) and normal (HaCaT) cells (300 cells/well) were exposed to different GNP concentrations for 3h and left to grow for 6 days to form colonies.
- At the end of day 6, cells were briefly rinsed with water, stained and fixed with 2% methylene blue in 50% ethanol.
- The staining/fixation solution was removed and plates were washed with distilled water and dried. Colonies with >50 cells were counted.

3. Silver Staining
- After acute exposure of cells to GNP for 3h, cells were washed with PBS and fixed in 4% PFA for 15 min. The cell membrane was permeabilized with 0.05% TX-100 in PBS.
- The silver staining developer and enhancer solutions were mixed at 50:50 ratio and applied to cells for 5-10 min in the dark. Cells were then washed 2x with PBS and samples were stored at 4°C for subsequent imaging. Samples were photographed by bright-field and phase contrast microscopy.

Preliminary Data

In this work, 2nm GNP surface functionalised with a 50:50 ratio of a thiocacetolate derivative and a thiol PEG amine were examined for their toxicity towards normal (HaCaT) and cancerous (HSC-3) human skin cell lines in vitro.

Using this simple ligand structure, GNP are toxic to HSC-3 cells without affecting the normal cell line (HaCaT).

A. Effects between the same 50:50 GNP synthesized for different times (1h, 2h, 5h, overnight*).

<table>
<thead>
<tr>
<th>Time</th>
<th>% Control Colonies</th>
</tr>
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<tbody>
<tr>
<td>15 min</td>
<td>10 μg/ml</td>
</tr>
<tr>
<td>15 min</td>
<td>1 μg/ml</td>
</tr>
<tr>
<td>15 min</td>
<td>0.1 μg/ml</td>
</tr>
<tr>
<td>15 min</td>
<td>0 μg/ml</td>
</tr>
</tbody>
</table>

* Overnight synthesis: resulted in bigger NPs >185 nm.

Fig 3: Cell survival assessed by clonogenic assay for GNP toxicity. Number of cell colonies (% of no-drug control) counted six days after acute (3h) exposure to various concentrations of GNP, made with the same ligand mixture but with different synthesis durations (1hr, 2hr, 5hr and overnight). Cell types tested were skin cancer (HSC3) and normal skin (HaCaT) cells lines.

B. GNP accumulate in endosomes/lysosomes.

Fig 4: Localization of 50:50 2h synthesis GNP in skin cancer (HSC3) and normal (HaCaT) cells after acute (3h) exposure at 10 μg/ml. EM data show large differences in uptake between HSC and HaCaT.

C. Does toxicity correlate with uptake?

Fig 5: Uptake data on GNP synthesis times (1h, 2h, 5h and overnight) in HSC cells. Silver staining appears stronger as the synthesis time increases.

D. Changes in surface charge don't fully explain changes in toxicity between different NPs

Fig 6: Clonogenic assay of different charged GNP.

- GNP 50:50 α-gal:AM, 4nm, +ve charge
- GNP α-gal, 4nm, neutral charge
- PAMAM G4 dendrimer, 4nm, +ve charge

Conclusions / Future Directions

A notable cancer cell only selective toxicity occurs that depends upon the synthesis duration of the GNP, probably due to differences in ligand density.

Future Directions: Batch standardisation for the optimum synthesis time. Elucidation of the mechanisms of selective uptake & toxicity in various cancer and normal cell lines.

In detail:

1. Explain differences between selective effect observed in cancer ≠ normal cell lines
2. GNP visualization for uptake assays and unravelling of internalization route
3. Comparison of GNP amount/cell for different cell types by ICPMS

Acknowledgements:

Nadia Tzelepi is supported by a PhD studentship from Midatech Phama.