Nanocytotoxicity and genotoxicity in human blood and nucleic acid

Nanocytotoxicity

Nanoparticles have received much attention because they are used in many fields especially in bio-applications. TiO_2, Ag and Au NPs have been the subject of different studies relevant to antimicrobial agents, therapeutics, fluorescent labels, drug delivery, medical imaging and transfection vectors. There has been evidence about the growing of ecotoxicological effects (the effects of toxic chemicals on biological organisms) of NPs.

A few studies have been reported concerning the interaction of NPs with living cells because of the main effects of NPs in the human body, such as:

(i) NPs inducing oxidative stress inflammation, and indirect DNA damage in a cell body, and

(ii) NPs causing size/shape-dependent cellular damage in living systems; these NPs have similar sizes to many cellular components like DNA, RNA and proteins,

where the physico-chemical properties of the NPs modulate their dynamic interaction with biomolecules and cellular organelles, and, possibly their toxicity.

NPs may by-pass the cell membrane and lead to harm in living cell and cause inverse effects in living cells. When DNA is exposed to UV light, excited levels are created in DNA leading to mutagenic photoproducts. This is where, single-stranded DNA can transfer an electron between stacked bases. The ability to observe and study photoinduced DNA offers exciting opportunities to explain the fundamental principles that govern energy and charge migration in multi-chromophoric systems made of organic building blocks. Systems play a central role in biological and biomimetic energy harvesting and photocatalysis.

The ways for drugs to interact with DNA

(i) drugs interact with protein which binds to the DNA,

(ii) drugs interact during RNA binding to DNA to form RNA hybridization,

(iii) small molecules electrostatically interact with DNA via intercalation between base pairs in DNA and the minor DNA grooves (where the two DNA strands are near (deep-narrow) to each other) and the major DNA grooves (where the two DNA strands are far (shallow-wide) from each other (see Fig.1).

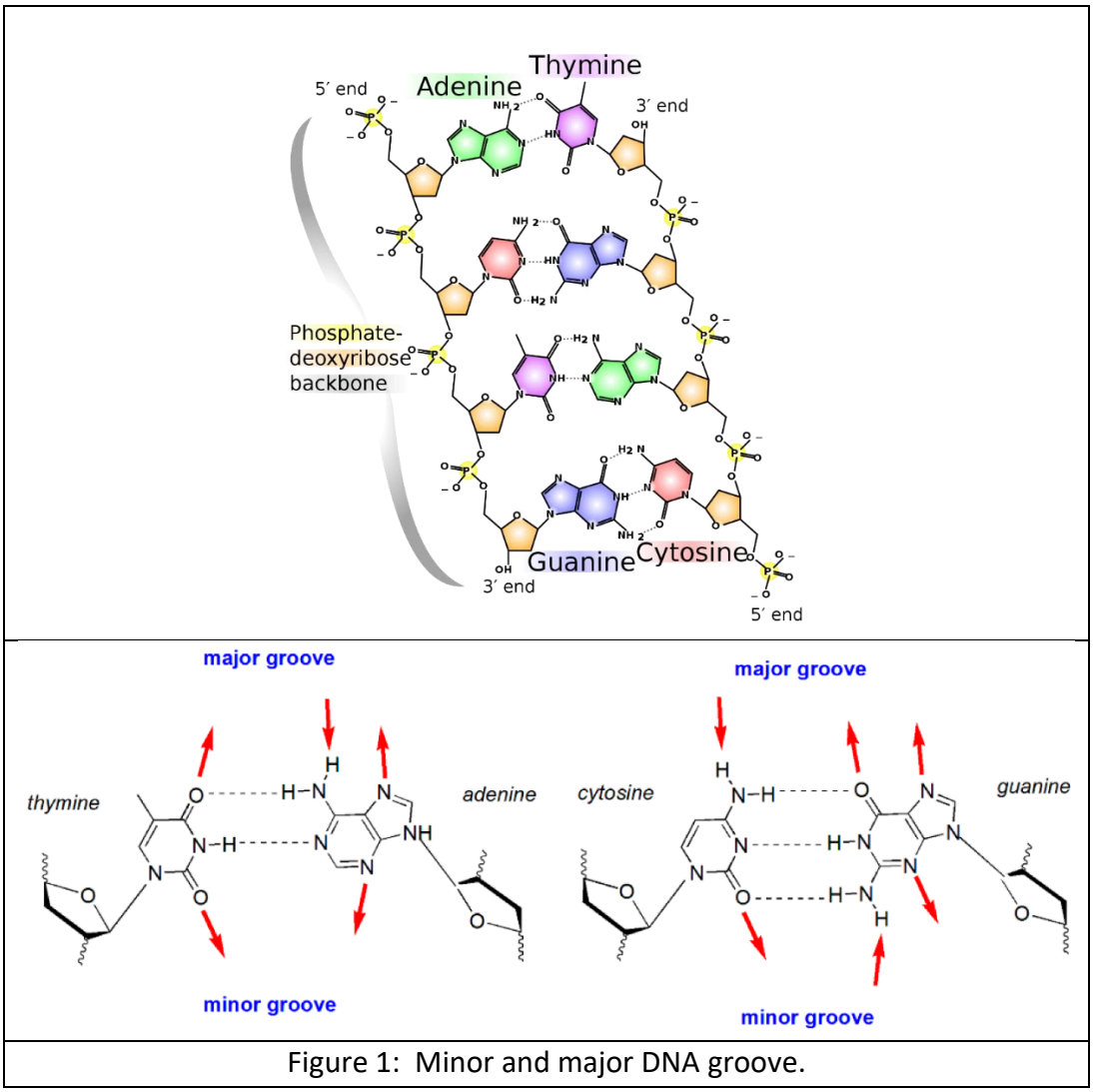

Figure 1: Minor and major DNA groove.

Types of drug-DNA interaction

1- **covalent binding** is invariable (and irreversible causing the inhibition of DNA processes completely). Then, it subsequently leads to cell death, see cis-Pt for example (Fig.2). This type of inhibition involves an alkylating agent because it attaches an alkyl group to guanine in DNA. It is considered to be relatively toxic.

2- non-covalent binding is reversible (fixed). Hence, it is sometimes preferred over covalent adduct formation and involves lower toxicity. The significant effects of non-covalent binding involve the DNA conformation, related structure perturbation and interaction with normal DNA protein-like topoisomerase due to affect the function of mitochondrial DNA and DNA strand breaks.

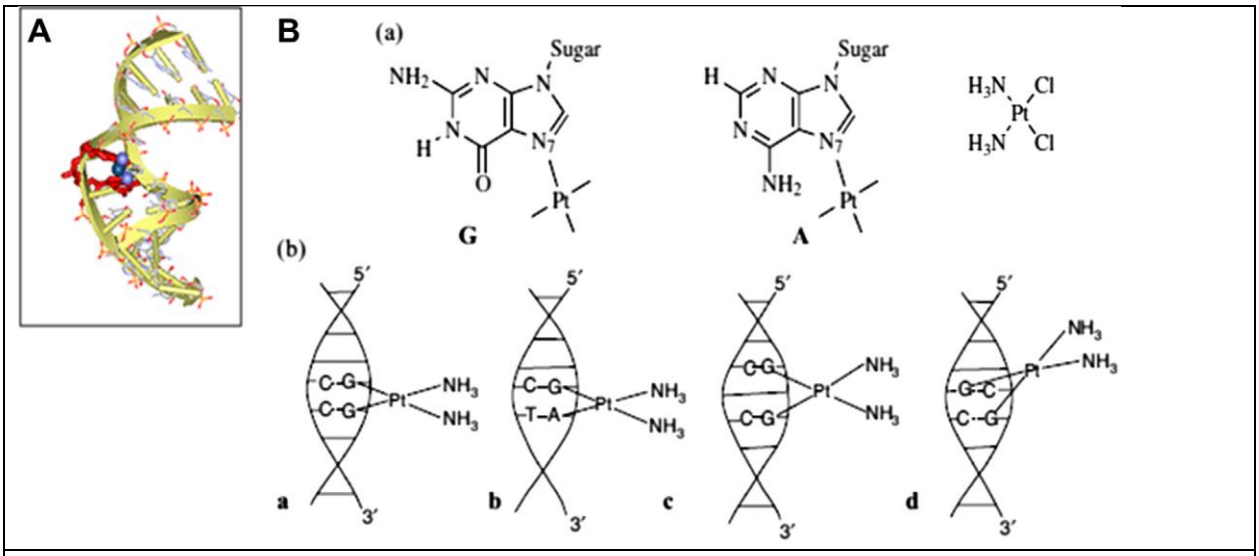

Figure 2: Covalent binding cis-Pt with DNA with guanine-cytosine (G-C) and adenine-thymine (A-T) nucleobases.

Classification of non-covalent binding

(i) **Intercalation**: this is when a molecule stacks perpendicular to the DNA backbone without forming covalent interaction or it cleaves the hydrogen bond between the two base pairs in DNA. Depending on the intercalator, the DNA must open the space between two base pairs dynamically by a varying degree of unwinding. For example, the ethidium cation (using in gel electrophoresis) that unwinds DNA, this is about 26°. There are many forces that sustain the stability of DNA-intercalator complex (hydrogen bonding, Van der Waal's forces, charge transfer forces and hydrophobic interactions). This mode is preferred by the presence of an extended fused aromatic ligand. Thus, the complex is stabilized by π-π^* stacking interaction and it is less sensitive to ionic strength. When the aromatic system is less extended, the intercalation is generally prevented during the clashing of the additional ligands with phosphodiester backbone. The intercalation of a planar ligand of the Co and Cu complexes in the DNA base pairs stack forming a covalent interaction or cleaves the hydrogen bond (Fig. 3).

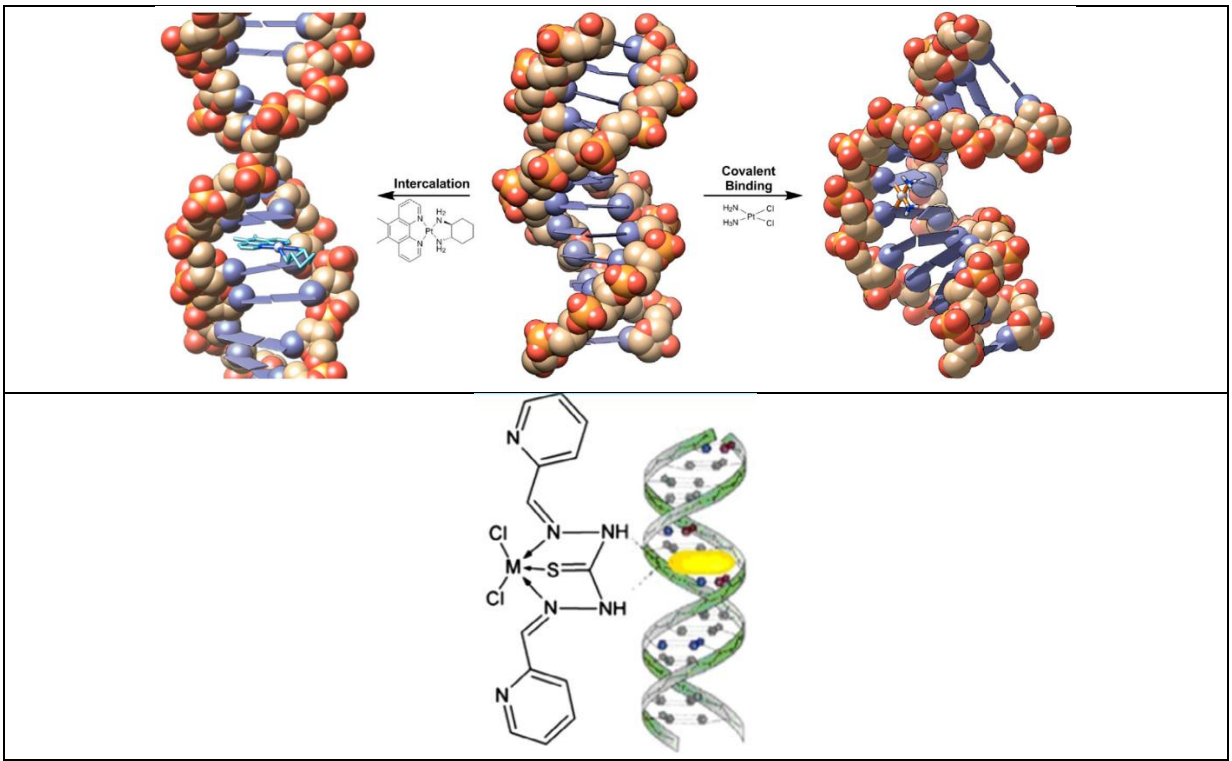

Figure 3: Intercalation of metal as Co and Cu complexes into the DNA base pairs forming covalent interaction or cleaves the hydrogen bond.

(ii) **Groove Binding:** Small ligands bind to the minor groove of the DNA by hydrogen bonding with bases (usually to O No.2 of thymine and N No.3 of adenine) and Van der Waal's forces. This binding is usually specific to adenine-thymine (AT) rich sequences because:

(a) AT regions are close and narrower than (GC) groove regions,

(b) The stereochemistry that is presented by the C-atom No.2 amino group of the guanine base.

This priority, in addition to the designed tendency for the electro-negative pockets of (AT) sequences, is due to a better Van der Waal's interaction between groove walls and the ligand in this place. Unlike the intercalator, groove binding drugs induce no structural rearrangement of DNA helix like Ni and Zn complexes, consider intercalation of Ni and Zn complexes into the DNA base pairs as adenine-thymine (AT) which is close and narrower than (GC) groove regions(Fig.4).

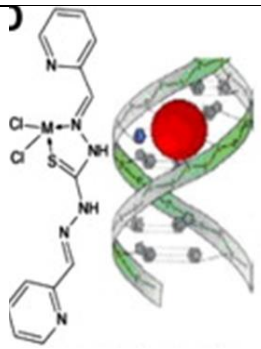

Figure 4: Intercalation of Ni and Zn complexes into the DNA base pairs as adenine-thymine (AT) which is close and narrower than (GC) groove regions.

(iii) **External Binding:** The ligands interact with DNA phosphate backbone where the ligand self-associates to form higher-order aggregates (it is electronic in nature). Here, the ligand stack on the anionic DNA backbone reduces charge-charge repulsion between the ligands. Mg and Ru complexes, which have divalent positive charge, bind with phosphate sugar back-bone in the DNA (negatively charged) dependent on ionic strength.

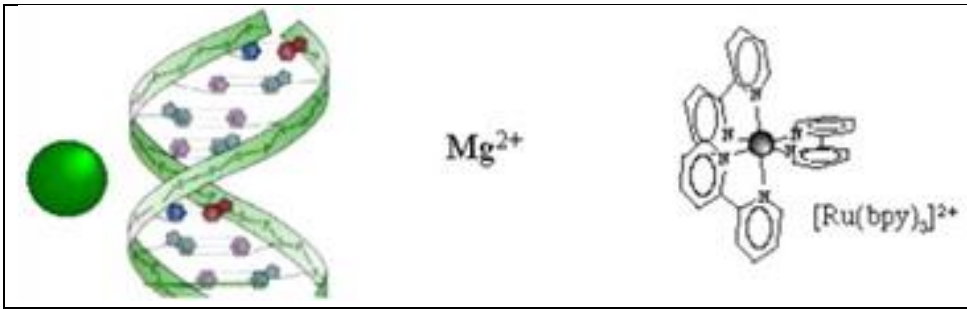

Figure 5: Intercalation of Mg complexes into the DNA base pairs.

Au NPs have higher cytotoxicity compared to Ag NPs because the charged Au NPs may adsorb serum proteins and enter cells by the more complicated endocytosis pathway.

Endocytosis and exocytosis and effect nanoparticles physicochemical properties on biological system

The understanding of the endocytosis and exocytosis mechanism of NPs is significant to reducing the toxicity of NPs. Thus, one can design NPs to be safer, and control their efficient entry into/exit from cell of human and tissues.

1- Endocytosis of NPs

Endocytosis of NPs is the uptake of small proteins or ions into the cell where special transport channels enable their translocation across the cell membrane.

When macro molecules and proteins are too large to enter through the plasma membrane, the cell has a different mechanism for their uptake from media. These uptake mechanisms refer to

endocytosis (see Fig. 6) and these mechanisms depends on: (i) changing the size of the transport vesicle, (ii) internalization machinery (tool), (iii) properties of the cargo (load).

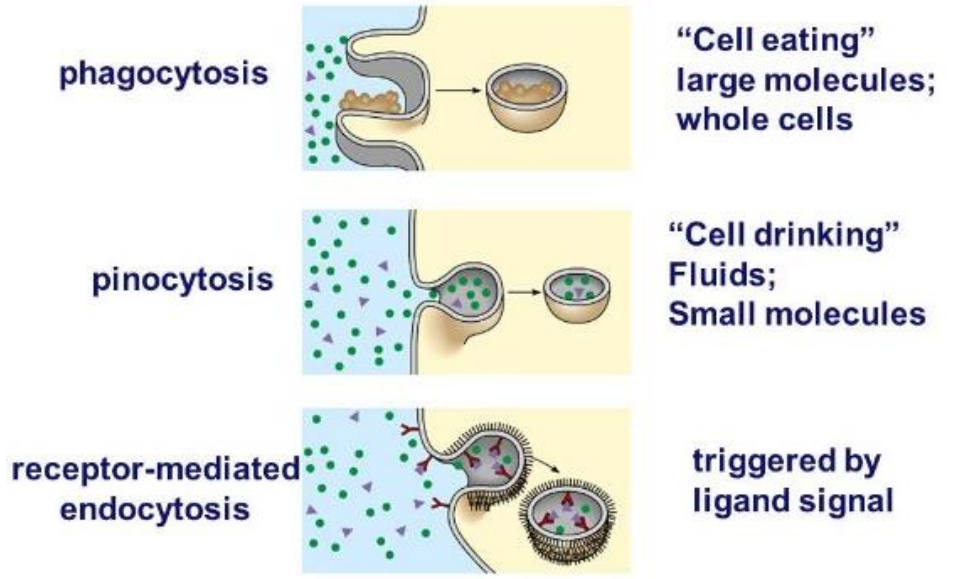

Figure 6: Endocytosis pathways (subdivided into four categories: receptor–mediated endocytosis, pinocytosis and phagocytosis).

2- Exocytosis of NPs

It is responsible for their systemic elimination and toxicity**.** The cellular uptake may be considered because of completion between the receptor diffusion kinetics and thermodynamic driving forces wrapping.

factors can explain nanotoxicity of NPs and effect nanoparticles physicochemical properties on biological system:

(i) surface chemistry can be determined by the chemical composition on the NP's surface and charge. The surface charge of NPs can influence their pathway of cellar uptake and efficiency because biomolecules in a biological system have various charges. Different surface charges are important as they influence uptakes by macrophages. For example, positively charged NPs exhibited a higher phagocytic uptake than neutrally or negatively charged NPs.

(ii) Size is an important factor that affects the interaction of NPs with cell in the same composition. It is critical *in vivo* functions of NPs which are dependent on size such as internalization, targeting and clearance. Generally, Au NP's cellular maximum uptake was observed with size 50nm and this size makes Au NPs nontoxic. Further, Au NPs with 5 -15nm inhibited colony formation in mouse fibroblast cells above

50μM. It was reported that the intercellular uptake of Au NPs depends on size, shape, surface coating, concentration and aggregation. Also Au NPs at 50nm diameter showed the highest efficient cellular uptake compared with other sizes. On the other hand, Au NPs of size 4nm showed the highest uptake in the macrophages depending on the number of NPs taken up per cell comparing with 11, 19, 35 and 45nm. Moreover, Au NPs with size less than 100nm were phagocytosed through scavenger receptor mediated phagocytosis.

(iii) Shape: Rod-shape nanoparticles exhibit the highest uptake in human cancer cell followed by spheres, cylinders and cubes. Studies reveal that the uptake of the rod-shaped NPs by macrophages were more efficient than with spherical NPs. However, the spherical NPs were taken up by cancer cells more efficiently than were rod–shaped NPs.