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Inhibition of Human Cytochrome P450 Enzymes by Metallic Nanoparticles: A Preliminary to Nanogenomics

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Abstract: The effect of nanoparticles on the biological functions or the relative toxicity is not well-understood and becomes the major public concerns. This study determined the *in vitro* effect of gold and silver nanoparticles as the two most frequently used metallic nanomaterials for therapeutics and diagnostic on the microsomes containing wild-type cDNA expressed human CYP450 enzymes CYP1A2, 2C9, 2C19 and 3A4. Results demonstrated that all of the CYP450s activities were down-regulated by metallic nanoparticles. These findings suggest the inhibition of oxidation based biological process by penetration of metallic nanosized particles across the microsomal membrane.

Key words: Cytochrome P450, gold nanoparticles, silver nanoparticles, metabolism

INTRODUCTION

It is widely recognized that nanomaterials produce extensive influences in general public including economical, technological and medicinal purposes (Farokhzad and Langer, 2006). The concern of negative consequence on health and environment of nanomaterials is continuously raised, whereas the knowledge on their biological effects remains unclear. The study related to the toxicity or biological activities of the Synthetic Nanoparticles (NPs) is an urgent need if such materials are aimed to further implement in human.

Gold and silver nanoparticles are the metal-based nanocarriers commonly used in various applications including drug delivery, diagnosis and therapy (Biju *et al*., 2008). Recently, the self-assembly metallic nanoparticles were reported to alter or inhibit the biological functions (Fischer *et al*., 2002; Mukherjee *et al*., 2005). Gold nanoparticles (AuNPs) were found to inhibit the chymotrypsin activity and vascular endothelial growth factor of endothelial cells (Fischer *et al*., 2002; Mukherjee *et al*., 2005). Importantly, AuNPs might play a role in cell cycle by upregulating the p21 and p27 proteins expressed in the G1 phase (Bhattacharya *et al*., 2007). Additionally, silver nanoparticles (AgNPs) showed ability to bind with the glycoprotein subunits of HIV-1 leading to deactivation of HIV-1 virus (Elechiguerra *et al*., 2005).

To explore whether AuNPs and AgNPs can interfere with the metabolic or biotransformation process of drug and xenobiotics, we performed an inhibition assay of NPs on some cDNA expressed human cytochrome P450 monooxygenases (P450), namely CYP1A2, CYP2C9, CYP2C19 and CYP3A4, as key oxidative enzymes in a first stage of metabolism (Coleman, 2005).

MATERIALS AND METHODS

Materials: Tetrachloroauric acid ($HAuCl_4$) was purchased from Acros Organics (Morris Plains, NJ). Trisodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$), silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) were supplied from Merck (Darmstadt, Germany). Microsomes from baculovirus-infected cells containing wild-type cDNA expressed human CYP1A2, CYP2C9, CYP2C19 or CYP3A4 and NADPH-cytochrome P450 reductase (P450 Baculosomes®), NADPH regeneration system containing

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glucose-6-phosphate dehydrogenase (G-6-PD) and glucose-6-phosphate (G-6-P), $NADP^+$, fluorogenic Vivid® EOMCC substrate (7-ethyloxymethyloxy-3-cyanocoumarin) for CYP1A2/2C19 or Vivid® BOMCC substrate (7-benzyloxymethyloxy-3-cyanocoumarin) for CYP2C9/3A4 and potassium phosphate buffer (reaction buffer) were from Invitrogen (Madison, WI). All other chemicals used were the highest commercial grade available.

Synthesis and characterization of AuNPs and AgNPs: AuNPs were synthesized from $HAuCl_4$ using $Na_3C_6H_5O_7{\cdot}2H_2O$ as a reducing agent and AgNPs were made by reducing $AgNO_3$ with $NaBH_4$ as earlier described (Enüstün and Turkevich, 1963; Van Hyning and Zukoski, 1998). The final concentration of Au atom in AuNP solution was 44 ppm. The AgNP solution was 20-fold diluted to obtain 50 ppm of Ag atom prior to use. The NPs were characterized using Transmission Electron Microscopy (TEM) H-7650 (Hitachi, Japan) and UV-visible spectroscopy (Shimadzu, Japan). The zeta potential or the surface charge of NPs was determined by Zetasizer NanoZS (Malvern, UK).

CYP P450 inhibition assay: The inhibition assay was conducted in black, flat-bottom, 96-well plate (PerkinElmer, Waltham, MA) for a 100 μL reaction per well according to the standard protocol described (http://www.invitrogen.com/contents/sfs/panvera/L0504.pdf). For inhibition assay of CYP1A2 or CYP2C19, a 50 μL buffer solution (pH 8.0) containing P450 Baculosomes® at assay concentrations of 5 nM CYP450, 0.3 U mL^{-1} G-6-PD and 3.33 mM G-6-P was added to 40 μL of the solution of AuNPs (44 ppm) or AgNPS (50 ppm). The plate was incubated for 20 min at room temperature before adding 10 μL a mixture of EOMCC substrate (3 μM for CYP1A2 or 10 μM for CYP2C19) and 100 μM $NADP^+$ solution. The same procedure was used for inhibition studies of CYP2C9 or CYP3A4 except for 10 μM BOMCC substrate was used and the assay concentrations of CYP2C9 and CYP3A4 were 10 and 5 nM, in orderly. The enzymatic reaction was stopped by addition of 5 μL of 0.5 M Tris base (pH 10.5) to all wells after 30 min incubation at room temperature. The relative fluorescence unit (RFU) was read on a Victor^3 V plate reader (PerkinElmer, Waltham, MA) using the fluorescent excitation filter at 390 nm and a bandwidth of 20 nm and emission wavelength at 450 nm and a bandwidth of 10 nm. Percent inhibition was then calculated by comparison of RFU to those wells using water instead of sample.

RESULTS AND DISCUSSION

Characterization of the metallic NPs: The visual appearance of synthesized AuNPs was in deep red color and AgNPs was in yellow color (Fig. 1a). The characteristic Surface Plasmon Resonance (SPR) bands of AuNPs at 525 nm and AgNPs at 440 nm were clearly observed by UV-Vis suggesting the formation of spherical NPs (Fig. 1b). The TEM images showed the average particle diameter of 9 nm for AuNPs and 15 nm for AgNPs (Fig. 1c, d). The zeta potential (Mean±SD, n = 3) of AuNPs were -30.8±2.4 mV. The AgNPs had slightly negative zeta potential, -14.4±1.1 mV.

P450 inhibitory potency of metallic NPs: The P450 metabolism cleaves the quenched substrates, resulting in a highly fluorescent metabolite of 3-cyano-7-hydroxycoumarin. P450 inhibitory effects of AuNPs and AgNPs were identified by their ability to prevent formation of fluorescent signal in the assay. The wild-type P450 isozymes were chosen to be studied as being dominant genes in the population (Coleman, 2005). The results clearly showed that the P450s were down-regulated by AuNPs and AgNPs with the greater extent being seen with the AgNPs (Table 1).

So far, there are no any evidences on how the NPs interact with the P450 proteins. We propose here in the possible ways in which the metallic NPs inhibit the P450 activity, NPs having the binding affinity to P450 due to the lipophilicity of the NPs and the hydrophobicity of the haem environment in P450 molecule, hence being the competitor of enzyme substrate (Lewis *et al*., 2004). NPs forming mixed micelles with the microsomal membrane leading to the change in membrane integrity and the inactivity of the enzyme.

From the results, the metallic NPs were surface charged as seen from the zeta potential; hence, the NPs might be less lipophilic than the value required for the P450 binding affinity. However, the data obtained from molecular modeling such as hydrogen bond and π-π stacking interaction energy of the NP molecule are needed for proper justification of the first hypothesis. For the assumption on membrane perturbation of NPs, it is known that the inactivation of P450 is well succeeded with the amphiphilic molecule (i.e., surfactants). The earlier study reported that the formation of micelles of glycocholic/phospholipid or taurocholate/phospholipid caused 80 to 100% inhibition of human CYP3A4 *in vitro* (Mountfield *et al*., 2000). Metallic NPs were considered to be amphiphilic, thus being able to fluidize

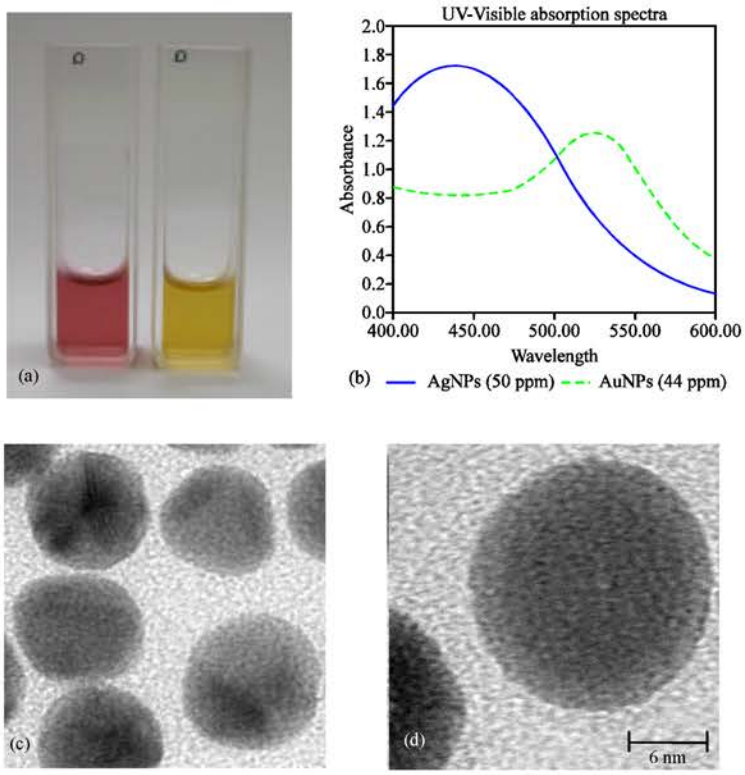

Fig. 1: Synthetic colloidal gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs); (a) Visual detection of the AuNPs (left) and the AgNPs (right), (b) UV absorption spectrum of AgNPs and AuNPs and (c, d) Electron micrographs of AuNPs (c) and AgNPs (d) at the same magnification (scale bar = 6 nm both)

Table 1: Inhibitory effect of gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) on wild-type human P450 (Mean±SD, n = 4)

	Inhibition (%)	
CYP 450 isozymes	AuNPs	AgNPs
1A2	6.3±2.6	95.0±0.8
2C9	28.5±2.9	83.5±2.4
2C19	32.0±5.4	97.8±3.2
3A4	26.0±5.9	98.7±0.5

and penetrate the microsomal membrane. Additionally, other nanomaterials such as carbon nanotubes were capable of passing through a transmembrane configuration (Lopez *et al.*, 2004).

CONCLUSION

Conclusively, to our knowledge, this is the first report of AuNPs and AgNPs influence on human P450 proteins. The preliminary findings implied that metallic NPs had an inhibitory potential on the oxidative metabolism. Any changes in P450-NP interaction due to the polymorphic variances of P450 isozymes are under our further investigations.

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REFERENCES

Bhattacharya, R., C.R. Patra, R. Verma, S. Kumar and P.R. Greipp *et al.*, 2007. Gold nanoparticles inhibit the proliferation of multiple myeloma cells. Adv. Mater., 19: 711-716.

Biju, V., T. Itoh, A. Anas, A. Sujith and M. Ishikawa, 2008. Semiconductor quantum dots and metal nanoparticles: Syntheses, optical properties, and biological applications. Anal. Bioanal. Chem., 391: 2469-2495.

Coleman, M.D., 2005. Human Drug Metabolism. 1st Edn., John Wiley and Sons, Chichester, ISBN-13 978-0-470-86353-6.

Elechiguerra, J.L., J.L. Burt, J.R. Morones, A. Camacho-Bragado and X. Gao *et al*., 2005. Interaction of silver nanoparticles with HIV-1. J. Nanobiotechnol., 3: 1-10.

Enüstün, B.V. and J. Turkevich, 1963. Coagulation of colloidal gold. J. Am. Chem. Soc., 85: 3317-3328.

Farokhzad, O.C. and R. Langer, 2006. Nanomedicine: Developing smarter therapeutic and diagnostic modalities. Adv. Drug Deliv. Rev., 58: 1456-1459.

Fischer, N.O., C.M. McIntosh, J.M. Simard and V.M. Rotello, 2002. Inhibition of chymotrypsin through surface binding using nanoparticle-based receptors. Proc. Natl. Acad. Sci. USA., 99: 5018-5023.

Lewis, D.F.V., M.N. Jacobs and M. Dickins, 2004. Compound lipophilicity for substrate binding to human P450s in drug metabolism. Drug Discov. Today, 9: 530-537.

Lopez, C.F., S.O. Nielsen, P.B. Moore and M.L. Klein, 2004. Understanding nature's design for a nanosyringe. Proc. Natl. Acad. Sci. USA., 101: 4431-4434.

Mountfield, R.J., S. Senepin, M. Schleimer, I. Walter and B. Bittner, 2000. Potential inhibitory effects of formulation ingredients on intestinal cytochrome P450. Int. J. Pharm., 211: 89-92.

Mukherjee, P., R. Bhattacharya, P. Wang, L. Wang and S. Basu *et al*., 2005. Antiangiogenic properties of gold nanoparticles. Clin. Cancer Res., 11: 3530-3534.

Van Hyning, D.L. and C.F. Zukoski, 1998. Formation mechanism and aggregation behavior of borohydride reduced silver particles. Langmuir, 14: 7034-7046.