Nano-silver Particle Effects On Drug Metabolism in Vitro.

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Abstract
Nano-silver particles are present in consumer and health care products. Their effects on human microsomal cytochrome P450 activities and induction in luciferase reporter-engineered Caco-2 (MDR1.C) and HepG2 (DPX2 and 1A2DRE) cells have been investigated. The LD$_{50}$ was $\sim$4 $\mu$g silver/ml for HepG2 and 5 $\mu$g/ml for Caco-2 cells. At silver concentrations showing no viability decrease ($<$1 $\mu$g silver/ml), the PXR-driven 4.5-fold induction response of MDR1.C cells to 50 $\mu$M omeprazole was unaffected. In DPX2 cells, the PXR-driven 5.5-fold and 6.5-fold induction responses to omeprazole and 10 $\mu$M rifampicin were attenuated to 4-fold and 3.5-fold, respectively. Nano-silver particles alone showed no induction. In 1A2DRE cells, the AhR-driven 5.5-fold induction response to omeprazole was attenuated to 4-fold. In 1A2DRE cells, nano-silver alone elicited slight induction at 1 $\mu$g/ml. The inhibition of human CYP-selective activities by nano-silver particles in vitro was proportional to the silver:microsomal protein ratio. At a fixed (0.5 mg/ml) protein concentration, CYP-selective activities differed in sensitivity (IC$_{50}$ value). Coumarin 7-hydroxylation and 7-ethoxy-4-trifluoromethylcoumarin O-deethylation exhibited the highest IC$_{50}$ values (33.5 and 31.9 $\mu$M respectively) and S-mephenytoin 4-hydroxylation the lowest (6.4 $\mu$M). Other IC$_{50}$ values were, in ascending order, 8.0 - 9.3 $\mu$M (testosterone 6$\beta$-hydroxylation, 7-benzyloxyquinoline debenzylation and diclofenac 4-hydroxylation), 16.0 $\mu$M (chlorzoxazone 6-hydroxylation), 21.2 $\mu$M (7-methoxy-4-(aminomethyl)-coumarin O-demethylation), and 24.4 $\mu$M (7-methoxyresorufin O-demethylation). Investigated at 70 $\mu$M nano-silver, microsomal NADPH cytochrome c reductase activities were inhibited $<$12%. Extrapolating from in vitro observations, nano-silver particles reaching the liver can be anticipated to be a potential source of drug-drug interactions.
Introduction

Silver has long been known to be a potent antimicrobial agent. In recent years, silver, much of it in nanoparticle form, has seen increased use in such items as children’s toys, babies’ bottles, textiles, cleaning agents, refrigerators, dishwashers, washing machines, air conditioners, air purifiers and vacuum cleaners. There are also a variety of nano-silver-containing dermatological preparations and cosmetics available, promoted for their antibacterial properties. Solutions containing nano-silver particles 5-15 nm in size (mean size, 10 nm) comprising an interior of elemental silver and an exterior of ionic silver oxide are commercially available as mineral supplements and are promoted for immune support. Recommended adult dosing for one such product, the one utilized in the present study, is 150 μg of silver, orally, three times per day. Information on the biodistribution of ingested nano-silver particles, and concentrations reached within the human body is not known. Orally ingested nano-silver particles obviously first impact cells in the GI tract, and these are therefore the first potential source of drug-drug interactions. If they enter the circulation from the intestine, the next cells impacted are those of the liver. In experimental animals, mice fed 13 nm silver nanoparticles show signs of inflammation in the liver indicating that the intestine is indeed a portal of entry for this size of nano-silver particle (Cha et al., 2008). Mice fed 30 nm silica particles showed evidence of liver toxicity indicating that it is also a portal of entry for non-metallic nanoparticles (So et al., 2008). Organically modified 20 nm silica (ORMOSIL) particles conjugated with a fluorophore in mice (Kumar et al., 2010) and 20 nm gold nanoparticles in rats (Balsubramanian et al., 2010) were both predominately localized to the liver within 1 day following intravenous injection. Studies with galactose-PEGylated nanoparticles showed that following tail vein injection, 50 nm particles
specifically accessed hepatocytes whereas larger 140 nm particles deposited in the Kupfer cells (Popielarski et al., 2005). Large silver nanoparticles (56 nm) given to rats at doses of 30, 125 and 500 mg/kg by gavage for 90 days resulted in dose-dependent accumulation of silver in the liver of 4 to 69 ug/g (males) and 9 to 99 ug/g (females) (Kim et al., 2010)

The effects of nano-silver particles on intestine and liver cells is therefore important to investigate since these are the tissues that are exposed to high concentrations of nanoparticles and also the major location of enzymes and transporters affecting the disposition of a wide range of drugs and other xenobiotics. Changes in, and effects on these enzymes and transporters are often an important source of unanticipated drug-drug interactions. In this study, the effect of nano-silver particles on commercially available luciferase reporter-engineered (human epithelial colorectal adenocarcinoma) Caco-2 cells (MDR1.C) and (human hepatocellular carcinoma) HepG2 (DPX2 and 1A2DRE) cells in culture and their ability to respond to xenobiotic inducing agents has been investigated. Studies have shown that 10 nm silver nanoparticles gain access to all subcellular compartments of HepG2 cells in culture (Kim et al., 2009) so contact with drug metabolizing enzymes localized in the endoplasmic reticulum is to be expected in vivo. Therefore the direct effects of nano-silver particles on human microsomal cytochrome P450 activities in vitro have also been evaluated.

Reports on P450 induction and inhibition by silver particles, or nanoparticles of any description, are scarce. With respect to induction, fish exposed to 10 nm silver particles show induction of CYP1A2 in their gill tissue (Scown et al., 2010). Rats injected with 20 nm gold nanoparticles showed induction of CYP1A1 mRNA after two months (Balsubramanian et al., 2010). With
respect to inhibition, silver in the form of 15 nm nanoparticles, showed similar inhibition (95-98% at 50 ppm) of activities of human CYP1A2, CYP2C19 and CYP3A4 expressed in insect cell membrane preparations. Expressed 2C9 was slightly less inhibited (84%) (Sereemaspun et al., 2008). Non-metallic carboxyl polystyrene particles (20-60 nm) inhibited the enzymatic activity of several P450 enzymes (CYP3A4, CYP2D6, and CYP2C9) in both insect cell membrane preparations and liver microsomes (Fröhlich et al., 2010), suggesting that “inert” nanoparticles in and of themselves can produce unexpected effects on liver cell function. The present study sought to evaluate whether nano-silver particles also carry the potential to affect the important liver cell function of detoxification and elimination of drugs, and so could be a potential source of unanticipated drug-drug interactions in the human population.
Materials and Methods

The nano-silver solution was obtained from American Biotech Labs (Alpine, Utah) as a 30 ppm solution in distilled water. Luciferase reporter-engineered Caco-2 (MDR1.C) and HepG2 (DPX2 and 1A2DRE) cells were obtained from Puracyp Inc (Carlsbad, CA) and maintained in the cell-appropriate media purchased from Puracyp Inc. Cell viability and luciferase activity was determined using a Multitox-Fluor Multiplex Cytotoxicity Assay and a Bright-Glo Luciferase Assay System, respectively (Promega, Madison, WI). Nano-silver toxicity (LD50) values were obtained following 24 h exposure. Induction by rifampicin and omeprazole was determined 24 h after the addition of the inducer, and for nano-silver effects on induction, the cells had been pre-exposed to nano-silver in the medium for 24 h. Experiments were repeated on several batches of cells. The results reported are the mean ± SEM of quadruplicate determinations on a single batch. Inter-batch induction responses were within 25% of each other. The fold induction was in comparison to vehicle (DMSO)-treated controls. Statistical analyses of cell viability and induction responses were undertaken using ANOVA and differences, considered significant at p < 0.05, were assessed by Fisher’s Partial Least Squares Difference multiple range test. Human liver microsomes were purchased from Human Biologics Inc (Scottsdale, AZ), Celsis (Chicago, IL) and Puracyp Inc (Carlsbad, CA). Cytochrome P450 monooxygenase activities towards 7-methoxyresorufin, coumarin, 7-ethoxy-4-trifluoromethylcoumarin, 7-methoxy-4-(aminomethyl)-coumarin and benzylxoyquinoline were determined in kinetic assays from the rates of fluorescence increase arising from the formation of the hydroxyl containing metabolites. Monooxygenase activities towards diclofenac, S-mephenytoin, chlorozoxazone and testosterone were determined by HPLC analysis of the microsomal incubation extract and quantified by
integration of peak areas. All microsomal assays were performed at 37°C in the presence of excess NADPH. Reported IC₅₀ values were the mean of three independent determinations, each with duplicates at each data point. Assay details and original references on CYP-selectivity with two exceptions were reported previously (Franklin and Constance, 2007). Assay details and CYP-selectivity for 7-methoxy-4-(aminomethyl)-coumarin demethylation and 7-benzyloxyquinoline debenzylation were reported by Ondenwater et al., (1999) and Stresser et al., (2002), respectively. The P450 substrates, metabolites, HPLC internal standards, NADPH, and cytochrome c were purchased from Sigma-Aldrich (Milwaukee, WI) with the exceptions of 7-methoxy-4-(aminomethyl)-coumarin, 7-hydroxy-4-(aminomethyl)-coumarin, benzyloxyquinoline and hydroxyquinoline (BD Biosciences, San Jose, CA), and S-mephenytoin, 4-hydroxymephenytoin and 6-hydroxychlorzoxazone (Ultrafine, Manchester, UK).

Throughout the manuscript, nano-silver particle concentrations are given as total silver concentration. For cell-based results, silver concentrations are given in μg/ml while in describing effects on enzyme activities, concentrations are given as μM to fit most readily with existing literature in the area.
Results

For both luciferase reporter-engineered HepG2 cell lines (DPX2 and 1A2DRE) in culture, the LD$_{50}$ determined after 24 h of exposure to the nano-silver particles in the medium was ~4 μg silver/ml (Table 1). For reporter-engineered Caco-2 cells (MDR1.C), the LD$_{50}$ was ~5 μg silver/ml. The ability of nano-silver in the cell-medium to affect the induction response to drugs was investigated at silver concentrations of 0.1 - 1.0 μg/ml. At 1 μg silver/ml, none of the three cell lines showed a significant decrease in cell viability (Table 1). In cells that were exposed to the nano-silver 24 h prior to the addition of inducing agent, the 24-h PXR-driven 4.5-fold induction response of MDR1.C cells to 50 μM omeprazole was unaffected (Figure 1). In the absence of drugs, nano-silver particles did not elicit any induction. In HepG2 cells however, the presence of silver depressed the induction response. In DPX2 cells, the pregnane X receptor (PXR)-driven 5.5-fold induction response to 50 μM omeprazole was attenuated to a 4-fold response and appeared to be largely independent of the silver concentration over the range investigated (Figure 2). The PXR-driven induction by 10 μM rifampicin was attenuated to a much greater extent, from 6.5-fold to ~3.5-fold (Figure 2). In 1A2DRE cells, the arylhydrocarbon (Ah) receptor-driven 5.5-fold induction response to 50 μM omeprazole was attenuated to a 4-fold response (Figure 3) and again the attenuation was largely independent of the silver concentration. In 1A2DRE cells, nano-silver particles at 1 μg silver/ml (9.3 μM) showed a slight induction response.
Nano-silver inhibition of all human microsomal CYP-selective activities was proportional to the silver:protein ratio. Examples shown are for the inhibition of testosterone 6β-hydroxylation a reaction with a relatively low IC₅₀ value (Figure 4-upper panel), chlorzoxazone 6-hydroxylation (Figure 4-center panel), and 7-ethoxy-4-trifluoromethylcoumarin O-deethylation (Figure 4-lower panel), reactions with intermediate and a relatively high IC₅₀ values respectively. When a battery of CYP-selective reactions was examined at a fixed protein concentration (0.5 mg protein/ml) and near-Km substrate concentrations, the activities showed differences in sensitivity to inhibition by nano-silver (Table 2). S-Mephenytoin 4-hydroxylation exhibited the lowest IC₅₀ (6.4 ± 0.1 μM) and coumarin 7-hydroxylation and 7-ethoxy-4-trifluoromethylcoumarin O-deethylation exhibited the highest IC₅₀ values (33.5 ± 2.3 and 31.9 ± 2.5 mM, respectively). Other IC₅₀ values were, in ascending order, 8.0 ± 0.3, 8.8 ± 0.5 and 9.3 ± 0.2 μM (testosterone 6β-hydroxylation, 7-benzyloxyquinoline debenzylation and diclofenac 4-hydroxylation), 16.0 ± 1.4 μM (chlorzoxazone 6-hydroxylation), 21.2 ± 1.3 μM (MAMC [7-methoxy-4-(aminomethyl)-coumarin] O-demethylation), and 24.4 ± 1.1 μM (7-methoxyresorufin O-demethylation). As anticipated from the protein dependency of the degree of inhibition, the mechanism of inhibition was non-competitive. From a four substrate concentration/six inhibitor concentration evaluation, the nano-silver Ki value for testosterone 6β-hydroxylation was 6.6 μM.

Human liver microsomal NADPH cytochrome c reductase activity in the various microsomes employed in the CYP-selective activity determinations was inhibited less than 12% at 70 μM silver (Table 3), a concentration well in excess of the IC₅₀ values for monooxygenase reactions.
Discussion

Silver nano-particles are far from biologically inert, and have significant effects on drug metabolizing enzymes systems in vitro. They are toxic to both Caco-2 (human epithelial colorectal adenocarcinoma) cells and HepG2 (human hepatocellular carcinoma) cells in culture, with 50% decreases in viability occurring around 4 - 5 μg/ml (37 - 47 μM) with 24 h of exposure (Table 1). The toxicity values obtained in the present study for reporter-engineered HepG2 cells using the live/dead cell protease activity as a monitor are considerably higher than the 0.5 to 3.4 μg/ml IC\textsubscript{50} values reported for cytotoxicity for HepG2 cells following 28-h exposure to 10 nm nano-silver particles (Kim et al., 2009). The 0.5 - 3.4 range arose from the different cytotoxicity tests employed; LDH leakage, Alamar Blue reduction, and MTT reduction. The mechanism of toxicity in the reported study was concluded to be via oxidative stress, a mechanism that had been reported earlier for 15 nm and 100 nm silver particles in rat liver derived BRL 3A cells (Hussain et al., 2005).

The ability of nano-silver particles to affect the induction response of colon- and liver-derived cells to drugs was conducted at concentrations at and below 1 μg/ml, concentrations where no decrease in cell viability was apparent. In addition to affecting induction by the drugs, omeprazole and rifampicin, it was of interest to determine whether nano-silver particles themselves are able to elicit induction, either through the PXR/PXRE (DPX2 and MDR1.C cells) or through the AhR/XRE (1A2DRE cells) mechanisms. At 1 μg/ml (9.3 μM), nano-silver particles caused a small (1.8-fold) induction in 1A2DRE cells (Figure 3), an observation perhaps consistent with a reported effect of gold nanoparticle treatment in rats inducing CYP1A1
(Balsubramanian et al., 2010). While competition for the Ah receptor might explain the attenuation of induction elicited by omeprazole by nano-silver particles at 1 μg/ml, it would not explain the equally large attenuation of omeprazole induction at lower nano-silver concentrations that did not show induction (0.1 μg/ml). The similarity of the nano-silver attenuation of omeprazole induction in both 1A2DRE and DPX2 cells (Figures 3 and 2 respectively) could suggest some sequestering of the drug by nano-silver particles to prevent omeprazole interacting with receptors. If this was the case, however, it would be expected to show a dose response, with ever increasing attenuation with increasing nano-silver concentration and this was not seen. In fact, in DPX2 cells, there is less attenuation at the highest (1 μg/ml) nano-silver concentration. Additionally, there was no attenuation of omeprazole induction in MDR1.C cells (Figure 1) which would have been anticipated if the “effective” concentration of omeprazole was lowered by sequestration with nano-silver particles. Both omeprazole and rifampicin can induce via the PXR/PXRE mechanism present in these cells (Yueh et al., 2005) but the attenuation of induction in the same DPX2 cell line is much greater with rifampicin as the inducer, than with omeprazole (Figure 2). In whatever manner nano-silver is attenuating induction, it appears that omeprazole is more able to override the block. A peculiar feature of the attenuation is the ~0.1 μg/ml (~1 μM) threshold phenomenon which is apparent with both inducers and in all cell lines (Figures 1 - 3). Nano-silver concentrations 7-10 fold higher produce little further change in the attenuation. The lower limit of this threshold, and the range over which attenuation is dose dependent has yet to be explored but our current interpretation is that nano-silver is affecting one of many components that control the extent of induction over the time frame investigated (24 h) and the other components continue to allow induction but at a reduced rate or to a reduced extent.
The possibility of a second mechanism of unanticipated drug-drug interactions and altered drug disposition \textit{in vivo} arises from the inhibition of microsomal P450 catalyzed oxidations. Using substrates that are preferentially metabolized by different forms of P450, it is apparent that CYP2C9, CYP2C19 and CYP3A4 activities are most sensitive to inhibition by nano-silver (Table 2). Together these three P450s are responsible for the metabolism of about 60% of the drugs in current use that undergo oxidative metabolism. CYP1A2, CYP2A6 and CYP2B6 activities are least subject to inhibition, and CYP2D6 and CYP2E1 activities fall into an intermediate range. Using heterologously expressed human P450s in insect cell membranes, Sereemaspun et al., (2008) found CYP2C9 to be less sensitive to 15 nm silver nano-particle inhibition than CYP1A2, CYP2C19 and CYP3A4. Because the size of the nano-silver particles far exceeds the dimensions of even the largest substrate pockets of P450s (Ekroos and Sjögren, 2006, Otyepka et al., 2007, Ohkura et al., 2009), the particle inhibition appears to be at a surface site. A non-competitive best-fit mechanism for the inhibition determined for testosterone 6β-hydroxylation supports this interpretation. While physical disruption of the electron supply to support the P450 oxidation cannot be totally eliminated as a possibility, the insensitivity of the P450-reductase to inhibition, (albeit using cytochrome c as the artificial acceptor), with less than 10% inhibition at silver concentrations 2- to 10-fold greater than concentrations causing 50% inhibition of the P450 oxidation reactions, indicates that electron flow through the flavoprotein itself is not the locus of silver inhibition. Physical disruption from particles interacting at surface sites would be supported as a mechanism from observations with small (20-60 nm) non-metallic carboxyl polystyrene particles which also inhibited the enzymatic activity of CYP3A4, CYP2D6, and CYP2C9, in insect cell preparations and normal liver microsomes. (Fröhlich et al., 2010).
With these non-metallic particles, CYP1A2 was much less susceptible to inhibition than the other P450s, as was the case in the present study for nano-silver particles.

This study provides documentation of the ability of nano-silver particles to affect cell function as it relates to the metabolism and elimination of drugs and other xenobiotics. The most sensitive process affected, in terms of the nano-silver concentration at which it occurs, appears to be the ability of the cells to upregulate cytochrome P450s, a response regarded as a protective mechanism to promote metabolism and excretion when the cell is challenged with high or continuous levels of drugs and other xenobiotics. At higher concentrations, the inhibition of P450 enzyme activity is a process that is affected. It appears that not all P450s are affected equally, since IC₅₀ values of model reactions range from ~6 to ~34 μM. Ironically, the P450 responsible for the largest proportion of drugs that undergo P450-dependent metabolism, CYP3A4, is among the enzymes with the lowest IC₅₀. Thus ingested nano-silver particles, dependent on the amount ingested and the amount that reaches the liver, can be anticipated to be a potentially frequent source of drug-drug interactions in vivo and the practice of regular ingestion regarded with some caution.
References


Figure Legends

Figure 1: The effects of nano-silver in MDR1.C cells.

Plated MDR1.C cells were exposed to nano-silver-containing medium for 24 h prior to the addition of fresh medium with or without 50 μM omeprazole. 24 H later, cells were evaluated for viability and luciferase activity as described in Materials and Methods. Luciferase activity was corrected for cell viability. Data points are mean ± SEM for quadruplicate wells. Fold induction was calculated relative to omeprazole-vehicle (DMSO) controls. Statistically significant (p < 0.05) changes from both DMSO (a), and from silver-alone controls (b) are indicated. No nano-silver & omeprazole value was statistically different from omeprazole alone (0 μg/ml nanosilver).

Figure 2: The effects of nano-silver in DPX2 cells.

Plated DPX2 cells were exposed to nano-silver-containing medium for 24 h prior to the addition of fresh medium with or without either 50 μM omeprazole or 10 μM rifampicin. 24 H later, cells were evaluated for viability and luciferase activity as described in Materials and Methods. Luciferase activity was corrected for cell viability. Data points are mean ± SEM for quadruplicate wells. Fold induction was calculated relative to drug-vehicle (DMSO) controls. Statistically significant (p < 0.05) changes from DMSO (a), from silver-alone controls (b), and from inducer in the absence of nano-silver (c) are indicated.

Figure 3: The effects of nano-silver in 1A2DRE cells.
Plated 1A2DRE cells were exposed to nano-silver-containing medium for 24 h prior to the addition of fresh medium with or without 50 μM omeprazole. 24 H later, cells were evaluated for viability and luciferase activity as described in Materials and Methods. Luciferase activity was corrected for cell viability. Data points are mean ± SEM for quadruplicate wells. Fold induction was calculated relative to omeprazole vehicle (DMSO) controls. Statistically significant (p < 0.05) changes from DMSO (a), from silver-alone controls (b), and from omeprazole in the absence of nano-silver (c) are indicated.

Figure 4: The variation of nano-silver inhibition of testosterone 6β-hydroxylation (upper panel) chlorzoxazone 6-hydroxylation (center panel) and 7-ethoxy-4-trifluoromethylcoumarin O-deethylation (lower panel) when determined at different microsomal protein concentrations.

Human liver microsomes, with relatively high activity for the CYP-selective substrate (Human Biologics Int'l. #501 and #227 for testosterone and chlozoxazone respectively, Celsis M008085-JTA for 7-ethoxy-4-trifluoromethylcoumarin) were incubated at three or four protein concentrations with a range of nano-silver concentrations and the monooxygenase activity relative to activity in the absence of nano-silver was expressed as %. Data points are the mean of three independent determinations, each of which was a mean derived from duplicate incubations.
Table 1. The effect of 24 h exposure to nano-silver on cell viability.

Plated cells were exposed to nano-silver-containing medium for 24 h and evaluated for viability with a Multitox-Fluor Multiplex Cytotoxicity assay (Promega). Values are mean ± SEM for quadruplicate wells. Viability was expressed relative to cells exposed to nano-silver-free medium controls and statistically significant (p < 0.05) changes from controls (\(^\ast\)) are indicated. Derived LD\(_{50}\) values were determined by interpolation.

<table>
<thead>
<tr>
<th>Nano-silver (μg/ml):</th>
<th>MDR1.C</th>
<th>DPX2</th>
<th>1A2DRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100 ± 1.2</td>
<td>100 ± 1.3</td>
<td>100 ± 1.4</td>
</tr>
<tr>
<td>1</td>
<td>113 ± 4.7</td>
<td>98 ± 1.2</td>
<td>105 ± 4.0</td>
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<td>2.5</td>
<td>95 ± 1.9</td>
<td>91 ± 2.0 (^\ast)</td>
<td>75 ± 6.2 (^\ast)</td>
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<td>5</td>
<td>52 ± 8.9 (^\ast)</td>
<td>24 ± 0.2 (^\ast)</td>
<td>23 ± 1.9 (^\ast)</td>
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<td>6</td>
<td>24 ± 1.7 (^\ast)</td>
<td>28 ± 0.2 (^\ast)</td>
<td>15 ± 1.9 (^\ast)</td>
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<td>18 ± 5.6 (^\ast)</td>
<td>26 ± 0.4 (^\ast)</td>
<td>27 ± 1.9 (^\ast)</td>
</tr>
<tr>
<td>10</td>
<td>5 ± 0.3 (^\ast)</td>
<td>24 ± 0.4 (^\ast)</td>
<td>10 ± 0.7 (^\ast)</td>
</tr>
</tbody>
</table>

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Derived LD\(_{50}\): 5.1 μg/ml  4.0 μg/ml  3.7 μg/ml
Table 2. Nano-silver inhibition of human CYP-selective monooxygenase activities.

Human liver microsomes, with relatively high activity for the CYP-selective substrates were incubated at 0.5 mg protein/ml and a range (0-70 μM) of nano-silver concentrations. IC\textsubscript{50} values were determined by interpolation in three independent experiments with each data point derived from duplicate incubations. IC\textsubscript{50} values are given as mean ± SEM (n = 3). P450 concentrations in the assays were derived from data provided by the microsome supplier.

<table>
<thead>
<tr>
<th>P450 (CYP-selective) Reaction</th>
<th>Human Liver Microsomes</th>
<th>[P450] in assay(^3) (μM)</th>
<th>[Substrate] in assay (μM)</th>
<th>nano-silver IC\textsubscript{50} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Methoxyresorufin O-demethylation (CYP1A2)</td>
<td>Celsis F008085-CST</td>
<td>0.283</td>
<td>0.5</td>
<td>24.4 ± 1.1</td>
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<td>Coumarin 7-hydroxylation (CYP2A6)</td>
<td>Celsis X008064-LSL</td>
<td>0.202</td>
<td>2.5</td>
<td>33.5 ± 2.3</td>
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<tr>
<td>7-EFC(^1) O-deethylation (CYP2B6)</td>
<td>Celsis M008085-JTA</td>
<td>0.279</td>
<td>6.25</td>
<td>31.9 ± 2.5</td>
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<td>Diclofenac 4-hydroxylation (CYP2C9)</td>
<td>Human Biologics Int'l. 112</td>
<td>0.235</td>
<td>50</td>
<td>9.3 ± 0.2</td>
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<td>S-Mephenytoin 4-hydroxylation (CYP2C19)</td>
<td>Puracyp 011</td>
<td>0.138</td>
<td>50</td>
<td>6.4 ± 0.1</td>
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<tr>
<td>Substrate and Enzyme Action</td>
<td>Supplier</td>
<td>Microsomal Protein (μg/ml)</td>
<td>Incubation Time (min)</td>
<td>Activity (μmol/min/mg)</td>
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<tr>
<td>-----------------------------</td>
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<tr>
<td>7-MAMC&lt;sup&gt;2&lt;/sup&gt; O-demethylation (CYP2D6)</td>
<td>Celsis M008085-JTA</td>
<td>0.279</td>
<td>25</td>
<td>21.2 ± 1.3</td>
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<td>Chlorzoxazone 6-hydroxylation (CYP2E1)</td>
<td>Human Biologics Int'l. 227</td>
<td>0.190</td>
<td>100</td>
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<td>Testosterone 6β-hydroxylation (CYP3A4)</td>
<td>Human Biologics Int'l. 501</td>
<td>not supplied</td>
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<td>8.0 ± 0.3</td>
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<td>7-Benzyl-4-quinoline O-debenzylation (CYP3A4)</td>
<td>Human Biologics Int'l. 501</td>
<td>not supplied</td>
<td>80</td>
<td>8.8 ± 0.5</td>
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</table>

1. 7-ethoxy-4-trifluoromethylcoumarin
2. 7-methoxy-4-(aminomethyl)-coumarin
3. 0.5 mg microsomal protein/ml
Table 3: Nano-silver effect on human liver microsomal NADPH cytochrome c reductase activity

The NADPH cytochrome c reductase activity of the human liver microsomes (0.5 mg protein/ml) in the presence of 70 μM nano-silver was determined and % change calculated from the activity in the absence of nano-silver (values are shown as mean ± SEM of 3 independent determinations with duplicate incubations in each).

<table>
<thead>
<tr>
<th>Human Liver Microsomes</th>
<th>NADPH cytochrome c reductase&lt;sup&gt;1&lt;/sup&gt; (nmol/mg/min)</th>
<th>70 μM nano-silver effect (% change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celsis F008085-CST</td>
<td>97.9</td>
<td>-11.4 ± 1.1</td>
</tr>
<tr>
<td>Celsis X008064-LSL</td>
<td>89.4</td>
<td>+3.9 ± 4.6</td>
</tr>
<tr>
<td>Celsis M008085-JTA</td>
<td>85.6</td>
<td>-5.4 ± 3.0</td>
</tr>
<tr>
<td>Human Biologics Int'l. 112</td>
<td>108.3</td>
<td>-2.7 ± 2.7</td>
</tr>
<tr>
<td>Puracyp 011</td>
<td>47.6</td>
<td>+0.8 ±2.4</td>
</tr>
<tr>
<td>Human Biologics Int'l. 227</td>
<td>86.4</td>
<td>+4.4 ± 2.0</td>
</tr>
<tr>
<td>Human Biologics Int'l. 501</td>
<td>70.5</td>
<td>-0.5 ± 3.6</td>
</tr>
</tbody>
</table>

<sup>1</sup> assayed at 0.5 mg microsomal protein/ml
Figure 2

Induction in DPX2 cells (fold)

Induction in DPX2 cells (fold)

nano-silver & rifampicin
nano-silver & omeprazole
nano-silver

Figure 2
Figure 4