Cytotoxicity of Copper Oxide Nanoparticles and Associated Ions on Human Epithelial Lung Cells (A549)

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Abstract:

The rapidly advancing field of nanotechnology offers many practical applications in consumer goods. As this area of science continues to grow and its uses become more widespread human contact with nanoparticles increases in likelihood, whether it is in consumption or production. This study focuses on determining the mechanism of toxicity caused by copper oxide (CuO) nanoparticles (NP) and the ions released from them on the A549 line of human epithelial lung cells. This is an *in vitro* representation of metal oxide nanoparticles being inhaled and accumulating in the lungs. Toxicity was assayed via deoxyribonucleic acid (DNA) microarray to determine up-regulated and down-regulated genes followed by gene ontology to classify those genes into categories such as cellular component and biological process. A mechanism of cytotoxicity was then proposed.

Experimental Procedure:

Treatment medium for cells was prepared by ultra-sonicating a stock solution of CuO nanoparticles [1] and preparing a 25 μg/ml solution of nanoparticles in normal cell culture medium. Ion medium was prepared by shaking the 25 μg/ml metal oxide medium for four days at medium speed, followed by centrifugation at 22,000 xg for five minutes at 4°C to remove the nanoparticles from solution. Inductively coupled plasma (ICP) was then used to measure ion concentration in medium.

For DNA microarray assay cells were plated at 5,000 cells/cm^2 and incubated for 72 hours until cells reached about 90% confluence. After incubation time elapsed cells were treated with 25 μg/ml CuO-NP or Cu^{2+} medium and incubated for 24 hrs. Following incubation RNA was extracted, amplified, purified, and dyed using the Amino Allyl MessageAmp™ II aRNA Amplification Kit: RNA Amplification for Array Analysis by Ambion. Microarray samples were then hybridized, washed, and scanned. Data was filtered to remove duplicate genes, and then compared with a second set; reproducible genes were calculated by averaging genes from both sets when the absolute value of the difference in fold change was less than one. Significant up and down regulation was determined to be genes with a fold change greater than absolute value 1. These affected genes were organized using gene ontology and the mechanism in Figure 1 was proposed.

Cell cycle arrest was tested by plating cells at 5,000 cells/cm^2 and incubating them for 48 hours after which they were exposed to 25 μg/ml CuO nanoparticle medium for 24 hours. Cells were washed twice with PBS, removed from culture dish, counted, and re-plated in a new culture dish at 5,000 cells/cm^2 in normal cell culture medium and incubated for 72 hours. The wash procedure was then repeated and these second generation cells were counted. They were again re-plated at 5,000 cells/cm^2, incubated for 72 hours in normal cell culture medium, and counted for the third generation.

Results and Conclusions:

A count of viable cells using Cell Counting Kit-8 (CCK-8) after exposure to nanoparticles or associated ions yielded the results in Figure 2. About 40% of cells died when exposed to Cu^{2+} and about 80% died when exposed to CuO-NP. Therefore we can conclude that about 50% of CuO toxicity is attributable to effects of the Cu^{2+} ion.

The up-regulation of metallothionein expression in cells exposed to both Cu^{2+} and CuO nanoparticles indicates the generation of reactive oxygen species (ROS). In cells exposed to CuO nanoparticles heat shock proteins were also up-regulated indicating a more intense environmental stress causing proteins to unfold. ROS have been known to cause damage to DNA, which in turn up-regulates the GADD45G/B genes which can inhibit PCNA, CDC2, and CCNB1 genes that control cell cycle. The GADD45G/B genes can also interact with MTK1 and active the JNK p38 pathway leading to the formation of dimeric transcription factor AP-1 suggesting that CuO nanoparticles affect the expression of genes regulated by AP-1 transcription factor

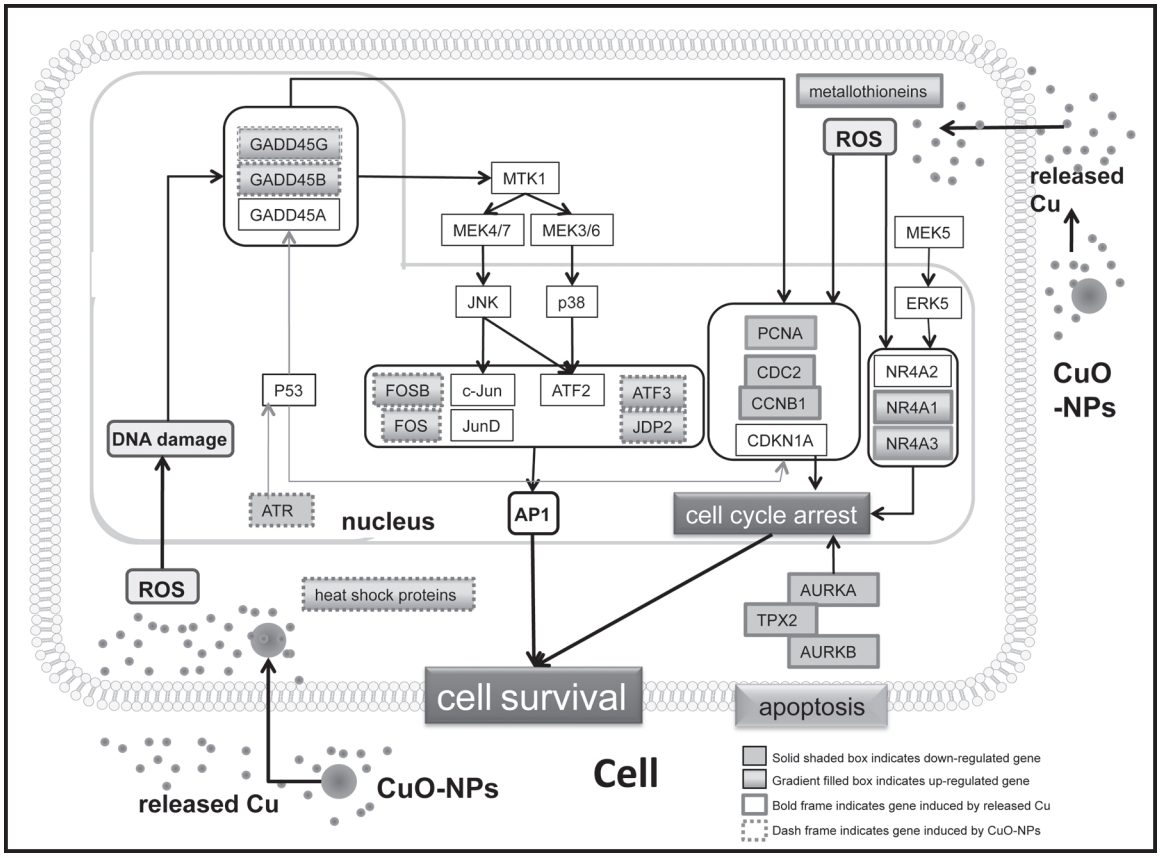

Figure 1: The proposed mechanism of cytotoxicity for CuO nanoparticles and Cu^{2+} ions.

[2]. AP-1 has been known to play roles in cell survival as well as apoptosis [3, 4] so more experiments are needed to confirm its role in this context. A future experiment would include inhibiting the JNK p38 pathway. If more cells are viable after this assay it can be determined that the pathway leads to apoptosis; the reverse is true if fewer cells are viable.

The down regulation of cell cycle regulating genes PCNA, CDC2, and CCNB1 indicate an arrest in cell cycle and the down regulation of AURKA/B and TPX2 indicate an arrest in cell division. Cell division experiments confirm that second generation cells whose parent generation was treated with CuO nanoparticles did not experience apoptosis, but did encounter an arrest in cell cycle and division. In the third generation recovery was evident as the cells resumed division.

Our hypothesis is that cell cycle arrest is a means by which cells can buy extra time to repair damaged DNA before dividing, ultimately resulting in cell survival instead of apoptosis.

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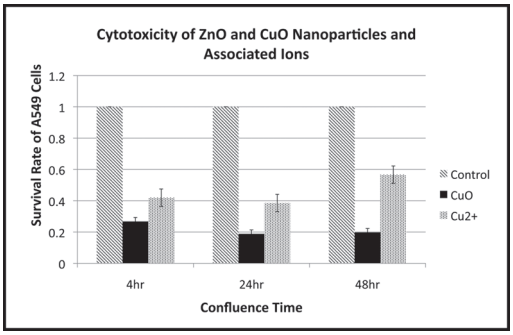

Figure 2: Cu^{2+} is responsible for about half of the toxicity of CuO nanoparticles.