Effect of Nano-Silver on Cell Division and Mitotic Chromosomes: A Prefatory Siren

K Babu, M Deepa, S Shankar, S Rai

Abstract

In vivo cytogenetic assay in Allium root meristems has been carried out to study the effect of nano-silver on cell division and mitotic chromosomes. Nano-silver is a potential anti-bacterial agent recommended as a preservative in personal care and consumer products. Nano particles owing to their size can enter freely into the cells and can interfere in cell's normal function. Studies to justify the safety of nano particles in living systems are inadequate. The present study was planned to understand the possible genotoxic effects of nano-silver in Allium meristems. The germinated onion root tips were treated with different concentrations i.e. 10, 20, 40 and 50 ppm of nano-silver for different time intervals i.e. 0.5, 1, 2 and 4h. The treated root samples exhibited increase in the frequency of chromosomal aberrations and decrease in mitotic index. These abnormalities were observed in a dose and duration dependent manner. The treated meristem cells showed various types of chromosomal and mitotic abnormalities such as fragments, C-metaphase, stickiness, laggard, anaphasic bridge and disturbed anaphase. The present investigation reveals that the nano-silver may cause potential damage to the genetic material and therefore the use of nano-silver in consumer products warrants a detail toxicological investigation to justify its safety.

INTRODUCTION

Silver is relatively rare metal that occurs naturally in the earth. Silver has been in medical use for decades and was used in systemic drugs before the advent of antibiotics. Today, silver is used routinely in antibacterial salves. Some of the more common silver compounds used in medical devices and industries are nitrate, chloride, bromide, acetate, oxide, sulfate and cyanide (Stokinger 1981, Quinn 2002). Silver is also used in the form of colloidal state (Colloid silver or silver mineral water) and nano particles (Nano-silver). The microbicidal effects involve both altering the function of the cell membrane and linking to the cell's DNA, disrupting cell reproduction. The bactericidal action of silver ions is effective against a broad spectrum of bacteria, including the common strains, which cause infection, and the more virulent antibiotic-resistant strains (Russell 1994). Silver is known to kill more than 650 kinds of bacteria and suppress the viral activity (Raymond Wai-Yin Sun et al., 2005). Human exposure to silver and silver compounds can occur orally, dermally or by inhalation. Silver is found in most tissue, but has no known physiological function (Rosemarie 1992). In long-term oral studies with experimental animals, silver compounds produced slight thickening of the basement membranes of the renal glomeruli, growth depression, shortened lifespan, and granular silver containing deposits in skin, eyes and internal organs (Matuk et al. 1981). Nano-silver is pure de-ionized water with silver (Ag) in suspension. Recently nano-silver has been used in cosmetics and textile fabrics as an antibacterial agent (Marcato et al., 2005). Adequate studies for evaluating the mutagenicity and carcinogenicity of nano-silver to humans or animals by ingestion, inhalation or other routes of exposure are not available.

A number of plant bioassay techniques had been developed for the detection of environmental mutagens because plant chromosomes are relatively large and respond to treatment with mutagens in a similar way to mammals and other eukaryotes (Grant 1978, 1994, WHO 1985). Among these assays, the Allium root meristems is routinely used for studying the effect of toxic materials on chromosomes and mitotic cell division and has been recommended as a standard assay for environmental monitoring (Fiskesjo 1985). Hence, in the present research work Allium root meristem was used as an in vivo test system to evaluate the toxicity of nano-silver.
MATERIALS AND METHODS
Commercially available onions, Allium cepa L. (2n = 16) were used as test system. Healthy bulbs of A. cepa were set for germination in sandy soil to obtain roots. The root tips from the germinated bulbs of onion were treated with nano-silver at different concentrations ranging from 10, 20, 40 and 50 ppm for different periods i.e. 0.5, 1, 2 and 4 h. The root tips treated in distilled water was used as control. After treatment, the root tips of control and experimental samples were thoroughly washed in distilled water and fixed in acetic-alcohol (1:3). Chromosome preparations were made following haematoxylin squash technique (Sharma and Sharma 1980). Minimum of 10,000 cells from 10 root tips from 5 bulbs were analyzed to score the frequency of mitotic index (MI) and chromosomal aberrations (CA). The values are expressed as mean ± SE. The statistical significance between control and experimental data were analyzed using one-way analysis of variance (ANOVA).

RESULTS
Experimental results on the effects of nano-silver on cell division and mitotic chromosomes of Allium cepa are presented in Tables 1&2.
Effect of Nano-Silver on Cell Division and Mitotic Chromosomes: A Prefatory Siren

**Table 2: Frequency of chromosomal aberrations observed in root meristems treated with Nano-silver solution. Types of aberrations (%)**

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Duration (hrs)</th>
<th>CM</th>
<th>FG</th>
<th>LG</th>
<th>ST</th>
<th>AB</th>
<th>DA</th>
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<td>14.43</td>
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<td>10</td>
<td>2</td>
<td>9.28</td>
<td>10.89</td>
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<td>10</td>
<td>4</td>
<td>6.97</td>
<td>17.27</td>
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<td>3.87</td>
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<td>31.73</td>
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**EFFECT ON MITOTIC INDEX**

Nano-silver treated cells showed significant reduction in the frequency of mitotic index during all doses and durations when compared to control sets (P<0.05, P<0.001). This divisional frequency was found to reduce even at 1h exposure during all treatments indicating that the nano-silver has an immediate effect on dividing cells. The frequency further got reduced when dose and duration increased (Table 1).

**EFFECT ON CHROMOSOMAL ABERRATION**

Nano-silver induced structural aberrations of chromosomes such as C-metaphase (Fig – 1), disturbed metaphase (Fig – 2), fragments (Fig - 3&8), sticky metaphase (Fig – 4), laggards (Fig – 5), anaphasic bridge (Fig – 6), disturbed anaphase (Fig – 7) and micronuclei (Fig – 9). The chromosomal aberrations observed in the treated cells were found to be of higher frequency during longer exposure. A distinct dose dependent increase in chromosomal anomalies was observed in all treatments (Table 1). This data is found to be statistically highly significant when compared to control (P<0.001). Fragments, C-metaphase, disturbed metaphase and disturbed anaphase were found to be in higher frequency in all treatments (Table 2). The frequency of anaphasic bridge was found to be higher during 0.5 and 1h exposures where as in 2 & 4 h exposures, this was reduced (Table 2). Rare instances of micronuclei were also observed in the treated cells.

**Figure 3**
Figure 1: C- Metaphase

**Figure 4**
Figure 2: Disturbed metaphase
**Effect of Nano-Silver on Cell Division and Mitotic Chromosomes: A Prefatory Siren**

**Figure 5**
Figure 3: Cell showing metaphasic fragments

![Figure 5](image)

**Figure 6**
Figure 4: Sticky metaphase

![Figure 6](image)

**Figure 7**
Figure 5: Laggard

![Figure 7](image)

**Figure 8**
Figure 6: Anaphasic bridge

![Figure 8](image)

**Figure 9**
Figure 7: Disturbed anaphase

![Figure 9](image)

**Figure 10**
Figure 8: Anaphase showing multiple fragments

![Figure 10](image)
DISCUSSION

Nano-silver is a new product of the emerging nanotechnology and is a new age preservative. Nano silver is used in personal care and consumer products owing to its high anti-microbial activity. Commercial Nano-silver is usually available as a suspension in pure de-ionized water with nanosized silver (Ag). Approximately 80% of the silver is in the form of metallic silver nano-particles. The remaining silver is in ionic form. Nano silver particles are much more smaller (2 nm in diameter) and exists in a stable form than colloidal silver (10 nm to 1 micron in diameter).

Nano-silver, though an effective antibacterial (She and Zhang 2003, Lok et al. 2006), may also cause genotoxic impact. Case histories indicate that dermal exposure to silver or silver compounds for extended periods can lead to generalized skin discolouration. Mild allergic responses attributed to dermal contact with silver or silver compounds was also reported (ATSDR 1990). The most commonly discussed side effects from excessive in take of elemental silver is a condition known as argyria. The excess silver usually gets deposited in the skin, organs and other tissues. The deposition of silver in the skin may lead to the change of skin colour to a gray or bluish gray (East et al. 1980). Nano-silver, is smallest enough to enter the skin pores if applied on the skin and it may result in damage to the skin cells. Research on the nano particles has revealed the ability of these particles to pass through cell walls and damage DNA (ATSDR 1990).

In humans, less than 1% of topically applied silver compounds are absorbed through the skin (Snyder et al. 1975). Nano-silver, incorporated in cosmetics or consumer products may enter the human cells easily through both intact and damaged skin. Silver particles once deposited in the layers of skin of humans, it accumulates throughout the ageing process (Hostyn et al. 1993).

Earlier reports showed that high concentrations of nano-silver based inorganic antibacterial agents have cytotoxic effects on rat's fibroblasts L-929 (Zhand et al. 2005). Histopathological studies on the effect of pure silver showed accumulation of granules in the cytoplasm and showed little cytotoxicity. It is supported by the study of Schmahl and Steinhoff (1960) that colloidal silver injected subcutaneously, into rats resulted in tumour in 8/26 rats, that survived longer than 14 months.

In proteomic studies nano-silver was shown to destabilize the outer membrane, collapse the plasma membranes' potential and deplete the levels of intracellular ATP of the bacterial cells (Lok et al. 2006). Similar studies on the effect of nano-silver on plasma membranes and ATP in mammalian and other eukaryotic systems are lacking.

In the present preliminary toxicity assay, treatments with 10, 20, 40 and 50 ppm of nano-silver produced a dose and duration-dependent MI and CA in Allium root meristems. The reduction of MI during all treatments clearly indicates the mitodepressive and cytotoxic effect of nano-silver. This might have been achieved by the inhibition of DNA synthesis at S-phase (Sudhakar et al. 2001). The high frequency of mitotic abnormalities such as C-metaphase, disturbed metaphase and disturbed anaphase induced by nano-silver primarily reflects its effects on mitotic spindles, altering the orientation of chromosomes at various stages of the cell cycle. Impairment of mitotic spindle function is probably due to the interaction of nano-silver with tubulin-SH group (Kuriyama and Sakai, 1974). Observation of chromosome stickiness was another type of abnormality induced by nano-silver. This stickiness presumably is due to intermingling of chromatin fibres, which leads to sub chromatic connections between chromosomes (McGill et al. 1974, Klasterska et al. 1976). The induction of chromosomal breaks and micronuclei by nano-silver indicates the clastogenic potential of the test chemical, which may lead to a loss of genetic material and these have been regarded as an indication of mutagenicity of the inducers (Raun et al. 1992). In conclusion, the results of present study in Allium root meristems suggest that nano-silver possesses mitodepressive, mitoclassic and clastogenic properties.

The present study provides an evidence for the possible genotoxic effect of nano-silver on plant system. Though the
results in the plant system cannot be extrapolated directly to animal systems, the findings of the present study raise the need for elaborate evaluations to ensure the safety of nano-
silver.

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