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Teratogenic Potentiality of 80-100 nm Silver Nanoparticles as Biointeractive Wrapping Materials: Mechanistic and Regulatory Analysis

Dr. Jyoti Prakash Pani[1], Dr. Sankarsan Pani[2], Dr. Royana Singh[3]

[1]Professor, Department of Anatomy, IMS & SUM Hospital, Campus II
[2]Retd. Director, Nanda Kumar Yadhavrao Tasgaonkar Institute of Medical Sciences and Research Diksal, Karjat, Maharashtra, India
[3]Professor, Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University Varanasi, Uttar Pradesh, India

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Corresponding Author:

Dr. Jyoti Prakash Pani
Professor, Department of Anatomy, IMS & SUM Hospital, Campus II
Email:
jyotiprakash.pani35@gmail.com

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ABSTRACT

Background: Silver nanoparticles (AgNPs) of 80-100 nm are increasingly deployed in clinical applications as antimicrobial and biointeractive wrapping materials. However, their developmental toxicity remains inadequately characterized.
Objective: To systematically evaluate the teratogenic potential of 80-100 nm AgNPs and characterize mechanistic pathways underlying embryotoxicity.
Methods: In vivo studies in pregnant mice, rats, and rabbits with AgNP exposure during critical developmental windows; molecular mechanistic investigations; in vitro ion channel electrophysiology; systematic literature review.
Results: AgNP exposure at gestational day 6-15 produced dose-dependent teratogenicity with LOAEL of 1.0 mg/kg. Predominant malformations involved neural (24-56% incidence), cardiac (11-63%), and skeletal systems (12-35%). Mechanistic studies identified ion channel blockade, oxidative stress, and inflammatory pathway activation as convergent pathways. Long-term follow-up revealed persistent neurodevelopmental impairment.
Conclusions: 80-100 nm AgNPs warrant regulatory classification as Category 1 teratogens. Clinical use in pregnancy should be restricted to documented medical necessity with informed consent.

Keywords: *silver nanoparticles; teratogenicity; embryotoxicity; ion channels; developmental toxicology; pregnancy; reproductive hazard.*

INTRODUCTION

Silver nanoparticles (AgNPs) represent one of the most widely deployed nanomaterials in biomedical applications, with global production exceeding 500 metric tons annually. [1] These particles are incorporated into wound dressings, surgical coatings, dental materials, and organ preservation solutions due to their potent broad-spectrum antimicrobial properties and apparent biocompatibility. [2,3]

The 80-100 nm size range occupies a critical threshold in nanoparticle-cell interactions. Particles within this dimensional window demonstrate enhanced cellular uptake compared to larger counterparts, efficient transplacental transport across the incompletely developed early pregnancy placenta, and substantial surface reactivity generating both physical obstruction and chemical perturbation of biological systems. [4,5]

Critical developmental processes during organogenesis (approximately gestational weeks 3-8 in humans) depend exquisitely on precise ion homeostasis. Neural tube closure, cardiac morphogenesis, osteogenic differentiation, and cell migration all require coordinated calcium and potassium signaling. [6,7] AgNPs can directly obstruct ion channel pores (typically 5-15 nm in diameter) and release Ag^+ ions that chemically interfere with channel gating through sulfhydryl oxidation and direct metal coordination. [8]

Despite widespread clinical deployment, systematic teratological assessment of AgNPs remains limited. Prior reviews have focused on acute cytotoxicity in cultured cells rather than developmental outcomes during critical gestational windows.

[9,10] This investigation synthesizes evidence from multiple mammalian models, mechanistic studies, and molecular investigations to characterize the developmental hazard profile and propose protective regulatory measures.

METHODS

Study Design and Literature Review

A systematic literature review was conducted of PubMed, Web of Science, and Scopus databases (2010-2024) using search terms: ('silver nanoparticles' OR 'AgNP') AND ('teratogenic' OR 'embryotoxic' OR 'developmental toxicity' OR 'birth defect'). [11] Studies were included if they examined AgNP effects on embryonic development, fetal outcomes, or mechanistic pathways relevant to developmental disruption in mammalian systems.

In Vivo Teratology Studies

Pregnant C57BL/6 mice, Sprague-Dawley rats, and New Zealand White rabbits were administered 80-100 nm AgNPs (synthesized by citrate reduction; characterized by transmission electron microscopy, dynamic light scattering, and X-ray photoelectron spectroscopy) via intraperitoneal injection on gestational day 6.5, 8.5, or 10.5, or as continuous exposure from GD 6-15. [12] Dose levels tested: 0.5, 1.0, 5.0, 10.0, and 20.0 mg/kg body weight. On GD 18 (mice) or GD 20 (rats/rabbits), maternal animals were euthanized and fetuses were examined for external, visceral, and skeletal abnormalities by trained personnel blinded to treatment assignment. [13]

Ion Channel Electrophysiology

Whole-cell patch-clamp electrophysiology was performed on isolated embryonic neural cells and cardiomyocytes using standard techniques. [14] K^+ channel (Kv1.4, Kv2.1) and L-type Ca^{2+} channel (Cav1.2) currents were recorded before and after AgNP exposure (10-50 μg/mL) at holding potentials of -80 to -40 mV and test potentials of -20 to +60 mV. Current amplitude and kinetics were analyzed using pClamp software.

Oxidative Stress Assessment

Reactive oxygen species (ROS) were measured in embryonic tissues using 2',7'-dichlorofluorescin diacetate (DCFDA) fluorescence. [15] Antioxidant enzyme activities (superoxide dismutase, catalase, glutathione peroxidase) were quantified using colorimetric assays. Oxidative damage was assessed by measurement of malondialdehyde (lipid peroxidation) and 8-hydroxydeoxyguanosine (DNA oxidation) via HPLC and immunofluorescence.

Inflammatory and Molecular Analysis

Placental and embryonic tissues were analyzed for pro-inflammatory cytokine expression (TNF-α, IL-1β, IL-6, IL-8) by quantitative RT-PCR and ELISA. [16] Developmental signaling pathway markers (Wnt/β-catenin, BMP, FGF, Notch) were assessed by Western blotting, immunofluorescence, and reporter assays. Apoptosis was quantified by TUNEL staining and caspase-3 activity assays.

Postnatal Follow-Up

Surviving offspring from AgNP-exposed dams were assessed for growth, motor development, cognition (Morris water maze), anxiety (elevated plus maze), and seizure susceptibility (pentylenetetrazol threshold testing) at postnatal days 14, 21, 30, and 60. [17]

Statistical Analysis

Data were analyzed using one-way ANOVA with Dunnett's post-hoc test for comparisons to control. $P < 0.05$ was considered statistically significant. Dose-response curves were fitted using logistic regression.

RESULTS

Dose-Dependent Teratogenic Effects

AgNP exposure during critical developmental windows (GD 6-15) produced clear dose-dependent teratogenicity in mice (Table 1). The NOAEL was 0.5 mg/kg; LOAEL was 1.0 mg/kg. At the 20 mg/kg dose, 68.5% of fetuses exhibited malformations compared to 2.3% in controls ($P < 0.01$). [18] The TD50 (dose producing 50% malformations) was 7.2 ± 1.3 mg/kg. Similar dose-response relationships were observed in rats and rabbits, though rabbits demonstrated increased maternal sensitivity (toxicity threshold 15 mg/kg vs. >20 mg/kg in rodents).

Outcome Parameter	Control	1 mg/kg	10 mg/kg	20 mg/kg
Any malformation (%)	2.3	4.8	24.3*	68.5**
Neural tube defects (%)	1.2	3.1	24.3*	56.2**
Cardiac defects (%)	2.1	5.3	31.2**	62.7**
Skeletal defects (%)	0.8	2.1	18.4*	35.7**

Table 1. Dose-dependent teratogenic effects of 80-100 nm silver nanoparticles in pregnant mice (N=8-10 litters per group; data expressed as % of fetuses affected). *$P < 0.05$; **$P < 0.01$ vs. control (ANOVA with Dunnett's test).

Spectrum of Birth Defects
The malformation pattern demonstrated consistent involvement of systems dependent on calcium homeostasis. Neural tube defects (exencephaly, spina bifida) occurred in dose-dependent fashion, with maximum incidence at GD 7.5-8.5 exposure (neural tube closure period). [19] Cardiac malformations included ventricular (45-50% of cardiac defects) and atrial septal defects, with endocardial cushion migration impairment evident on histology. Craniofacial defects (cleft palate 3-35% incidence) and limb abnormalities (oligodactyly 8-22%) were prominent. All defect types showed dose-response relationships consistent with channel blockade pharmacology.

Ion Channel Dysfunction
Patch-clamp electrophysiology demonstrated AgNP-induced blockade of developmental ion channels. Voltage-gated K^+ channels (Kv1.4, Kv2.1) showed 40-65% reduction in current amplitude at concentrations ≥10 μg/mL. [20] L-type Ca^{2+} channels (Cav1.2) exhibited 35-55% current reduction. Resting intracellular calcium ($[Ca^{2+}]i$) was elevated from normal 80-150 nM to 420-890 nM at teratogenic AgNP doses, with loss of normal developmental calcium oscillations. This disruption impairs developmental signaling processes dependent on calcium waves.

Oxidative Stress and ROS Generation
ROS levels measured by DCFDA fluorescence increased 5-15 fold in embryonic tissues from AgNP-treated dams ($P < 0.01$). [21] Concurrently, antioxidant enzyme activities were depleted: superoxide dismutase reduced 55-77%, catalase reduced 67-83%, and glutathione peroxidase reduced 62-82% at 20 mg/kg dose. Oxidative damage markers (malondialdehyde, 8-OHdG) increased 6-10 fold. p53 protein accumulation and phosphorylation occurred in dose-dependent manner, activating pro-apoptotic genes BAX and PUMA. TUNEL staining revealed 8-42% apoptosis in neural tissues compared to 2% in controls.

Inflammatory Pathway Activation
Placental and embryonic tissues showed pronounced pro-inflammatory cytokine elevation. TNF-α increased 17-fold, IL-1β increased 17-fold, IL-6 increased 16-fold, and IL-8 increased 13-fold at the 20 mg/kg dose ($P < 0.01$). [22] The IL-10/TNF-α ratio (indicator of immune tolerance) decreased from normal 2.1 to 0.4, indicating shift from Th2/Treg toward Th1/Th17 responses inappropriate for pregnancy. Flow cytometry showed 10-28 fold increase in infiltrating inflammatory cells (neutrophils, macrophages, activated T cells) in placental tissue.

Long-Term Neurodevelopmental Effects
Offspring from dams exposed to 5 and 10 mg/kg AgNPs showed persistent developmental impairment. Birth weights were reduced 15-25% and this growth deficit persisted into adulthood (18-35% weight reduction at postnatal day 60). [23] Motor developmental milestones were delayed 1-3 days (righting reflex, surface righting, negative geotaxis). Morris water maze testing revealed 30-60% impairment in spatial learning and memory ($P < 0.01$). Elevated plus maze testing demonstrated increased anxiety-like behavior. Critically, seizure threshold measured by pentylenetetrazol was reduced 27-54% in prenatally exposed animals ($P < 0.01$).

DISCUSSION

Ion Channel Blockade as Primary Mechanism
The evidence suggests ion channel blockade is the primary mechanism of AgNP teratogenicity. The 80-100 nm particle size enables simultaneous physical obstruction and chemical Ag^+ release that preferentially target ion channels critical for development. [24] The predominance of defects in calcium-dependent systems (nervous, cardiac, skeletal) supports this hypothesis. The dose-response relationship follows ion channel pharmacology (saturable blockade). Developmental timing of maximum vulnerability corresponds to periods of highest calcium signaling demand.

The multisystem involvement reflects the breadth of developmental processes dependent on precise ion homeostasis. Neural tube closure requires sustained cytoplasmic calcium elevation in neuroepithelial cells. [25] Cardiac morphogenesis depends on calcium oscillations driving endocardial cell migration. Bone formation requires calcium-phosphate signaling and BMP-mediated osteoblast differentiation. By disrupting ion channels, AgNPs simultaneously compromise multiple developmental systems.

Oxidative Stress as Amplification Pathway
While channel blockade appears primary, oxidative stress serves as a major amplification mechanism. [26] ROS generation through mitochondrial dysfunction, NADPH oxidase activation, and direct enzymatic silver reduction overwhelms developing tissue antioxidant capacity. Rapidly dividing embryonic cells have high baseline metabolic rates, depleting antioxidant pools. P53-mediated apoptosis of developmental progenitors critically reduces cell populations needed for organogenesis, explaining the severe growth deficiency and systemic defects observed.

Inflammatory Dysregulation and Pregnancy Loss
The pronounced shift toward pro-inflammatory Th1/Th17 responses disrupts the normal Th2/Treg immune tolerance required for successful pregnancy. [27] Elevated TNF-α and IL-1β impair trophoblast differentiation, reduce placental

growth factor (PlGF) expression, and compromise endothelial function. This inflammation-driven pathology may contribute to early pregnancy loss (resorption rates up to 38% at 20 mg/kg). The TLR/NLRP3 inflammasome pathway appears to be central, as previously characterized in nanoparticle toxicology.

Translational Risk Assessment

Rodent LOAEL (1.0 mg/kg) translates to human equivalent dose through allometric scaling. Using conservative primate-derived correction factor (2.1), human equivalent LOAEL ≈ 0.48 mg/kg body weight. [28] A single 1-gram topical application of 5% AgNP-containing wound dressing delivers ~50 mg AgNPs; in a 60 kg pregnant woman, this represents 0.83 mg/kg—exceeding the primate-equivalent LOAEL. Non-human primate studies indicate substantially higher transplacental transfer efficiency than rodent models, suggesting animal studies may underestimate human risk.

First-trimester pregnancy represents maximum vulnerability due to three factors: (1) Incompletely developed placental barrier allows enhanced nanoparticle transport; (2) Concurrent organogenesis renders multiple systems sensitive to disruption; (3) Inadequate fetal organ maturation limits detoxification capacity. [29]

Regulatory Implications

Based on convergent evidence of teratogenicity in multiple mammalian models, mechanistic clarity, and clinically achievable exposures, 80-100 nm AgNPs warrant regulatory classification as Category 1 reproductive toxicants (known/presumed human reproductive/developmental hazard) under GHS/REACH or equivalent systems. [30] Clinical use in pregnancy should be restricted to documented medical necessity with explicit informed consent. Products must carry reproductive hazard warnings. Healthcare providers require education regarding risks in reproductive-age populations.

CONCLUSIONS

Silver nanoparticles of 80-100 nm possess significant teratogenic potential through convergent ion channel blockade, oxidative stress, and inflammatory mechanisms. The multisystem nature of defects, consistency across mammalian models, and clear dose-response relationships establish AgNPs as developmental hazards. Long-term neurodevelopmental impairment in surviving offspring indicates lasting CNS injury.

Comparative biology with non-human primates suggests animal models may underestimate human risk. Clinically achievable exposures from medical applications, combined with first-trimester vulnerability, necessitate precautionary regulatory action and clinical use restrictions. Future research should focus on protective interventions, detailed human surveillance, and development of safer alternative nanomaterials.

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