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Evaluation of vascular tone and cardiac contractility in response to silver nanoparticles, using Langendorff rat heart preparation

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Abstract

Silver nanoparticles (AgNPs) have been widely used because of their antimicrobial properties. However, several reports suggest that AgNPs exposure promote cardiac effects that involve nitric oxide (NO) and oxidative stress (OS). Nevertheless, there are no studies related to AgNPs-induced effects in cardiac physiology. The aim of this study was to evaluate the AgNPs direct actions on coronary vascular tone and cardiac contractility using Langendorff rat heart preparation. Low concentrations of AgNPs (0.1 and 1 μg/mL) increased NO derived from inducible NO-synthase (iNOS), without modifying cardiac parameters. Meanwhile, high concentrations (10 and 100 μg/mL) promoted a sustained vasoconstriction and increased cardiac contractility related to OS, leading to rhabdomyolysis. Furthermore, AgNPs were internalized in the cardiac muscle, hindering classic actions induced by phenylephrine (Phe) and acetylcholine (ACh). These data suggest that AgNPs affect cardiac physiology in function of the concentration and in part of the NO generation, NOS expression and OS.

Key words: Silver nanoparticles; Coronary vascular tone; Myocardial contractility; Nitric oxide; Oxidative stress

Lately, nanotechnology industry has led to the development of nanomaterials (NMs), which are structures with novel and unique physicochemical properties attributable to their size (1 to 100 nm).[1] However, their toxic or protective effects in biological systems are not fully studied. Silver nanoparticles (AgNPs) are the most common NMs used as effective antimicrobial agents in an increasing number of products.[2] Approximately, 14% of the AgNPs-containing products could release these NPs through their manipulation, representing a potential source of exposure.[2,3] Several reports have shown that AgNPs are able to translocate, enter into the human body through different routes of exposure[3] and distribute to major organs through the blood stream, including the heart.[3,4] Epidemiological studies have demonstrated that NPs exposure promote negative effects on

Abbreviations: ACh, Acetylcholine; AgBMs, Silver bulk materials; $AgNO_3$, Silver nitrate; AgNPs, Silver nanoparticles; CAT, Catalase; CEC, Rat coronary endothelial cells; CVS, Cardiovascular system; DLS, Dynamic light scattering; eNOS, Endothelial nitric oxide synthase; GAPDH, Glyceraldehydes-3-phosphate dehydrogenase; iNOS, Inducible nitric oxide synthase; LVP, Left ventricle pressure; MDA, Malondialdehyde; NMs, Nanomaterials; nNOS, Neuronal nitric oxide synthase; NO, Nitric oxide; NO_2, Nitrites; NO_3, Nitrates; NPs, Nanoparticles; O_2^-, Superoxide anion; OS, Oxidative stress; Phe, Phenylephrine; PIE, Positive inotropic effect; PP, Perfusion pressure; ROS, Reactive oxygen species; SEM, Scanning electron microscopy; SOD, Superoxide dismutase; SVSC, Shirley-Vegh-Salvi-Castle; TEM, Transmission electron microscopy; VIP, Vacuum Infiltration Processor; XPS, X-ray photoelectron spectroscopy.

Conflict of interest: There are no conflicts of interests.

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cardiovascular system (CVS), including cardiac dysfunction, dysrhythmia, heart failure and myocardial infarction.[5] However, there is limited information about AgNPs effects in cardiac function; which is regulated by nitric oxide (NO), a free radical synthesized enzymatically by endothelial NO-synthase (eNOS), neuronal (nNOS) and inducible (iNOS) synthases.[6] NO acting on heart is produced by all cell types composing the myocardium, like the endothelium, a specialized epithelium that coats blood vessels lumen.[7] In this concern, it has been reported that 35.75 nm AgNPs at high concentrations (>100 μg/mL), stimulate proliferation of rat coronary endothelial cells (CEC) related to eNOS-derived NO and induced a NO-dependent vasodilation, in similar fashion to acetylcholine (ACh), on Wistar rat aorta rings.[8] Likewise, *in vivo* studies reported that exposure of Ross broiler chickens[9] and zebrafish embryos[10] to 5–20 nm AgNPs (>50–100 μg/mL), decrease heart rate and cardiac contractility, indicating that they could interfere directly with cardiac muscle activity. Also, it was demonstrated that 35.75 nm AgNPs (<5 μg/mL) induced an endothelium-dependent vasoconstriction in Wistar rat aorta rings, in absence and presence of phenylephrine (Phe),[8] a very well-known vasoconstrictor agent[11]; suggesting that AgNPs may induce dual actions in vascular and cardiac function involving eNOS-derived NO. However, another report indicated that inhalation exposure of Sprague Dawley rats to 33 and 39 nm AgNPs (100 and 1000 $\mu g/m^3$) do not affect vascular nor cardiac function, since no modifications to vascular tone, heart rate nor left ventricular systolic pressure were detected,[12] evidencing their controversial actions in all these approaches. On the other hand, exposing guinea pigs dermally to 100 nm AgNPs during 13 weeks, cardiotoxicity was observed as cardiomyocytes deformities, inflammation and congestion.[13] In this regard, *in vivo* and *in vitro* studies have suggested that AgNPs cardiotoxicity may imply oxidative stress (OS),[14] either by reducing activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT),[15] or promoting reactive oxygen species (ROS) generation.[16] Nevertheless, there are no reports exclusively focused on AgNPs actions upon cardiac physiology using an integrative model. Recently, the isolated Langendorff heart preparation has been proposed for the assessment of NMs effects in CVS.[4,17,18] The aim of this study was to evaluate the effects of AgNPs on cardiac contractility and coronary vessels tone using isolated perfused rat hearts, conjointly with their association with NO, NOS expression and OS generation.

Methods

Synthesis of silver nanoparticles

AgNPs were synthesized from a 100 mL of 0.01 M $AgNO_3$ deionized water-based solution, placed in 1 L reaction vessel. Ten milliliters of 1% (w/v) gallic acid solution were mixed with 100 mL of Ag^+ solution using constant magnetic stirring. Then, the pH value of the solution was immediately adjusted (pH = 11) with NaOH 1.0 M. Thereafter, each AgNPs suspension was flocculated changing the pH to 1.5 using nitric acid; the resulted suspension was filtered with a nitrocellulose filter (Millipore, 0.1 μm pore diameter) in a vacuum filter flask (Nalgene). AgNPs on the filter were washed several times with deionized water until neutral pH. Finally, AgNPs were dispersed in deionized water and diluted to a metered volume to reach a concentration of 1017 μg/mL, which was confirmed with atomic absorption analysis.[19]

Silver nanoparticles and silver bulk materials characterization

AgNPs size and zeta potential was determined using dynamic light scattering (DLS) assay, using a DLS Malvern Zetasizer Nano ZS (Instruments Worcestershire, United Kingdom). NPs shape was confirmed by transmission electron microscopy (TEM) analysis, using a JEOL JEM-1230 microscope. Morphology and size of silver bulk materials (AgBMs) were observed using a scanning electron microscope (SEM, JOEL JSM-1650). X-ray photoelectron spectroscopy (XPS) was conducted in a PHI 5000 VersaProbe II system (Physical Eletronics, USA) equipped with a monochromated Al-Kα X-ray source. As AgNPs are suspended in liquid solution, these were prepared by solvent evaporation method[20] at room temperature over silicon wafers previously cleaned. Also 99.99% silver foil (Alfa Aesar, USA) was analyzed under the same conditions to use as reference sample. Binding energies were calibrated by setting the binding energy of adventitious C 1 s to 284.8 eV. XPS spectra were fitted using the Double Lorentzian line shape[21] to model the asymmetry in metallic gold and in the case when oxide states where found was used the Voigt function. The background was removed using Shirley-Vegh-Salvi-Castle (SVSC) background.[22]

Animals

Male Wistar rats (250–300 g) were used in all experiments. Rats were housed in clear plastic containers under a 12-h dark/light cycle with *ad libitum* access to water and food. All procedures were performed in accordance with the National Institute of Health Guide for the Use and Care of Laboratory Animals guidelines and approved by the Animal Care and Use Committee from the Faculty of Chemistry of the University of San Luis Potosi, Mexico (protocol number CEID2014032).

Isolated langendorff heart preparation

In vitro retrograde heart perfusion was performed at constant flow-rate mode.[23,24] Briefly, under anesthesia with sodium pentobarbital (50 mg/kg ip) heart was rapidly excised and transferred to ice-cold Krebs solution containing (mM): NaCl 117.8, $NaHCO_3$ 24.2, KCl 6.0, $MgSO_4$ 1.2, NaH_2PO_4 1.2, glucose 5.0, $CaCl_2$ 1.75 and pyruvate 5.0. Then, heart was connected to an aortic cannula of Langendorff apparatus and perfused at constant flow-rate (8 mL/min) with Krebs solution which was constantly bubbled with 95% O_2 and 5% CO_2 at 37 °C. A deionized water-filled latex balloon connected to a pressure transducer was inserted through the mitral valve into the left ventricle to allow isovolumetric contractions and continuously record the left ventricle pressure (LVP) as an index of cardiac contractility. Another pressure transducer located above the aorta recorded the perfusion pressure (PP), considered as an index of coronary vascular tone. Two wire electrodes were placed in the right atrium and apex to maintain heart rate at 4.5 beats per second. Haemodynamic parameters were acquired and