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Changes in Cardiopulmonary Function Induced by Nanoparticles

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Abstract

Nanoparticles (NP) are highly applicable in a variety of technological and biomedical fields due to their unique physicochemical properties. The increased development and utilization of NP has amplified human exposure and raised concerns regarding their potential to generate toxicity. The biological impacts of NP exposures have been shown to be dependent on aerodynamic size, chemical composition, and the route of exposure (oral, dermal, intravenous, and inhalation), while recent research has demonstrated the cardiovascular (CV) system as an important site of toxicity. Proposed mechanisms responsible for these effects include inflammation, oxidative stress, autonomic dysregulation, and direct interactions of NP with CV cells. Specifically, NP have been shown to impact vascular endothelial cell (EC) integrity, which may disrupt the dynamic endothelial regulation of vascular tone, possibly altering systemic vascular resistance and impairing the appropriate distribution of blood flow throughout the circulation. Cardiac consequences of NP-induced toxicity include disruption of heart rate and electrical activity via catecholamine release, increased susceptibility to ischemia/reperfusion injury, and modified baroreceptor control of cardiac function. These and other CV outcomes likely contribute to adverse health effects promoting myocardial infarction, hypertension, cardiac arrhythmias, and thrombosis. This review will assess the current knowledge regarding the principle sites of CV toxicity following NP exposure. Furthermore, we will propose mechanisms contributing to altered CV function and hypothesize possible outcomes resulting in decrements in human health.

Introduction

Since the development of engineered nanoparticles (NP), their use has increased dramatically in an assortment of fields, including consumer goods, structural engineering components, electrothermal conductors, and a variety of proposed biomedical applications. NP have aerodynamic diameters at <100 nm, large surface area/mass ratios, high capacities for electrothermal conduction, and diverse chemical compositions. While these physicochemical properties increase their applicability, they also raise concerns for potential adverse human exposure outcomes. Despite apprehensions, the broad application of NP has increased human exposures, especially in occupational settings.¹ While dermal, oral/gastrointestinal, and inhalation exposures to NP are all relevant to the occupational setting,

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pulmonary exposures have been the principle route of NP administration in toxicological studies.² These investigations have shown that despite minimal pulmonary outcomes, in many cases, the cardiovascular (CV) system may be a key site of NP-induced toxicity.^{3,4}

The toxicity of NP is thought to increase as particle size decreases, resulting in increased surface area/mass ratios, enhanced transport across epithelial and endothelial barriers, and interactions with subcellular components, all of which could potentially induce inflammation.¹ Exposure to NP may also exacerbate CV disease, and perhaps even disrupt CV homeostasis in otherwise healthy individuals. These concerns are derived from epidemiological studies demonstrating that individuals with chronic CV diseases exhibit increased CV-related mortality and morbidity following exposure to ambient environmental particulate matter.⁵ We propose that NP inhalation may also accelerate disease processes and result in similar increases in mortality and morbidity.

Recent studies have investigated the impacts of NP exposure on the CV system, yet the specific mechanisms of toxicity remain in question. Thus, the focus of this review will be to highlight potential CV endpoints associated with pulmonary and intravenous routes of NP exposure and discuss plausible mechanisms of toxicity. We will critically evaluate recent studies that have examined NP-induced toxicity in vascular endothelial cells (EC), macrovessels, small conduit arteries, microvessels, cardiac myocytes, and the heart. We will further emphasize the current knowledge gaps in these areas, and postulate on how NP-induced CV toxicity may alter CV function and affect overall human health.

Routes of Exposure and Mechanisms Responsible for Adverse CV Outcomes

The important routes of NP exposures include occupational inhalation and intravenous injection for medical purposes. Both exposure routes have been shown to increase NP burden within the lungs. Due to their extremely small size, inhaled NP are able to deposit deep within alveolar regions of the lung and may evade mechanisms which clear larger particles.⁶ During intravenous delivery, since the entire cardiac output passes through the pulmonary circulation, NP pass through pulmonary capillaries and have been found to accumulate in the lung.⁷ Various inhalation, instillation, and aspiration models of NP exposure in rodents, including CeO₂, TiO₂, SiO₂, NiO, Fe₂O₃, ZnO, single-walled (SWCNT), and multi-walled carbon nanotubes (MWCNT), have been shown to cause pulmonary inflammation, apoptosis, oxidative stress, fibrosis, damage to the epithelial barrier, and eventually reductions in function.^{8,9,10,11,12,13,14,15,16} Specifically, intratracheal instillation of carbon black NP (CB) demonstrated increases in pulmonary and hepatic transcription of genes related to inflammation and acute phase response.¹⁷ This evidence demonstrates the potential for pulmonary exposures to generate extrapulmonary responses. Also, repeated intravenous injection of silica and gold NP demonstrated accumulation of gold NP within the lung and liver, while the inflammation following silica NP exposure resulted in DNA damage in the lung and liver.⁷ These findings suggest that intravenous injection of certain NP may accumulate within the lung and result in pulmonary toxicity, while pulmonary accumulation may in some cases result in adverse systemic effects, implying the ability for NP to enter and exit the vascular compartment and disseminate throughout the circulation.

Mechanisms of CV toxicity from the inhalation of ambient particulates have previously been proposed^{18,19} and the mechanisms of extrapulmonary toxicity from NP inhalation have recently been extensively reviewed.²⁰ Based upon the ability for NP to translocate from exposed tissues, and the fact that NP are now being developed for intravenous injection, we propose that the mechanisms of NP-induced CV toxicity include: 1) direct NP interaction

with cells of the CV system, either by intravascular delivery or NP translocation, 2) tissue injury at the site of exposure with generation of inflammatory/oxidative responses that propagate throughout the circulation, and 3) interaction of NP with sensory nerve endings at the site of exposure and subsequent dysregulation of autonomic CV reflexes (see Fig. 1).

Direct NP Interactions

Many NP are being developed as drug delivery systems and contrast agents for medical imaging. These NP are designed for direct injection into the circulation, allowing for immediate NP-CV cell interactions. It has been demonstrated that silica NP and SWCNT accumulate and remain in the spleen and liver of mice weeks after a single intravenous injection, suggesting the ability of NP to interact with CV cells while being cleared from the circulation.^{21,22} NP are also being incorporated into CV implants, such as stents and synthetic heart valves, which may release NP directly into the bloodstream over time and potentially produce negative CV outcomes.²³ Also, following pulmonary exposure a fraction of pulmonary deposited NP have been shown to translocate systemically.^{24,25} By inhalation, CeO₂ has been found in the testis, liver, spleen, kidney, brain, and epididymis as early as 6 hrs after a single exposure in rats.²⁶ Extrapulmonary translocation has been shown to be related to NP size, charge, and surface modifications^{27,28} and could occur due to increases in pulmonary epithelial permeability, allowing NP to pass directly into alveolar capillaries or the lymphatic system.²⁹ An additional concern is the use of highly-reactive metals such as Co, Fe, and Ni in the production of NP which could potentially leach off the surface of NP, translocate into the circulation, and facilitate CV toxicity. The translocation of NP or their leachable components may allow for direct interactions with EC, circulating platelets, and/or cardiac myocytes. These interactions may possibly initiate CV oxidative stress, inflammation, fibrosis, and prothrombosis, ultimately impairing CV function.

Autonomic Dysregulation

Pulmonary inhalation of NP could result in autonomic dysregulation which may influence CV health effects. NP could modulate autonomic regulation through the stimulation of vagal sensory nerves within the lung via direct interactions with NP or via inducible pulmonary inflammation similar to other forms of inhaled particulate matter.^{30,31,32} This nerve stimulation could be exacerbated by NP compared to larger inhalable particles due to their potential to evade clearance mechanisms and possibly result in sustained level of pulmonary inflammation.^{33,9} To date no studies have examined autonomic dysfunction resulting from routes of exposure other than inhalation.

Propagated Inflammatory/Oxidative Responses

Finally, the release of various cytokines and prothrombotic factors into the circulation after NP exposure could mediate CV toxicity. Specifically, a 4 week inhalation exposure of Sprague-Dawley rats to carbon black NP demonstrated a lack of translocation of particles from the lung and increased pulmonary mRNA expression of the inflammatory cytokines IL-6 and MCP-1, while causing elevations in systolic blood pressure and circulating levels of the pro-inflammatory markers IL-6, MCP-1, and C-reactive protein.³⁴ These findings support the possible release of cytokines produced in the lung in response to NP exposure to subsequent systemic effects. Intravenous injection of silica NP have also been demonstrated to cause increased levels of the circulating pro-inflammatory cytokines IL-6 and TNF- α .⁷ These inflammatory cytokines have been linked to CV disease processes and may increase both cardiac and vascular inflammation.

Vascular Outcomes

Apart from the generalized paradigm of oxygen and nutrient delivery, the vascular system is critical in the maintenance of blood pressure, hemostasis, coagulation cascades, immune system function, humoral transmission, fluid/ion balance, angiogenesis, and thermoregulation. Cellular components of the vascular system primarily include vascular EC and smooth muscle cells, but other components include autonomic nerve endings, resident mast cells, macrophages, circulating neutrophils, monocytes, perivascular fat, connective tissues, and extracellular matrices. These components, and the complex mechanisms by which they assert physiological vascular function, can potentially be influenced by NP exposure through direct NP-cell interactions, autonomic dysfunction, and the release of inflammatory mediators. *In vitro* studies have demonstrated the direct impact of NP exposure on vascular EC, while *in vivo* exposures have investigated alterations in physiological functions of selected vascular beds.

EC Responses to NP Exposure In Vitro

Direct NP Interaction—Approximately 60 trillion EC line the human vasculature and perform a diverse array of physiological functions, including influences on leukocyte adherence/recruitment, altering vascular permeability, contributing to innate and adaptive immunity, and regulation of vascular smooth muscle tone.³⁵ In terms of immune function, vascular EC have the ability to act as sentinel cells by interacting with and reacting to foreign substances, such as, NP in the circulation.³⁶ As discussed previously, NP have demonstrated an ability to translocate from sites of exposure into the systemic circulation and, therefore, *in vitro* studies have been utilized to understand direct NP interactions with vascular EC. Recent reports on EC responses to NP exposure generally have shown increased oxidative burden, changes in actin dynamics, and/or general cytotoxicity/apoptosis.

MWCNT have been proposed for use as intravenous drug delivery systems and as medical contrast imaging agents.³⁷ On this basis, M. Pacurari et al. (2012) sought to determine if direct exposure to MWCNT would alter permeability and migration of microvascular EC. They found that exposure to MWCNT concentrations relevant to *in vivo* imaging studies (2.5 $\mu\text{g/ml}$) induced increases in microvascular EC monolayer permeability as early as 1 hr, peaked at 8 hrs, and persisted for a minimum of 50 hrs without causing cell death. They further showed that MWCNT exposure increased reactive oxygen species (ROS)-dependent cell migration and initiated actin filament remodeling. Together these findings support the possibility that MWCNT can interact with vascular EC directly to produce oxidative stress, and that MWCNT when administered intravenously have the potential to translocate out of the vascular compartment.

Propagated Inflammatory/Oxidative Responses—Further *in vitro* studies showing that MWCNT exposure can generate increases in oxidative stress have been conducted on human umbilical vein EC, in which changes in cellular redox state were investigated along with assessing DNA damage as a determinate of general cytotoxicity.³⁸ Specifically, Guo et al. (2011) demonstrated that 20 $\mu\text{g/ml/day}$ of MWCNT resulted in ROS generation, while a significantly lower concentration of 5 $\mu\text{g/ml/day}$ caused lipid peroxidation. This study also noted that glutathione peroxidase and super oxide dismutase activity was significantly increased with exposures as low as 0.5 $\mu\text{g/ml/day}$. The induction of these ROS scavenging systems provides a buffer to any measured ROS production and may explain why it took a 20 $\mu\text{g/ml}$ MWCNT dose to measure an increase in ROS generation at 24 hrs. In terms of the general cytotoxicity of these MWCNT, all doses examined (0.5–20 $\mu\text{g/ml/day}$) were capable of causing a statistically significant apoptotic response. In general, these findings were

consistent with increased oxidative burden in microvascular EC after exposure to MWCNT.³⁷ Collectively these results suggest that MWCNT can cause increased ROS burden upon direct interaction with vascular EC.

Silver NPs (Ag-NP) have been identified as an antimicrobial agent, and thus have been incorporated into consumer products to combat bacterial growth.³⁹ Based on reports that Ag-NP introduced into the blood stream produce adverse impacts on blood-brain barrier function *in vivo*, Trickler et al. (2010) investigated the mechanisms by which Ag-NP induced toxicity in primary rat brain microvessel EC. In general, their study showed both size-dependent (25 nm > 40 nm > 80 nm) and time-dependent (8 hrs > 4 hrs > 2 hrs) increases in the release of the pro-inflammatory mediators TNF- α , IL-1 β , and PGE₂. The release of pro-inflammatory mediators also correlated with significant increases in EC monolayer permeability. Interestingly, polyethylene glycol protected Ag-NP have been shown to maintain their antimicrobial and anti-platelet properties while decreasing their EC cytotoxicity,^{40,41} but the impact on permeability and cytokine production was not evaluated.

The prevalence of CV disease in the United States often dictates the medical use of stents and other CV implants.²³ These devices are often derived from various metal alloys, like Nickel-Titanium (NiTi) or Cobalt-Chromium (CoCr). In cases of production failure, wear, and ablation of these CV implants, NPs and metal ions can be unintentionally released into circulation. Hahn et al. (2012) performed a study in which human primary coronary EC (hCAEC) and vascular smooth muscle cells (hCASMC) were cultured and exposed to various molar concentrations (0.1–1000 μ M) of physically characterized Ni, Ti, Ni₅₀Ti₅₀, Co, or Ni₄₈Fe₅₂ NPs (size 5–250 nm diameter) for 72 hrs.²³ To account for protein corona formation at the particle surfaces *in vivo*, they suspended particles in 5 mM citrate or 5 mM cysteine before being added to cell cultures. The results showed that hCAEC were generally more sensitive to Co, Ni, NiTi, and NiFe NPs than hCASMC, but both cell types showed dose-dependent responses and a similar order of sensitivity (Ni $\approx$ Co > NiFe $\approx$ NiTi > Ti). Since Ti was not shown to generate adverse effects, the primary conclusion drawn was that Ni or Co content may be the elements responsible for cytotoxicity. These findings also suggest that EC may be increasingly susceptible to metal ions released from NP as compared to vascular smooth muscle cells.

In general, NP appear capable of increasing EC oxidative burden, inflammation, and cytotoxicity; however, the exact mechanisms are likely complex and multifaceted. Many characteristics of NP may be responsible for these effects including chemical composition,²³ surface area/mass, and biopersistence of the particle.³⁹ As reviewed in Buyukhatipoglu and Clyne (2011),⁴² NP can increase cellular oxidative burden via ROS generation by transition metal mediated Fenton-type reactions and/or mitochondrial dysfunction following NP uptake. Furthermore, NP-induced ROS can disrupt actin dynamics and lead to increased vascular permeability.⁴² In ECs impaired actin dynamics can decrease their ability to detect and transmit changes in shear stress, resulting in decreased nitric oxide synthase (NOS) release,⁴² possibly promoting enhanced vasoconstriction.

Vascular Responses to NPs

Direct NP Interactions and Autonomic Dysfunction—The potential for NP to influence vascular EC through direct interaction is quite evident, but the overall susceptibility of the vascular system should also be evaluated at the level of the intact organ and the whole animal. This is important because NP exposures have been shown to indirectly produce adverse vascular impacts. This is thought to occur either through the generation of local/systemic inflammatory responses, oxidative stress, and/or changes in autonomic outflow. Increases in circulating inflammatory markers like TNF- α , IL-1 β , and IL-6 are known to disrupt vascular function in several pathophysiological states, and could

also augment vascular function following NP exposure.⁴³ Oxidative stress can be systemically propagated through the release of oxidized proteins and lipids into circulation, potentially leading to activation of receptors for advanced glycation endproducts or leptin-like oxidized low-density lipoprotein receptor-1 on the surface of vascular cells.²⁰ Furthermore, autonomic outflow can be altered after pulmonary exposure to NP by stimulating pulmonary reflex arches that modulate basal sympathetic and parasympathetic tone.⁴³ These outcomes may impact vascular physiology via augmentation of G protein-coupled receptor responses, alterations in Ca^{2+} handling/sensitivity, and activation of cyclooxygenase (COX) pathways, thus impairing vascular reactivity.

Propagated Inflammatory/Oxidative Responses—Macrovasculature, like the aorta, contain microvessel networks associated with the vascular wall that have been shown to intensely regulate vascular inflammation,⁴⁴ suggesting the importance of assessing macrovessel function following NP exposure. These mechanisms could include mast cell-mediated endothelial cell activation and nucleoside release.^{45,46} In the context on NP exposure, we reported that aortic vascular reactivity responses to adenosine were altered 24 hrs following pulmonary instillation of 10 μg and 30 μg of CeO_2 (70–90 nm diameter) in C57BL/6J mice.⁴⁷ We further showed that pulmonary instillation of CeO_2 increased IL-6 and MIP-1 α expression in lung tissue and osteopontin levels in bronchoalveolar lavage fluid. These aortic and pulmonary effects were not duplicated in B6.Cg-*Kit*^{W-sh} mast cell deficient mice. We concluded from this study that CeO_2 inhalation exposure could result in vascular outcomes and that the mast cell may mediate inflammation. Vesterdal et al. (2010), demonstrated the vascular impacts of intratracheally instilled CB in young *apoE*^{-/-} mice.⁴⁸ Specifically, they found that instilling two doses of CB at 0.5 mg/kg, 24 hrs apart, and collecting aortas 2 hrs after the final dose significantly impaired acetylcholine (ACh) mediated relaxation responses. The impaired relaxation was not found in responses to sodium nitroprusside, a direct NO donor, suggesting an endothelial-dependent mechanism. The blunted relaxation response was found in the absence of nitrosative stress or changes in EC adhesion protein expression. They concluded that multiple exposures to low doses of CB were critical in the development of impaired ACh relaxation, as these responses were not observed when larger single doses of CB were administered and animals were sacrificed after either 2 hrs or 24 hrs.

In order to understand how blood flow to specific regions of the body may be affected following NP exposure it is important to examine other arterial segments. Direct exposure of isolated pulmonary artery segments from Sprague-Dawley rats to carbon-derived NP (CNP) generated dose-dependent isometric relaxation after vessels were preconstricted with 10 μM $\text{PGF}_{2\alpha}$ or 30 mM KCl.⁴⁹ This suggested that CNP can preferentially bind to several pharmacological agonists, thus reducing the number of ligands able to bind their receptors. The authors also suggested that CNP may interfere with voltage operated Ca^{2+} channels. Courtois et al. (2010) concluded that these types of interactions should be considered when interpreting results from future vascular studies on NP exposure.⁴⁹ This finding may also be physiologically relevant as NP in the circulation could bind endogenous ligands and modify vascular receptor signaling throughout the vascular tree.

There are general concerns about adverse vascular outcomes associated with occupational inhalation of Ni-containing NP.⁵⁰ Cuevas et al. (2010) exposed C57BL/6 mice to multiple concentrations (100, 150, or 900 $\mu\text{g}/\text{m}^3$) of Ni hydroxide nanoparticles (NH-NP), with diameters of 5–40 nm, via whole body inhalation chambers for 1, 3, or 5 days (5 hrs/day).⁵⁰ One day following the final exposure, 2 mm segments of the carotid artery were isolated from each mouse and suspended on a wire myograph for vascular reactivity assessments using cumulative dose-response curves for phenylephrine (PE) and ACh. Both constrictor responses to PE and relaxation responses to ACh were depressed in a time-dependent and

dose-dependent fashion. While the causality of these findings were not investigated, they did suggest a variety of potential mechanisms including altered G protein-coupled receptor responses, changes in Ca^{2+} sensitivity, and/or COX induction.

Microvessels provide the largest resistance to blood flow in the vasculature and regulate tissue specific flow. LeBlanc et al. (2009) demonstrated that an inhalation model capable of depositing 10 μg of TiO_2 into the lungs of Sprague-Dawley rats was able to generate endothelial dysfunction in subepicardial arterioles.³ Twenty four hrs following inhalation exposures, coronary resistance arterioles (150 μm in passive diameter) were isolated from the left anterior descending artery distribution and mounted into a vessel chamber. Their findings showed that endothelial relaxation responses to shear stress, ACh, and the Ca^{2+} ionophore A23187 were diminished in animals exposed to TiO_2 . Furthermore, TiO_2 exposure increased the heart weights of exposed rats indicating a possible rise in microvascular permeability. This group has previously shown that inhalation of 10 μg TiO_2 also disrupted microvascular NO bioavailability and increased oxidative and nitrosative stress in microvessel walls of other systemic vascular beds.⁵¹ They also have demonstrated that TiO_2 exposure causes ROS-dependent impairment of endothelium-dependent relaxation in coronary arterioles.³ Taken together, these studies demonstrate that pulmonary exposure to TiO_2 NP increases spontaneous microvascular tone and impairs endothelium-dependent relaxation through a ROS-mediated mechanism.

While many of the effects of NP exposure have been strongly associated with endothelium-dependent mechanisms, Knuckles et al. (2011) demonstrated that some vascular outcomes may also be endothelium-independent.⁵² Briefly, 24 hrs following inhalation of TiO_2 NP (10 μg total lung deposition), male Sprague-Dawley rats were subjected to intravital microscopy of arterioles in the spinotrapezius muscle. The TiO_2 group showed heightened sensitivity to α -adrenergic blockade during perivascular nerve stimulation and blunted arteriolar dilation in response to active hyperemia. The dampened active hyperemia-mediated dilation was not exacerbated by the use of a NOS inhibitor, suggesting a loss of NO bioavailability. However, COX inhibition did further impair active hyperemia-induced vasodilation, suggesting that arterioles from the TiO_2 exposed animals had compensated for the reduced NO bioavailability by a COX-mediated mechanism. Further, the TiO_2 group demonstrated increased sensitivity to a TXA_2 mimetic and decreased sensitivity to a PGI_2 mimetic. This study provides evidence for NP-induced endothelium-independent mechanisms of vascular toxicity, but further investigation will be needed to identify its role in altering vascular homeostasis relative to endothelial-dependent mechanisms.

Direct NP-EC interactions have been shown to increase oxidative burden, decrease actin dynamics, and increase apoptotic responses. Pulmonary exposures to NP *in vivo* are known to produce consequences throughout the vascular tree. These outcomes have been associated with alterations in G protein-coupled receptor responses (adenosine, ACh, PE, etc.), changes in Ca^{2+} handling/sensitivity, loss of NO bioavailability, and activation of COX-mediated responses (see Fig. 2). All of these outcomes suggests that NP exposure can generate a relatively vasoconstricted state, which has strong implications for heightened vascular resistance throughout the body and diminished precision in the regulation of blood flow distribution throughout the circulation. The consequences of such phenomena may be benign in the relatively well perfused organs, but could be rather profound in organs with poor perfusion or high metabolic demand like the heart.

Cardiac Outcomes

The heart is responsible for generating pressure and driving the flow of blood through the body. To do so, the heart functions as an electrical-mechanical syncytium, relying on the

generation and cellular propagation of action potentials through nodal tissue, conductive fibers, and myocardial tissue by converting waves of electrical depolarization into intracellular Ca^{2+} release and cardiac contraction. While the heart can maintain this function without any nervous innervation, it is sensitive to various blood components and neurohumoral messages. These include activation of sympathetic and parasympathetic nerves, the renin-angiotensin system, or paracrine factors such as NO and endothelin. NP exposure can potentially influence these factors, via direct nanoparticle interactions, autonomic dysfunction, and/or inflammatory/oxidative stress responses. Despite the significant role the heart plays in CV homeostasis and the overwhelming prevalence of CV disease, only a few studies have examined changes in CV function induced by NP and fewer have investigated the outcomes of direct NP on cardiac myocytes.

Direct NP Interactions

The Langendorff heart (LH) model is an isolated beating heart model used to assess the autoregulatory function of the intact heart by isolating it from the systemic vasculature, pulmonary system, and neurohumoral influences. In an experiment designed to study direct delivery of TiO_2 , SiO_2 , or Printex90 NP via the perfusate in a LH model, results demonstrated NP exposure induced a significant increase in heart rate, with concurrent ECG changes, and arrhythmias.⁵³ Specifically, Stampfl et al. (2011) demonstrated that TiO_2 and Printex90, when added to the coronary perfusate and cycled through the heart, increased both heart rate and compensatory coronary flow.⁵³ The changes in ECG specifically were ST elevations and atrioventricular block. Interestingly, they found that a 30 min washout period with NP-free perfusate allowed heart rate to return to baseline, but the ECG changes persisted. Furthermore, the authors demonstrated that the NP-induced increased heart rate was due to the release of catecholamines from the sympathetic cardiac nerve endings. This study supports the postulated mechanism that NP exposure can affect heart rhythm potentially via autonomic influences and/or release of mediators.

Pulmonary exposure to NP has also been shown to alter cardiac ischemia/reperfusion (I/R) injury in both the LH model and *in situ*.^{47,54} Acid-functionalized single-wall carbon nanotubes (AF-SWCNT) instilled intratracheally in mice have been found to promote infarct expansion following I/R (20/120 min respectively) in a LH model, 24 hrs after exposure.⁵⁴ In this study by Tong et al. (2009), it was also demonstrated that AF-SWCNT exposure significantly decreased left ventricular developed pressure recovery during reperfusion, diminished the time until contracture, and enhanced focal myofibril degeneration.⁵⁴ Work from our own group has demonstrated that pulmonary exposure to CeO_2 promotes an exacerbation of cardiac I/R injury (20/120 min, respectively) in mice through a mast cell-dependent mechanism.⁴⁷

Autonomic Dysregulation

Few studies have investigated the ability of NP to influence cardiac autonomic regulation and pressure regulation. One such study utilized multiple intratracheal instillations of SWCNT in rats, which showed subsequent decreases in heart rate and increases in baroreflex sequences,³⁰ supporting the claim that pulmonary inhalation of nanoparticles can result in autonomic dysregulation, thus impacting regulation of blood pressure and CV variability in response to hemodynamic pressure changes. The observed change in baroreflex function suggested a loss of sensitivity to arterial pressure. Such impairments in baroreflex function are considered prognostic for cardiac mortality,⁵⁵ particularly in individuals affected with ischemic cardiomyopathies and myocardial infarction.

Normal stimulation of the sympathetic nervous system in response to emotional or physical stress causes an increase in heart rate. In order to compensate for the shortening of diastolic

action potentials, slow voltage-gated potassium channel I_{Ks} are up-regulated to prevent repolarization delays. Anomalies in the cardiac action potential channel $KCNQ1$, of I_{Ks} are known to predispose patients to arrhythmias.⁵⁶ It was postulated that intravenously injected magnetic- Fe_3O_4 NP could affect expression of the $KCNQ1$ channel during exercise and result in altered cardiac function.⁵⁶ Results showed that the magnetic- Fe_3O_4 NP had no significant effect on the expression profile of $KCNQ1$ in both rested and exercised mice. However, at baseline and within the first 24 hrs, the upregulation of $KCNQ1$ was consistently lower in the particle exposed group versus exercised-only group. These results illustrate that even in the absence of statistically significant changes following NP exposure, small physiological changes in the heart may occur that increase the propensity for cardiac arrhythmias via delays in cell membrane repolarization.

Propagated Inflammatory/Oxidative Responses

NP have been found to inhibit myocyte damage from cigarette smoke extract (CSE) *in vivo* through inhibition of ROS generation, nuclear factor-kappaB (NF- κ B) signaling cascade, and antioxidant depletion.⁵⁷ CeO_2 was specifically selected because of its dual oxidative states in order to test its potential ROS neutralization ability on pre-damaged cells. CeO_2 also inhibit CSE-induced phosphorylation of $I\kappa B\alpha$, the inhibitory subunit of NF- κ B. Another study demonstrated that TiO_2 NP had only minimal impacts on adult cardiac myocyte function, but showed higher toxicity in embryonic stem cell derived cardiac myocytes.⁵⁸ Collectively, these results demonstrate that NP such as CeO_2 may have different oxidative capacities depending on the exposed cell type and TiO_2 NP may have greater cardiac cytotoxicity in heart patients that have undergone stem cell grafts.

Taken together, NP exposure appears to exacerbate I/R injury and potentially disrupt the intricate cardiac conduction system which could adversely affect cardiac action potential repolarization, and thus the coordinated contraction and relaxation of the atria and ventricles. This could predispose individuals to cardiac arrhythmias and exacerbate underlying CV diseases. Specifically, NP may disrupt normal cardiac function through modulation of autonomic reflexes or direct myocardial inflammation/oxidative stress. The NP-induced cardiotoxic impacts seen both in the intact heart and *in vivo*, together with the few studies on direct cardiac myocyte toxicity to NP, suggest that adverse cardiac effects are the result of multiple contributing mechanisms and may not be a result of direct NP-myocyte interactions. Further investigation is required into the ability of NP to influence cardiac function and fully elucidate the possible mechanisms.

NP in Medicine

Beyond occupational exposures, NP are showing promise in medical applications as contrast agents for medical imaging,⁵⁹ drug delivery systems,⁶⁰ and cell/organ specific therapies.⁶¹ Magnetic resonance imaging has been enhanced with the use of superparamagnetic iron oxide NP as contrast agents and optical cellular imaging techniques using quantum dots are quickly outdating the use of conventional dyes and chemical staining techniques.⁵⁹ The use of NP as drug delivery systems have been proposed to improve the solubility of hydrophobic drugs, increase the circulating half-life of rapidly metabolized drugs, and release drugs in response to stimuli in the biological environment.⁶² Liposomes are spherical lipid vesicles that represent NP designed for drug delivery, which can be coated with inert biocompatible polymers like polyethylene glycol in order to increase the circulating half-life.⁶² NP designed to carry poorly soluble chemotherapy drugs are expected to passively target tumors due to the enhanced permeability and retention properties of tumors.⁶¹ Specific targeting of NP to certain cell/tumor types can be achieved by coating NP with biological ligands or antigens that recognize cell receptors or specific peptides, thus allowing for very specific and localized NP/drug delivery.⁶⁰ NP comprised of iron, nickel, and/or cobalt have magnetic

properties in the presence of magnetic fields and have shown promise with targeted drug delivery against tumors.⁶³ Using magnetic fields to move these magnetic NP to the site of interest is a noninvasive technique, making the use of magnetic NP for such applications highly attractive, and the magnetic properties of these NP also allows for easier monitoring of their biodistribution.⁶³ Outside of cancer therapies, magnetic NP have been proposed as cardiac and coronary targeted drug delivery devices during cardiopulmonary resuscitation, and also during the post-resuscitation period.⁶⁴ NP are also able to be modified in a manner that promotes immunosuppression or immunostimulation.⁶⁵ Given our understanding of how medical NP can be modified to alter their biological interactions, perhaps similar solutions can be identified in order to decrease the potential CV detriments associated with the NP exposures outlined in this review.

Conclusions

Human exposure to NP continues to increase, however, given their size, chemical compositions, and oxidative potential, the physiological/toxicological consequences may likely be profound. The CV system has been shown to distribute NP throughout the body, and is a key site of NP-induced toxicity. We have discussed alterations in CV function and an exacerbation of cardiac I/R injury following NP exposure in healthy animal models. This suggests that one generalized outcome of NP exposure is a narrowing of normal homeostatic ranges, thereby limiting the compensatory ability of the CV system to respond to perturbations. Identified changes in the vasculature following NP exposure includes increases in vascular permeability, loss of vascular reactivity, and shifts towards relatively vasoconstricted states. This alters perfusion in specific vascular beds and thus contributes to energy supply and demand mismatches in metabolically active organs. Such endpoints manifest in the heart as loss of electrical conductivity, initiation of cardiac arrhythmias, and expansion of infarcted tissue. Currently, the study of CV outcomes associated with NP exposure has largely been descriptive, while many of the mechanisms underlying these effects remaining unknown or poorly described. To date research has suggested that the CV effects occur via direct interactions of NP with the CV system, modified autonomic regulation, and/or immune-mediated inflammatory cytokine release into the circulation. Likely, the adverse CV outcomes are a result of some combination of these three mechanisms, thus elucidating the contribution of each mechanism remains a complex task. Another challenging factor is the inherent variability in the responses of individuals to NP, likely through genetic, epigenetic, and predisposition to disease susceptibility. In particular, given the tendency for NP exposure to drive enhanced vasoconstriction and thereby increased vascular resistance, individuals who are possibly at an increased risk include those with atherosclerosis, hypertension, and heart failure. Lastly, given the evidence that NP exposure increases pro-arrhythmic conditions, individuals predisposed to cardiac arrhythmias may also have a high susceptibility to NP exposure. Taking into account the aforementioned factors, along with the epidemiological evidence for ambient particulate-induced CV morbidity and mortality in individuals with CV disease, and the CV alterations following NP exposure, we believe that individuals with CV disease will likely be highly susceptible to NP exposure. Therefore, future research on CV toxicity to NP should be conducted in animal models of CV disease such as hypertension, heart failure, and aging.

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Figure 1.
Exposure routes and mechanisms leading to adverse cardiovascular effects of nanoparticles

Figure 2.
Potential cellular mechanisms that drive enhancement of vascular tone.