

Review Article

Cerium oxide nanoparticles: potential applications for cancer and other diseases

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Abstract: The diverse abilities of cerium oxide nanoparticles (CONPs) have encouraged researchers to pursue CONPs as a therapeutic agent to treat a number of diseases, including cancer. *In vitro* and *in vivo* studies have shown CONPs to be toxic to cancer cells, inhibit invasion, and sensitize cancer cells to radiation therapy. However, CONPs display minimal toxicity to normal tissues and provide protection from various forms of reactive oxygen species (ROS) generation. The antioxidant capabilities of CONPs, which enable radiation protection, have also resulted in the exploration of these particles as a potential treatment for other disorders characterized by ROS accumulation, such as diabetes and macular degeneration. While critical information regarding the uptake, retention, and clearance of these particles is incomplete and conflicting reports exist about *in vitro* toxicity, most research into the various applications of CONPs has yielded promising data. This review highlights the current research into cerium oxide nanoparticles as a novel therapeutic for the treatment of cancer and other diseases.

Keywords: Cerium oxide nanoparticles (CONPs), cancer, reactive oxygen species (ROS), pro-oxidant, anti-oxidant

Introduction

In recent years, nanotechnology has become a main focus of biomedical research. Nanoparticles applications include drug delivery systems, luminescent biomarkers, and tissue engineering, among others [1]. In particular, cerium oxide nanoparticles (CONPs), which consist of a cerium core surrounded by an oxygen lattice, have shown promise in a number of applications. Originally of interest for the ability of surface oxygen vacancies to interact with and modulate free radicals, CONPs have since been shown to display a number of antioxidant behaviors, including superoxide dismutase (SOD) activity [2], catalase mimetic activity [3], nitric oxide radical scavenging [4], and hydroxyl radical scavenging [5]. However, tissue or cell environmental conditions appear to play an important role in the determination of activity, as CONPs also possess direct oxidant behavior [6]. To date, pH is one of the few factors shown to drive whether CONPs act as oxidants or anti-oxidants [7, 8].

As cellular levels of reactive oxygen species (ROS) are tightly controlled in normal, healthy

cells [9], the ability to modulate the redox status of cells has applications in diseases where ROS levels have become de-regulated or are altered by treatment. Though more recently linked to cell proliferation and survival, ROS accumulation is generally associated with undesired effects, having been linked to neurodegenerative diseases, diabetes, atherosclerosis, and even aging [9]. In cancer, which causes over 500,000 deaths per year [10], ROS can drive both the initial development and progression, as well as down regulate antioxidant enzymes that normally combat radical production [11]. Studies have shown CONPs to possess innate cytotoxicity to cancer cells, anti-invasive properties, and the ability to sensitize cancer cells to radiation induced cell death, while protecting the surrounding normal tissues (**Figure 1**). Additionally, CONP treatment has been shown to prevent macular degeneration [12] and the formation of neovascular lesions in the retina [13], as well as decrease hepatic ROS levels linked to the progression of diabetes [14]. Thus, CONPs have extensive potential as a therapeutic agent for the treatment of cancer, as well as other diseases in which ROS have

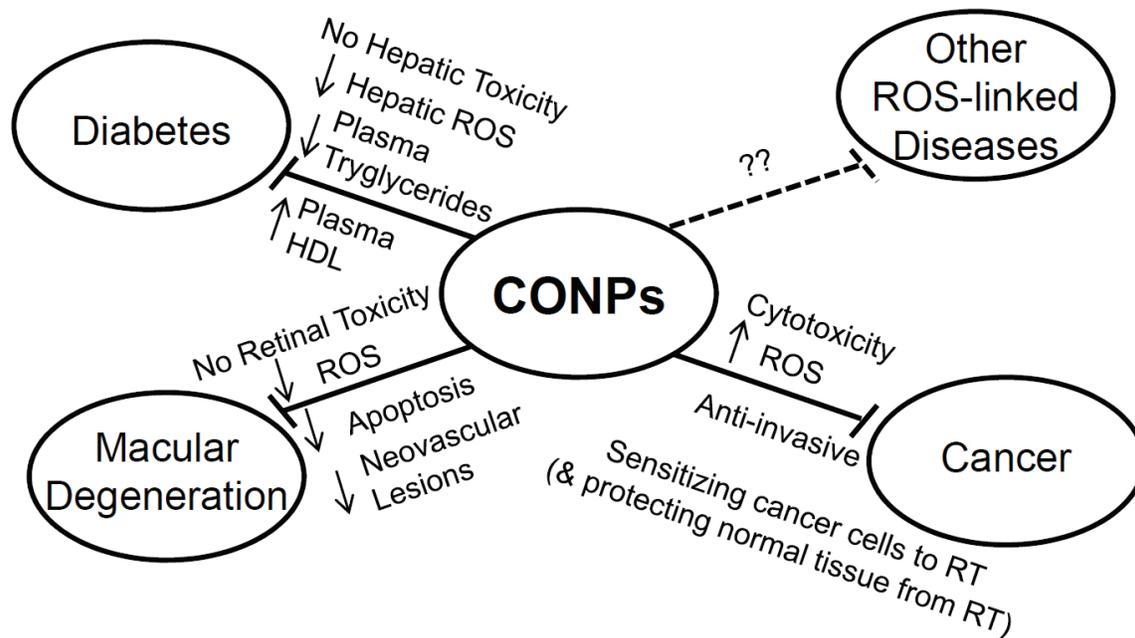


Figure 1. Potential therapeutic applications of CONPs. The various diseases in which CONPs have been tested and ROS-related therapeutic mechanisms are illustrated.

been implicated. Potential applications of CONPs are summarized in **Figure 1** with brief description of ROS-dependent mechanisms that are discussed in further details below.

CONP effect on cancer

Anti-invasive properties

In addition to CONPs' toxicity to cancer cells *in vitro* and *in vivo*, studies have shown polymer-coated CONPs to also manipulate tumor-stromal interactions to the detriment of tumor progression and invasion [15]. Polymer coating of CONPs increases aqueous solubility [7], yet does not appear to impact CONP redox activities [6, 15]. Epithelial/stromal signaling is largely mediated by myofibroblasts, which play a key role in the expression of extracellular matrix components, including α -smooth muscle actin and collagen, to facilitate tumor invasion and angiogenesis [16]. With the transition from fibroblast to myofibroblast driven by transforming growth factor beta 1 (TGF β 1)-induced ROS-dependent expression of α -smooth muscle actin, data shows CONPs possess the ability to modulate myofibroblast formation [15]. Pre-treatment with CONPs mitigated both TGF β 1-induced α -smooth muscle actin expression in fibroblasts and the corre-

sponding myofibroblast transition [15]. As some myofibroblasts localize to the invasion front of tumors, CONP treatment diminished the ability of myofibroblasts to induce invasion by squamous tumor cells *in vitro* [15]. Interestingly, CONPs were also able to decrease the intrinsic ability of cultured squamous tumor cells to invade, even in the absence of any myofibroblast stimulation [15]. Taken together, these data demonstrate the direct negative effects of CONPs on cancer cells, as well as their ability to modulate the tumor environment and indirectly inhibit tumor cell invasion.

Radio-protection and radio-sensitization

The ability of CONPs to modulate ROS has led to their exploration for the improvement of a current cancer treatment: radiation therapy (RT). In addition to surgery and chemotherapy, RT remains a mainstay in the treatment of cancer, with nearly half of all cancer patients receiving RT at some point during treatment [17]. Many harmful side effects are associated with the RT, including fatigue, nausea, and dermatitis, yet few radiation adjuvants are available to mitigate these painful outcomes. For example, Amifostine, which remains the only clinically available radio-protectant [17], is itself associated with nausea and hypotension [18].

The dual capabilities of CONPs to act as an antioxidant in normal cells, yet oxidant in cancer cells, supports the role of CONPs as an adjuvant for RT that could significantly impact patient quality of life.

In line with the protection from other methods of inducing oxidative stress, several publications have shown that treatment with CONPs prior to RT exposure decreases the RT-induced cell damage and death in normal tissues of the gastrointestinal tract [19], lung [20], breast [21], and head and neck [22]. Mechanistically, CONP radical scavenging inhibited the resulting caspase 3 activation in irradiated colonic crypt tissue [19], as well as caspase 3 and 7 activation in irradiated lung fibroblasts in culture [20]. CONPs also increased super oxide dismutase 2 (SOD2) expression up to two-fold in a dose dependent manner in normal human colon cells *in vitro*, while increasing SOD2 expression by 40% in colonic crypt cells from mice treated with CONPs [19]. Together, these data indicate that CONPs protect normal cells both directly, by scavenging cellular ROS, and indirectly, by priming cells to respond to ROS insult.

Conversely, in cancer cells with acidic pH, pre-treatment with CONPs has been shown to enhance the ability of RT to induce cell death. As predicted [7], a most recent publication demonstrated the ability of CONPs to drive RT-induced ROS levels in pancreatic cancer cells [8]. As acidic pH has been shown to inhibit the catalase activity of CONPs [15], it is suggested that CONPs in cancer cells are only capable of catalyzing the conversion of highly unstable superoxide to far more stable H_2O_2 . Without the ability to act as a catalase mimetic and remove H_2O_2 , CONPs actually enhance the toxicity of RT in cancer cells by encouraging the accumulation and stability of ROS in the cell [8]. These effects resulted in the radio-sensitization of pancreatic cancer, significantly decreasing cell viability *in vitro* [8]. In a pancreatic tumor bearing mouse model that received the combination therapy of CONPs prior to RT, significant decreases in tumor weight and volume correlated with an increase in the number of apoptotic cells in the tumors [8]. Overall, these data demonstrate that CONPs modulate ROS in cancer cells such that, not only are there direct toxic effects, but the therapeutic properties of CONPs extend to radio-sensitization of

cancer cells and potentially sensitization to other ROS-inducing therapies.

CONP effect on other diseases

Beyond cancer, several other diseases are characterized by ROS accumulation. The incidence and progression of neurodegenerative diseases is often linked with the buildup of ROS, which becomes detrimental as it overwhelms cellular mechanisms to combat oxidative stress [13]. Increased ROS provides the primary link between the cause of the disease and the associated neurodegeneration in Huntington Disease, Parkinson Disease, Alzheimer Disease, and age related macular degeneration (AMD) [13]. While researchers have yet to truly explore the potential therapeutic benefits of CONPs in many of these diseases, CONPs have been shown to scavenge ROS in mouse models of hereditary retinal degeneration, preventing deterioration of retinal function, as well as apoptosis in photoreceptor cells [12]. In a specific AMD model, CONPs scavenged ROS to prevent the associated increase in vascular endothelial growth factor (VEGF) expression in photoreceptor cells, thereby preventing intra-retinal and sub-retinal neovascular lesions which lead to blindness [13]. Excitingly, CONP treatment was also able to induce the regression of pre-existing pathologic retinal neovasculature suggesting the anti-angiogenic function of CONPs [13], and has been suggested to be applicable for the treatment of other ocular disorders, such as diabetic retinopathy [12, 13].

In the context of diabetes, increased ROS, especially in the liver which is responsible for removing free radicals, is established to be an important factor in the development and progression of the disease [14]. CONP treatment of diabetic rats was able to return hepatic ROS levels to levels comparable to those in non-diabetic rats, as well as decrease plasma triglycerides and increase plasma HDL [14]. As many patients with insulin dependent diabetes require pancreatic islet transplantation, CONPs have also been tested for their ability to improve pancreatic islet function during isolation and transplantation procedures, during which oxidative stress is an issue [23]. Pre-treatment with CONPs was shown to significantly increase viability and insulin secretion, while decreasing ROS levels in isolated, cultured pancreatic islet

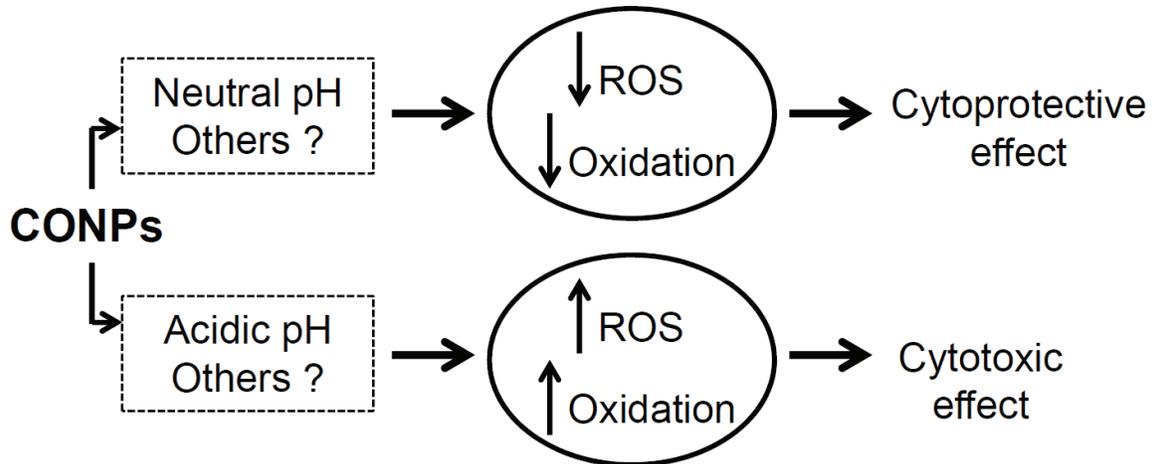


Figure 2. pH-dependent activity of CONPs as pro- or anti-oxidants. In a neutral pH environment, CONPs act as anti-oxidants that are cytoprotective. On the contrary, in an acidic pH environment, for example in cancer cells, CONPs function as pro-oxidants that are cytotoxic. Yet other factors likely exist that contribute to the switch between the pro- and anti-oxidant functions of CONPs.

cells [23]. Overall, CONPs show promise for addressing the various radical associated problems driving and resulting from diabetes.

Cellular uptake and localization of CONPs

CONPs have been shown to enter mammalian cells in both normal and diseased states [15, 24, 25], with significant uptake occurring within 3 hours of exposure in culture [26]. CONPs appear to take multiple routes into cells, as uptake has been suggested to occur via receptor-mediated endocytosis in both lung cancer and normal lung cell lines [27], while other studies have shown CONP uptake via clathrin-mediated and caveolae-mediated endocytotic pathways [26]. There is some debate about the fate of CONPs once inside the cell. Some studies show that CONPs accumulate in the cytoplasm without translocation to the nucleus [15, 24]. Other studies have demonstrated that CONPs accumulate primarily in the peri-nuclear space [28], while still others detected CONPs co-localized with the mitochondria, endoplasmic reticulum, lysosomes, as well as diffused throughout the cytoplasm and nucleus [26]. Particle size and surface charge appear to be determinants of CONP uptake and cellular localization [7]. As the differential pH of various sub-cellular localizations has been shown to be a determinant of CONPs' anti- or pro-oxidant activity [7], manipulation of CONPs to target specific cells or sub-cellular locations is a path

that has yet to be fully elucidated and exploited (Figure 2).

Biodistribution and biopersistence of CONPs

Several reports have shown CONPs (<10 nm) to be well tolerated by animals without inducing overt toxicity or an immune response across a range of doses [19, 20, 29]. When administered intravenously (i.v.) or intraperitoneally (i.p.), studies show that CONPs accumulate primarily in the spleen and liver, to a lesser extent in the lungs and kidneys, but not in the heart or brain [29, 30]. Tissues such as the breasts and pancreas have not been analyzed for retention, yet nearly half of the injected CONPs remained in undetermined locations within the body [30]. Further, CONPs were not readily cleared, persisting in the animals for at least 30 days without any appreciable CONP concentration in the urine or feces [29, 30], suggesting that other CONP destinations within the body have yet to be identified.

CONP toxicity

Despite the apparent lack of toxicity in animal models, reports provide conflicting data about the toxicity of CONPs *in vitro*, likely attributable to the impact of undetermined cellular and environmental factors on the manifestation of anti- or pro-oxidant behavior. CONPs are toxic to bronchial epithelial lung fibroblasts in culture [28], but non-toxic to mammary epithelial cells

[21], macrophages [31], immortalized keratinocytes [26], or immortalized pancreatic epithelial cells [8]. In normal cells to which they are not toxic, the physiological pH is an environment which enables canonical radical scavenging by CONPs. Therefore, CONPs introduced prior to ROS insult confer protection from the effects of oxidative stress *in vitro* and *in vivo* [13, 32-34].

However, published data indicates that CONPs are toxic to several types of human cancer cells *in vitro*, including squamous cell carcinoma [15], alveolar epithelial cancer cells [35], and pancreatic carcinomas [8]. Additionally, CONPs also display toxicity to pancreatic tumors *in vivo*, reducing tumor volume by almost 40% [8]. Cellular toxicity is attributed to the generation of ROS [8, 15] and the induction of oxidative stress [35], at least in part by the inherent oxidase activity of the nanoparticle core at acidic pH similar to that of cancer cells [6]. In particular, CONP treatment has been shown to induce glutathione oxidation, lipid peroxidation, and membrane damage in lung cancer cells [35]. Further experiments have demonstrated that generation of CONPs with a negative surface charge can induce preferential accumulation in acidic lysosomes within the cell, resulting in increased toxicity selectively in cancer cells [7].

Conclusions

Despite conflicting *in vitro* data regarding toxicity, *in vivo* data about the toxicity and application of CONPs for the treatment of numerous diseases remains overwhelmingly positive thus far. In contrast with currently available ROS modulators, which are characterized by a short half-life and usually require the introduction of one antioxidant molecule for each radical to be scavenged, CONPs persist in the body with a single particle scavenging many free radicals or inducing the oxidation of several targets through its auto-regenerative capacity [12]. With ROS and oxidative stress linked to so many conditions, the number of potential applications for CONP-based therapies appears countless. While current research into the therapeutic applications of CONPs leaves some questions unanswered, it provides a firm basis and evidence of a bright future for the pharmaceutical application of CONPs in cancer, diabetes, and other ROS-linked disorders that have yet to be pursued.

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