ToxEHS

Effects of Rare Earth Elements on the Environment and Human Health: A Literature Review

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Abstract

REEs are a group of metals comprised of yttrium, fourteen lanthanide elements, and scandium, which have been called 'industrial vitamins' and a 'treasury' of novel materials due to their dominant role in technical progress and in the development of traditional industries. Despite the growing interest, information that has become available over the last two decades regarding RREs is relatively premature and scarce, which has led to the current controversy regarding the health benefits *vs* toxic effects of these materials. There are many environmental and health issues associated the production, processing, and utilization of REEs. This review offers an examination of the roles of REEs in the onset of cellular oxidative stress in reference to the impact of REE exposure to cells, animals, and plants, in order to explain disease and occupational poisoning of local residents, water pollution, and farmland destruction. Conversely, a body of evidence has shown REE-associated antioxidant effects in the treatment of many diseases. The content herein is aimed at presenting the recent and pending developments in the field of REE with respect to environmental and human health implications. Multi-faceted updates on the roles of REEs focusing on different organisms and exposure routes, and several issues regarding environmental and biological research, are discussed. The current gaps in information raise a number of open questions that deserve *ad hoc* investigation.

Keywords: Rare earth element, Environment, Health, Review

Introduction

Rare earth elements (REEs) have been the subject of a limited number of books and technical reports since the 1980's to date, with a major or exclusive focus on REE-related chemistry, mineralogy, economy, and the development of technological applications using these elements[1-6]. Recent research achievements regarding REE-associated health effects are reported in the present review[4,5].

REEs are a group of metals comprised of yttrium, fourteen lanthanide elements, and scandium, which have been called 'industrial vitamins' and a 'treasury' of novel materials due to their dominant role in technical progress and in the development of traditional industries[7,8]. Despite the growing interest raised by REEs, the information that has become available over the last two decades regarding RREs is relatively premature and scarce (the attention seems to be focused only on a restricted number of REEs such as Ce, La, Gd, Nd, and Y), which has led to the current controversy regarding the health benefits *vs* toxic effects of these materials. There are many environmental issues associated with the production, processing, and utilization of REEs.

REEs, as members of the *f*-block in the periodic table, benefit from a series of unique physical and chemical properties that make them invaluable to a number of critical technologies. One of the main industrial uses for REEs, involving millions of tons of raw material each year, is in the production of catalysts for the cracking of crude petroleum. Yet, the widespread involvement of REEs in industry is demonstrated by their use in the production of strong permanent magnets for electromechanical devices, for screen displays, glass, and lenses, for laser technology, and in solid state microwave devices (for radar and communications systems), gas mantles, and the ceramic, photographic, and textile industries, as well as in medical x-ray and magnetic resonance image scanning systems[9-11].

Nevertheless, there are many environmental issues associated with the mining, isolation, recovery, and recycling of REEs. A few reports indicate that the chemicals used in the refining process have been involved in REE bioaccumulation and pathological changes in local residents. Given the recent toxicology results on

REE exposure, it is of utmost importance to elucidate the mechanisms of REE-associated damage. An established mechanism of REE-associated effects relates to the induction of oxidative stress as a result of the high redox potential exhibited by REE^{3+}/REE^{2+} ions. The redox behavior of REEs is also influenced by different factors including pH and oxic/anoxic conditions, making REEs in some cases process tracers in a variety of natural waters such as fresh groundwater, lakes, rivers, estuaries, oceans, and sediments. The present review offers an evaluation of the roles of REEs in the onset of cellular oxidative stress in reference to the impact of REE exposure to cells, animals, and plants, on the level of some of the most common enzymatic and non-enzymatic oxidative stress markers.

A body of evidence has reported that the chemicals used in the refining process of REEs are involved in disease and occupational poisoning of local residents, water pollution, and farmland destruction[12,13]. Occupational and public safety and health risks related to REEs may be addressed at several stages, such as mining and refining, transportation, processing, waste disposal, and decommissioning. The multiple contaminants (including radionuclides and heavy metals) cause negative effects on aquatic and terrestrial organisms, as well as on humans. Conversely, other evidence has shown REE-associated antioxidant effects in the treatment of many diseases[14].

Thus, it is recognized that REE-associated health effects have constituted a thriving area of research in recent years, though confined to journals based on individual laboratory studies, and with a limited number of review papers[15-17]. This review is aimed at presenting the recent and pending developments in the field of REE environmental and human health implications.

Results

Industry and Occupational Health

The majority of global REE ore extraction and refining takes place in China[2,3,5], and these activities constitute the majority of REE environmental pollution at mining sites and in the surrounding area. This environmental impact of REE ore mining is associated with bioaccumulation in residents at varying distances from mining sites[18,19]. Further implications of REE extraction and refining activities as relevant environmental issues arise from the use of strong acids at several stages of ore processing[5], with the consequent release of acidic effluents affecting downstream water bodies. Thus, the limited evidence for the combined toxicities of REEs and a decrease in pH[20-22], along with the long-established notion of multi-fold acid toxicity, raise substantial concern regarding the environmental impact downstream of mining sites and refining facilities. The current gap in information on this subject warrants field investigation and *ad hoc* experimental studies.

In addition to mining and refining activities, worldwide REE manufacturing activity also raises environmental concern relating to REE-polluted wastewater with consequent bioaccumulation, however, the effects on aquatic biota have not yet been thoroughly investigated[23,24]. The most widespread source of REE-related air and soil pollution is the global use of cerium oxide nanoparticles ($nCeO_2$) as a catalytic additive in diesel fuel. The limited available literature indicates $nCeO_2$ as a component of diesel exhaust particulate matter[25-28], prompting investigations into the possible health implications of diesel exhaust particulate matter following occupational and environmental exposure.

A few reports regarding occupational REE exposure have shown adverse health effects to the respiratory tract, along with REE bioaccumulation[29]. To the best of our present knowledge, this limited body of literature dates from 1982 to 2005, and most invariably consists of case reports[16]. Therefore, the major gap in knowledge with respect to the possible long-term effects of occupational REE exposure is due to the lack of current epidemiological studies, which represents an outstanding research priority in industrial medicine. A last, and very relevant, adverse effect of REEs has been appraised following the observation of a severe skin condition, nephrogenic systemic fibrosis, related to the use of gadolinium (Gd) as a contrast agent in magnetic resonance imaging[30,31]. Adverse effects of Gd-based contrast agents are regarded as a potential concern in dialysis patients undergoing magnetic resonance imaging[30].

Environmental Effects

REEs have been broadly neglected as xenobiotics until recent years, despite the unprecedented boost in their use in technological applications over the last two decades. Investigations into REE-associated health effects have been thriving in recent years, which include experimental and bioaccumulation studies involving a number of endpoints evaluated in cell, animal, and plant models. This growing database of REE toxicity has been reviewed recently[15-17]. A number of animal-specific damage such as organ and system effects, and plant-specific damage such as growth inhibition and decreased chlorophyll production, have been reported. A more general outcome of several toxicity studies consisted of redox imbalances induced by a number of REEs in cell systems, animals, and plants.

The application of REEs as feed additives for live-

stock and in crop improvement has long been practiced in China, and relevant results have been reported in the Chinese literature. Where applicable, these beneficial effects included an increase in body weight in cattle, pigs, chicken, fish and rabbits, as well as an increase in milk production in dairy cows and egg production in laying hens[32-34]. However, other studies have extensively investigated REE bioaccumulation and adverse effects on plant growth[35] and algae.

Further suggestions for REE-associated stimulating effects have been provided by several studies conducted in mammalian cells, algae, and microorganisms[36-39]. These reports suggest a role for low-level REEs in substituting essential elements[36], and suggest the novel concept that REEs may represent essential elements for some biota[39]. It should be noted that there are drugs and other commercial products already on the market that use the physicochemical characteristics of REEs to produce health or environmental benefits.

The recognized environmental disasters in agricultural areas and downstream waters of REE mining areas have been, and are likely to be, environmental emergencies in the regions affected by REE ore extraction and refining. The concomitant role of acidic pollutants should be highlighted, leading to the enhanced toxicity of inorganic chemical species, as is well-established for several cations and dissolved REEs. Beyond the extreme cases at REE mining areas, REE accumulation in marine coastal sediments and biota can occur downstream of REE processing facilities, and has been detected as shown in a few studies that are overdue forthcoming investigations. As "novel" sediment pollutants, REEs may play a role the impairment of sensitive ecosystems, thus, the determination of REE levels in sediments and benthic biota is warranted.

Favorable and Adverse Effects of REEs

Limited REE-related studies indicate antioxidant and protective effects, raising possible controversy regarding the actual roles played by REEs. Cerium nanoparticles (CeNPs) have been shown to inhibit ROS production in a human ovarian carcinoma cell line (A2780), attenuate the action of growth factors, and mediate cell migration and invasion of another human ovarian carcinoma cell line (SKOV3) without affecting cell proliferation, suggesting the use of CeNPs as novel anti-angiogenic therapeutic agents in ovarian cancer[14]. Cerium chloride has also been shown to possess antitumor effects by inhibiting the proliferation of gastric cancer and leukemia cells[49]. The role of redox mechanisms in the biological effects of REEs has been discussed for a limited group of elements, namely Y, La, Ce, Nd, Gd, Tb, and Yb, in terms of reactive oxygen species (ROS) formation, lipid peroxidation, and activity modulation of the most relevant oxidative stress-related enzymes (SOD, CAT, glutathione peroxidase (GPx)) (Table 1).

Table 1. REE-induced effects on oxidative stress endpoints

REE	Change in oxidative stress parameters	Reference
Yttrium	↑ SOD activity	40
Lanthanum	↓ SOD and CAT activity ↑ GPX activity ↑ GSH, malondialdehyde, ROS levels, and mitochondrial dysfunction ↓ Antioxidant capacity	41,42
Cerium	↑ ROS levels, lipid peroxidation, pro-inflammatory cytokines, and cyclooxygenase-2 ↓ Antioxidant capacity, SOD, and CAT activities	43-45
Neodymium	↓ Antioxidant capacity, SOD, and CAT activities ↑ GPX activity, GSH, and lipid peroxidation	41,44,45
Gadolinium	↑ Ferritin, transferrin oversaturation, lipid peroxidation, and ROS levels	46,47
Terbium	↑ Lipid peroxidation ↓ SOD, CAT, and GPx activities	48

Animal Studies

Several toxicity tests on REEs, mostly Ce, La, and Gd, have been conducted in animals, with the majority being mammals. The current database includes a number of toxicity endpoints in the liver, lungs, blood, and nervous system, following several administration routes and life stages (adult and fetal). The adverse effects at the cellular, organ, or system level were found to be associated with pro-oxidant states, including the modulation of antioxidant activities and oxidative damage. Two limitations of this database include: 1) the relatively low number of studies focusing on REEs other than Ce, La, and Gd, and 2) the relatively low number of studies conducted on other vertebrates or invertebrates. The most severe limitation of the current database is the lack of long-term REE exposure studies with life-long observations, which would allow verification of the likely effects in terms of lifespan, late onset of chronic diseases, and mortality. These as yet unexplored studies will provide essential and predictive information regarding the effects on human health[17].

Human Exposure

Limited information is available on occupational REE

exposure, and the references that are available are confined to case reports of individual workers affected by respiratory tract pathologies (mainly pneumoconiosis), with analytical evidence of REE bioaccumulation. Occupational REE exposure ranges from ore mining and refining to end users in the workforce of an extensive number of industrial applications. Thus, the global number of REE-exposed workers amounts to huge numbers, at least in the order of hundreds of thousands. To the best of our present knowledge, no epidemiological studies have been performed to date among REE-exposed workers, either in mining and refining activities or in the cascade of technological activities[15]. A major problem in view of planning epidemiological studies relates to the occurrence of other chemical and/or physical agents in the working environments. As two major examples, one should recall the occurrence of radioactive agents (uranium and thorium) in REE ores and, in diesel exhaust fumes, the occurrence of fuel combustion carbon particulates in addition to nanoceria particulates[17].

Nevertheless, this foreseeable bias due to confounding factors as concomitant exposure to other xenobiotics may be overcome by appropriate study design. REE-exposed groups must be identified in the workforce of intermediate or end-use facilities, which should be first characterized by means of REE dust levels in the air, followed by bioaccumulation endpoints through non-invasive sampling of peripheral blood and/or urine and radiological investigations. Once homogeneous groups have been characterized by exposure and bioaccumulation, classical epidemiological studies such as cohort or case-control studies should be subsequently planned and implemented. Based on the currently available data on occupational REE exposure, it is realistic to foresee that appropriate epidemiological studies will provide valuable information to fill the current gaps in information regarding the potential REE-associated effects on human health, especially in relation to respiratory pathologies[15].

Another research priority should be recognized in terms of evaluating and quantifying the health risks following environmental REE exposure, by extending the present information on resident populations in mining areas. Moreover, a working hypothesis may explore whether, and to what extent, other environmental REE exposure may occur. This may be the case, for instance, in urban areas with heavy exhaust release, with respect to the animal studies that indicate adverse effects of nanoceria in diesel engine exhaust fumes.

ROS-Associated Effects

It is recognized that redox imbalance is a relevant feature of REE-associated toxicity, as indicated by findings of excess ROS and nitrite formation, along with cytogenetic damage and transmissible damage from REE-exposed sperm to their offspring[50,51]. Beyond the database of REE-associated adverse effects, it should be noted however, that beneficial antioxidant mechanisms have also been reported in the scope of REE-associated effects.

The available literature on REE-associated toxicity is confined to a few REEs, mostly Ce, La, and Gd, requiring investigation into the comparative toxicities of other, as yet neglected, REEs. Animal studies are limited to short- to medium-term observation (mostly 1-3 months)[16], thus, studies of long-term REE exposure and life-long observations are currently lacking.

By analyzing the time trend of a number of publications on REEs, the growing interest of the scientific community regarding the mechanisms associated with REE-toxicity appears evident, and reflects the relevance of these elements in many industrial, agricultural, and medical technologies. This is the reason why human exposure to REEs is very high, and is essentially dependent on: therapeutic treatments involving REEs (iatrogenic exposure); REE accumulation (bioaccumulation and pollution-induced) in marine and freshwater, air, and soil, especially for individuals residing close to mining areas (environmental exposure); and REE exposure of specialized workers (occupational exposure)[52].

On the one hand, there is a high demand for REEs in several applications in the technological field, and on the other hand, there has been such poor information to date regarding their effects on human health and the environment. This situation has led to an increase in controversy between the favorable and adverse health effects of REEs, which still remains unsolved. The hormetic concentration-depending behavior of REEs has recently gained particular attention, since this may indicate that they induce a protective effect at low levels and a toxic effect at higher levels[53,54]. Two recent reviews[52,55] provide state of the art REE adverse health effects and toxicity mechanisms, discussing the different factors influencing the relationships between REEs and their biological targets.

The controversial body of evidence pointing to REE-associated pro-oxidant and toxic effects, as opposed to antioxidant and beneficial effects, should be disentangled with respect to both theoretical and applicative purposes. The most realistic interpretation of the oxidant/antioxidant dilemma resides in the recognized hormesis phenomenon. Far from being novel or specific to REEs, a concentration (or dose)-related trend from stimulation to inhibition has been well-documented in an extensive series of chemical and physi-

cal agents. A major challenge in verifying hormetic effects is the choice of suitably extensive concentration intervals. This study design foresees cumbersome workloads that may discourage several researchers. Nevertheless, elucidating a shift from stimulation to inhibition should be viewed as the prime goal in both the evaluation of REE-related mechanisms of action and in the definition of the borders between adverse and favorable health effects. This research strategy encompasses a number of applicative issues[15].

The recognized use of nanoceria and other REE nanoparticles for therapeutic purposes deserves extensive research efforts regarding the prospect of novel medical applications of REE nanoparticles. Another field of REE application relates to the possible use of REE-based stimulation in crop yield and animal husbandry. To date, these agronomical and zootechnical REE additives are known to be confined to China, however, it can be speculated that REEs are present in certain Chinese food exports. It may be daring to envision the possible extension of this practice at a global scale, provided that substantial and undisputed evidence were obtained, confirming benefits and ruling out any undesired effects on foodstuffs, agricultural soil, animal excreta, and/or wastewater. A relevant and as yet broadly unexplored subject may be the potential role of REEs in microorganisms as novel essential elements, and/or as protective factors *vs*. other environmental stressors. Despite the currently scarce database, this subject deserves a line of *ad hoc* investigation. In turn, the possible outcomes of these studies could shed light on REE-associated mechanisms of action extended to nutrient bioavailability and plant physiology[33].

REE Nanoparticles (NPs)

Since the early 2000's, biologists have expanded the use of nanomaterials from industrial applications to biological research. Nanomaterials have unique functions different from their bulk forms due to their minute size, measuring in 10^{-9} meters or nanometers. The dramatic increase in the ratio of surface molecules in nanomaterials is postulated to be the cause of the increase in reactivity of these nanoparticles[56,57]. Cerium oxide nanoparticles (CeNPs or nanoceria) belong to the redox-active class of nanomaterials. Other members include yttrium oxide nanoparticles and fullerene nanoparticles[58,59]. CeNPs are unique nanomaterials since they possess catalytic radical scavenging activities mimicking the two endogenous anti-oxidative enzymes, superoxide dismutase (SOD)[60] and catalase[61,62], which are ubiquitous in order to scavenge superoxide anions and hydrogen peroxide, respectively. Unlike dietary antioxidants, the redox capacity of CeNPs is greatly expanded due to their auto-catalytic properties[59,61].

Since the progression of many diseases such as neurodegeneration (Alzheimer's, Parkinson's, Amyotrophic Lateral Sclerosis), blinding (age-related macular degeneration (AMD), glaucoma, diabetic retinopathy, inherited retinal degeneration[63]), tumor growth, and aging, is tightly associated with oxidative stress and damage[64], biological researchers have turned to CeNPs as potential therapeutics for the treatment of these intractable diseases[64,65]. The rationale is that excess oxidative stress causes cells to malfunction, senesce, and eventually undergo apoptosis[66], therefore, lowering oxidative stress by the administration of antioxidants should lower oxidative damage and prolong the functional lifespan of cells, leading to a delay in disease progression. In addition to being potent antioxidants, CeNPs are unique, since the beneficial effects are observed for a few weeks to months following a single intravitreal application in animal models of retinal degeneration[63]. The fact that daily dosing is not necessary, is a huge advantage from a treatment perspective. With respect to the environmental and human toxic effects of cerium, it is necessary to be mindful that the properties of nanoparticles are unique and different from the ionic form(s) of the same metal in bulk.

Biological Effects of Nanoceria-Antioxidant *vs* Oxidative Stress

Cell culture systems are the most cost-effective method by which to assess toxic or beneficial effects of engineered CeNPs prior to testing in animal models. Ideally, non-cancer cell lines should be used to assess cytotoxic effects, since these cells should mimic the behavior of normal healthy cells. In general, cells are incubated for 24 to 72 hours with nanoceria at a range of concentrations, and then assayed for viability[67]. To test for protective effects against oxidative stress, cells are treated with specific oxidants or chemicals to induce oxidative stress, and then assayed for viability after a specified period of time. CeNPs are added at the same time as the oxidant or at an earlier or later time point. A popular method for measuring the levels of intracellular reactive oxygen species (ROS) is to load cells with 2′,7′-dichlorodihydrofluorescein diacetate (DCF-DA), which, upon entering cells and being oxidized by ROS, is converted to the fluorescent molecule DCF[68], and can be detected by flow cytometry or spectrophotometry. Understandably, our goal is to develop effective engineered CeNPs as therapeutic agents to treat diseases in which progression is tightly associated with oxidative stress.

Considering the three major biological effects of CeNPs, oxidative, antioxidative, and oxygen buffering, it could be hypothesized that the differential effects of CeNPs in cancer *vs* non-cancer cells may be

due to the fact that cancer cells are more sensitive to DNA damage (the genotoxicity effect of CeNPs as discussed above[69]), since cancer cells have a high intrinsic load of mutation and chromosomal abnormality. Any additional DNA damage would tip the balance and ultimately lead to cell death (apoptosis). In healthy cells, this mild damage is repaired, and thus allows the cells to survive and function normally.

Catalytic Activity of Nanoceria in Biological Tissues

Currently, tools are not readily available to assess the catalytic activity or pharmacodynamics of CeNPs once they are inside cells or in tissues/organs. It is assumed that the activities demonstrated in cell-free suspensions are active after uptake of CeNPs by cells in cell culture or in tissues/organs of animal models. The cellular effects of CeNPs are likely to involve signaling pathways that may be triggered by their antioxidative, oxidative, or oxygen buffering activities.

An attempt to address the length of time that CeNPs remain active once inside cells has been illustrated by Das and coworkers[70]. Firstly, it was established that the engineered CeNPs promoted viability of primary adult rat spinal cord neurons for up to 30 days in culture following a single application at a concentration of 0.0016 μg/mL or 0.01 μM on day 1 of culture. Secondly, the aim was to elucidate whether the neuroprotective effect of the CeNPs was still present 30 days following administration. The neurons were challenged with 100 mM hydrogen peroxide for 1 hour, after which the number of viable cells was measured. It was found that CeNP-treated neurons had a survival rate of 18.5% compared with 8.6% in the untreated control. The results of this study suggest that the neuroprotective effect of CeNPs persisted for at least 30 days in at least a small proportion of the treated cells.

With the use of a P23H-1 rat model, one of the most commonly used autosomal dominant RP models, the *in vivo* catalytic activity of CeNPs was assessed[63]. It was reasoned that rod photoreceptor (RPr) cells in degenerating retinas follow universal cell death (apoptosis) to be eliminated. Thus, the catalytic activity of CeNPs in the retina was indirectly determined by measuring the number of TUNEL+ cells (cells in the degradation phase of apoptosis) at intervals that are longer than the time required for clearing of TUNEL+ cells. In this manner, a snapshot of the health status of the retina was taken. Following CeNP (344 ng) delivery to the vitreous humor of P15 animals, the RPr cell death index (the number of TUNEL+ cells in the outer nuclear layer (ONL) of the retina) was determined on day 3, 7, 14, and 21 post-injection. A reduction in TUNEL+ cells by 46%, 54%, 21%, and 24%, respectively, was observed compared with the saline-injected control. From these results, it was concluded that CeNPs achieved maximal activity between day 3 and 7 post-injection, and the activity declined from day 14 to 21 post-injection in this autosomal dominant retinitis pigmentosa rodent model. As noted above, enhanced RPr cell function could be detected up to 35 days post-injection. Taken together, these results suggest that the net cumulative effects observed could be used as a gross estimate of the catalytic activity of CeNPs *in vivo*.

Since the oxidation state ($Ce^{3+}/^{4+}$ ratio) of CeNPs influences their redox activity, Szymanski and colleagues[71] decided to investigate whether the oxidation state of CeNPs changed with respect to their presence in different subcellular compartments. The oxidation states of CeNPs outside cells and inside specific subcellular compartments were measured by combining scanning transmission x-ray and super resolution fluorescence microscopy methods. A net reduction in CeNPs (higher $Ce^{3+}/^{4+}$) was detected following uptake into the cytoplasm from outside the cells. A similar oxidation ratio for CeNPs in the cytosol and in lysosomes was also found, thus, it was concluded that reduction must have taken place earlier in the internalization process. This study provides the first direct measurement of the oxidation state dynamics of CeNPs from the outside to the inside of cells and in different subcellular compartments.

Since the goal is to develop safe and effective CeNPs for therapeutic use, the future of nanomedicine must include imaging techniques to localize CeNPs in tissues, and to identify the oxidation states in different subcellular compartments. The use of Raman spectroscopy appears to be able to fill this gap in the near future[72,73].

Discussion

Evidence points to beneficial or safe effects of REEs, which have been found to exert antioxidative and neuroprotective actions[74-77]. The use of cerium oxide nanoparticles ($nCeO_2$) as antioxidants in biological systems has shown a protective effect by reducing oxidative stress in cell culture and animal disease models associated with oxidative stress. Ophthalmic therapeutics using $nCeO_2$ has been reported to slow the progression of retinal degeneration along with anti-angiogenic agents in rodent models. The authors suggest that the radical scavenging activity of $nCeO_2$ is mainly due to the increase in surface area to volume ratio of these nanocrystalline structures[77]. Another study reported that cerium or yttrium oxide nanoparticles protected nerve cells from oxidative stress, and that the neuroprotection

was independent of particle size[76].

Taken together, it can be recognized that antioxidant and potentially beneficial effects of REE nanoparticles have been shown with potential for use in therapeutic applications. This promising body of evidence awaits further investigation aimed at elucidating the mechanisms of action and validating this approach. The controversial literature referring to REE-associated toxicity and stimulatory action, also termed "dual effects"[5], is not novel. Since the earliest report by Hugo Schulz in 1888[78], a redoubtable body of evidence has supported the hormesis concept[79,80], implying that low levels of chemical or physical agents induce stimulatory effects in a broad number of biological endpoints, which are then inhibited by increasing the levels of the agent. Understanding the different, complementary or opposite, actions of dissolved species *vs.* nanoparticles, and the roles of nanoparticle size and geometry and of ligands, will allow forthcoming studies to evaluate and/or predict the biological actions of REEs with respect to the environment and human health.

Mechanism of Oxidative Stress

Biological researchers are fervently validating the beneficial effects of redox-active cerium oxide nanoparticles (CeNPs) in tissue culture and animal disease models. The benefits on the reduction of oxidative stress and the prolongation of function and/or cell/tissue health are undeniable. On the contrary, environmental/occupational toxicologists are diligently gathering evidence on the adverse health effects due to exposure of CeNPs by different routes of entry[17]. The negative health effects from CeNP exposure are equally indisputable. The present review focused on the biological effects and mechanisms of CeNPs that are intended specifically for biological applications. A brief discussion on the methodology of synthesis and characterization parameters for the well-defined nanomaterials was provided in order to lay a framework on which meaningful comparisons of different engineered CeNPs and their specific effects could be made. The catalytic activities of CeNPs that are currently known were highlighted, and examples of positive and negative biological effects and their proposed mechanisms were discussed. Since the radical scavenging activity of CeNPs has been shown to be self-regenerating in cell-free suspensions, studies that attempt to assess the catalytic activity of CeNPs were also discussed, as well as whether CeNPs act as direct antioxidants/oxidants in biological environments.

Molecular Mechanisms of Nanoceria

CeNPs are effective at extremely low concentrations (as low as 10-20 ng/mL), and the effective range appears to follow a biphasic or hormetic pharmacological response, such that positive effects are observed at low concentrations, and neutral or negative effects at high concentrations. Additionally, the effect requires a latent period of at least 1 to 2 hours following interaction of CeNPs with the biological system. These observations indicate that the action of CeNPs inside cells is the manifestation of signaling pathways triggered by CeNPs, and therefore will depend on the health status of the cell and the specific cell type used for assessment.

Different studies have demonstrated up- or downregulation of a number of genes that are involved in signaling pathways related to oxidative stress. Cai and coworkers[81] showed that the anti-angiogenic effect shown by CeO_2 NPs in the retinas of The very low-density lipoprotein receptor (*vldlr*) knockout mice correlated with downregulation of the ASK1-P38/JNK-NF-kB signaling pathway. It was hypothesized that reduction in the levels of cellular ROS by CNP1 was likely the cause.

In another study, von Montfort and coworkers[82] showed that the reduction in oxidative stress by dextran-coated CeNPs (25.8 μg/mL) in human dermal fibroblasts was not due to the increase in endogenous GSH or cellular GSH-peroxidase expression levels, as was the case for Na selenite-treated cells. It was hypothesized that the CeNPs acted as direct antioxidants, even though the effects were observed after 24 hours of CeNP incubation.

Park and coworkers[83] showed that CeNPs (40 μg/mL) generated by the heated solvent method caused oxidative stress in BEAS-2B (normal lung epithelial) cells. The CeNPs caused upregulation of oxidative stress-related genes including catalase, glutathione S-transferase, heme oxygenase-1, and thioredoxin reductase, after four hours of incubation. In spite of the upregulation of these genes, the GSH level was reduced by 25% following 24 hours of CeNP incubation.

To further delve into the molecular mechanisms of action of CeNPs, a systematic approach was warranted. Lee and coworkers[84] performed a gene expression profiling study of the mouse hippocampal neuronal cell line, HT22, regarding the effects of nanoparticle size and chemical composition. It was shown that the 6 nm HMT-coated CeNPs showed the highest number of uniquely expressed genes among the three groups of nanoparticle-treated cells following 8 hours of incubation (230 vs 26, and 20). Using the Ingenuity Pathway Analysis (IPA, Qiagen) software, the authors explored the relationships among these 230 differentially expressed genes. The major pathways that were postulated to be affected were 1) inhibition of G1/S transition, 2) induction of apoptosis, and 3) growth inhibition. From these results, it could be speculated

that the engineered CeNPs at a concentration of 20 μg/mL induced oxidative damage, most likely in the form of DNA damage, to disrupt DNA replication and initiate apoptosis in HT22 cells. It will be interesting to elucidate whether at much lower concentrations, such as 0.02 μg/mL, a similar or different set of genes will be detected.

Another study by Ciofani and coworkers[85] interrogated 84 genes using the Rat Oxidative Stress RT2 Profiler PCR array from Qiagen with respect to their expression in PC12 cells (a model mimicking dopaminergic neurons) upon incubation with CeNPs purchased from Sigma (code 544841). Previously, it had been shown that these 5-80 nm CeNPs had $Ce^{3+/4+}$ at roughly 23%, and were not toxic to PC12 cells at concentrations of 10 to 100 μg/mL for 72 hours. At 20 and 50 μg/mL, the CeNPs promoted neuronal differentiation and dopamine production in these cells[86]. In the gene expression study, the gene expression level was measured following 72 hours of incubation at 20 and 50 μg/mL. The results of the low and high concentrations of CeNPs treatment were relatively similar. The authors showed that the differentially expressed gene pattern was challenging to interpret due to the inconsistency of the observed pattern. To explain their observations, the differentially expressed genes were divided into three categories: 1) genes related to antioxidant defense, 2) genes involved in the metabolism of ROS, and 3) genes responsible for oxygen transport. It was found that genes in the first group (such as members of the glutathione peroxidase family) were mostly downregulated. However, in the second group, a mixed pattern of up- and downregulated genes was observed. More notably, Hspa1a (heat shock 70kD protein 1A), Ncf1 (p47phox, neutrophil cytosolic factor 1), and Sod3 (superoxide dismutase 3, extracellular) were upregulated. Finally, it was shown that Cygb (Cytoglobin), a member of the oxygen transport group, was upregulated. Since Cygb expression is regulated by the HIF pathway, CeNPs may induce mild hypoxic conditions in these cells. This is an exciting hypothesis since hypoxia is known to promote neurogenesis and neuronal differentiation[87], as well as angiogenesis.

It is evident that the technological advantages of REE utilization are well understood. As usually happens in these cases, the benefits gained from REE applications have partially shaded and slowed down the control processes aimed at checking for possible side effects of REEs. The lack of information is the main reason for the rise in the current controversy between stimulatory and inhibitory REE-associated health effects. This controversy is made more evident by a series of factors influencing the properties and chemical reactivity of REEs. The still-limited body of evidence available on REEs cannot fill this gap, but may represent part of a more complex picture that will be delineated in the future[15].

The elucidation of REE speciation is essential to understanding their effects on health. As mentioned above, the role of pH is well established in causing more reactive species in acidic compared with neutral wastewater. Another relevant issue in modulating REE toxicity relates to the comparative bioavailability of nanoparticles of different sizes and geometry *vs*. dissolved species. Research interventions relating to the effect of REE speciation on health will be of paramount relevance in future investigations.

Conclusions

This condensed review of the biological applications of CeNPs provides a framework to be able to continue to stitch pieces of fabric to this unfinished multilayered and multicolored quilt. As is apparent, the area covering the molecular mechanisms of CeNPs in different cell types is still quite sparse. However, there is exciting evidence that CeNPs elicit a brief and mild hypoxic environment inside cells[88]. Das and coworkers showed that the reduction in oxygen level was brief, from 30 to 60 minutes post CeNPs incubation in human umbilical vein-derived endothelial cells (HUVEC). By 2 hours post-incubation, the oxygen level had returned to the pre-incubation level. The data indicates that this brief hypoxia triggered cascading events for the stabilization of HIF1a and the upregulation of VEGF, and subsequent tube formation. Studies by Ciofani and coworkers[85,86] suggest that CeNPs may promote the differentiation of PC12 cells via the HIF signaling pathway. Taken together, if these observations are extrapolated to other cell types, many of the beneficial effects documented by various research groups can be explained. Hypoxia is a well-documented cell stressor that can stimulate an adaptive cellular stress response[89]. Systemically, ischemic pre-conditioning is also considered a beneficial phenomenon[90]. Together with the pre-conditioning effects of CNP1 discovered in the rat retina, there is a strong motivation to understand the molecular mechanisms underlying these transient and possibly periodic hypoxic conditions induced by CeNPs in biological systems.

This review has provided multi-faceted updates on the roles of REEs, focusing on different organisms and exposure routes, and raising several issues in environmental and biological research. The current gaps in

information raise a number of open questions that deserve *ad hoc* investigations.

Methods

In this extensive literature review, relevant articles in the field of rare earth elements including hazards evaluation, environmental as well as *in vitro* and *in vivo* toxicology studies, occupational hygiene, and epidemiology, were found using PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), Google Scholar (http://scholar.google.com), and ScienceDirect (www.sciencedirect.com). Keywords were used to search relevant articles, and the following is an example of a typical search: rare earth AND hazard OR toxicology AND environment OR worker OR occupation AND health.

These searches yielded more than 100 articles, which were further reviewed for health hazard or environmental factors. At the end of this review, 90 articles were considered relevant to this study, and they were inspected with particular focus on two topics: environment and human health upon exposure. The prospects for studies that depend on many toxicology tests and the significance of preventive measures are also discussed.

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