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Neuroprotective Potential of Cerium Oxide Nanoparticles for Focal Cerebral Ischemic Stroke

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Summary: During the previous years, with the emerging of nanotechnology, the enormous capabilities of nanoparticles have drawn great attention from researchers in terms of their potentials in various aspects of pharmacology. Cerium oxide nanoparticles (nanoceria), considered as one of the most widely used nanomaterials, due to its tempting catalytic antioxidant properties, show a promising potential in diverse disorders, such as cerebral ischemic stroke (CIS), cancer, neurodegenerative and inflammatory diseases. Overwhelming generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) during cerebral ischemia and reperfusion periods is known to aggravate brain damage via sophisticated cellular and molecular mechanisms, and therefore exploration of the antioxidant capacities of nanoceria becomes a new approach in reducing cerebral ischemic injury. Furthermore, utilizing nanoceria as a drug carrier might display the propensity to overcome limitations or inefficacy of other conceivable neuroprotectants and exhibit synergistic effects. In this review, we emphasize on the principle features of nanoceria and current researches concerning nanoceria as a potential therapeutic agent or carrier in improving the prognosis of CIS.
Key words: Cerium oxide nanoparticles; nanotechnology; reactive oxygen species; cerebral ischemic stroke

Cerebral ischemic stroke (CIS) is considered as one of the leading causes of severe disability, or even death in the world. According to the latest statistics, it ranks the fourth most common cause of death in the United States[1]. The high incidence of death and disability among CIS patients poses a significant burden on individual patients, their families and society as a whole. Under conditions of ischemia or reperfusion, the overwhelming production of free radicals often leads to a condition known as oxidative stress, which increases the susceptibility of cerebral tissues to further injury.

Although cerium oxide nanoparticles (nanoceria) have already been widely used in various fields, including solar cells, oxygen pumps and gas sensors[2], their applications on biomedical aspects still await more research. Nanoceria can reversibly bind oxygen and switch between Ce^{3+} (reduced) and Ce^{4+} (oxidized) states at surface of particles depending on external stimuli, thus producing a redox couple that exhibits catalytic antioxidant functions[2, 3]. A large number of diseases have also been demonstrated to be associated with dysregulation of reactive oxygen species (ROS) levels. Recent studies have implicated that naoceria might play an effective role in anti-cancer[4, 5], anti-inflammation[6], neuro-protection[7–9], cardio-protection[10] therapies. Therefore, nanoceria have drawn increasing attention from researchers throughout the world as a promising antioxidant agent in many medical fields. Herein we mainly summarized potential investigations and future prospects of nacoceria in the protection against CIS.

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1 ISCHEMIC STROKE AND ROLES OF OXIDATIVE STRESS

1.1 Ischemic Stroke

Stroke is one of the biggest killers in the world and accounts for nearly half of all hospitalized patients in acute neurological cases. More than 80% of all strokes are ischemic in origin. Presently, only recombinant tissue plasminogen activator (rt-PA) has been approved by the Food and Drug Administration (FDA) in US as the first option in the clinical practice of acute CIS. The main mechanism of rt-PA in treating acute CIS is its capability of converting plasminogen to plasmin, thus breaking down or dissolving blood clots so as to re-establish blood flow reperfusion and oxygen supply in the ischemic cerebral tissues, especially in penumbra areas. The new protocol guideline suggests intravenous administration of rt-PA be accomplished within 4.5 h from onset of symptoms[11]. However, in real clinical settings, only around one-quarter of patients with ischemic stroke got admitted to hospitals within this narrow therapeutic window[12, 13]. Additionally, other adverse effects such as increased risk of intracerebral hemorrhage, ischemia-reperfusion injury, and various contraindications have made the clinical application of this agent less feasible. Therefore, invention of alternative neuroprotective drugs that can efficiently go across blood brain barrier (BBB) and confer protection to damaged ischemic tissues has become an imminent issue.

Many attempts have been made so far, whereas few successes have been achieved. Limitation of application can be summarized as follows: (1) they are unable to cross BBB efficiently; (2) they have unsolved, lurking

toxicity to biological systems; (3) they possess a short half-life in blood circulation; (4) they lack the ability of being selectively taken up by targeted cells or tissues; (5) they have poor solubility in circulation and rapid elimination; and (6) some antioxidants such as SOD and catalase (CAT), can scavenge only one type of free radicals, whereas multiple types are generated during ischemia-reperfusion periods, thus restricted effects are expected.

1.2 Roles of Oxidative Stress

Various complicated, bizarre, poorly understood mechanisms such as excitotoxic injury, inflammation, oxidative stress, nitrative stress, membrane depolarization are associated with cerebral ischemia[14, 15]. When ischemia occurs, reactive oxygen species (ROS) and reactive nitrogen species (RNS), especially the most prevalent ones, including hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hydroxyl radical (·OH), nitric oxide (NO), are massively generated and accumulated, resulting in oxidative damage to cerebral tissues. Oxidative damage could further induce even worse consequences including cell apoptosis via nucleus damage, lipid peroxidation, protein oxidation and so on[7, 16–18].

Free radicals, as intermediates or byproducts generated from sophisticated reactions in living cells, causing the production of reactive oxygen and nitrogen species, normally remain in a homeostatic state[19]. Within a normal range, ROS/RNS can not only help eliminate biological intermediate metabolic wastes, but also act as essential mediators in various cell signal transduction pathways. However, ROS/RNS can also potentially pose deleterious effects on cells and generally be scavenged by endogenous antioxidant systems, like enzymes including CAT, superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px)[20, 21]. Under pathological circumstances, excessive accumulation of ROS and insufficient supply of antioxidants lead to a condition, namely oxidative stress. Oxidative stress has been proven to be involved in a great deal of human disorders, including neurodegenerative diseases, stroke, tumors, acute or chronic inflammatory diseases. Consequently, antioxidants have become a prevalent issue in the field of neurological science due to their potent and promising application potential in CIS therapy[18, 22, 23].

2 INTRODUCTION OF ANTIOXIDANT ACTIVITIES OF NANOCERIA

2.1 Antioxidant Activities of Nanoceria

Nanoceria, as one of the most noticeable catalysts, offer the possibility to be investigated as a novel approach for treatment of CIS and other diseases involved with oxidative stress. Cerium oxide, with specific fluorite crystalline lattice structure, exhibits powerful antioxidant capacity by reversibly binding oxygen and switching between Ce^{3+} (reduced) and Ce^{4+} (oxidized) states (fig. 1). The valence shift creates a redox couple with a highly reactive surface for scavenging free radicals, and this characteristic allows nanoceria to participate in various biological reactions[3, 24, 25]. It was reported that nanoceria could react with various types of ROS, in particular with H_2O_2 and O_2^-, and possessed so called SOD-mimetic and CAT-mimetic activities. Generally, SOD transforms the superoxide into H_2O_2 and oxygen under the catalytic effect of Cu^{2+} and Zn^{2+} while H_2O_2 is further eliminated by CAT into water and oxygen.

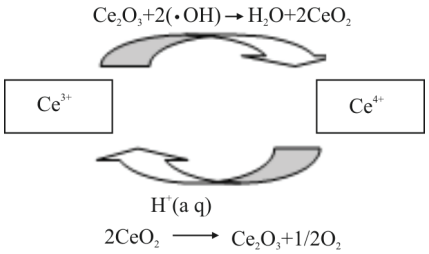

Fig. 1 The antioxidant mechanism of nanoceria

A very classic research carried out by Self. *et al*[26] showed a dramatically increased generation of H_2O_2 levels with the addition of nanoceria and the level of H_2O_2 was even greater with higher ratio of Ce^{3+}/Ce^{4+} in the nanoparticles. In order to test the SOD-mimetic activity of nanoceria, they also used ceria nanopaticles with different concentrations and Ce^{3+}/Ce^{4+} ratios to compete with ferricytochrome C, and found that a higher ratio displayed more efficacy in reducing superoxides, suggesting nanoceria might be a more efficient catalyst than SOD. Moreover, a recent study by Ganesana *et al*[27] found that 1 μg/mL of nanoceria was equivalent to 527 U of SOD in terms of superoxide scavenging activity. The same team led by Self[28] also confirmed nanoceria exhibited CAT-mimetic activity in a redox-state dependent manner, and a lower ratio of Ce^{3+}/Ce^{4+} was found to be more powerful in the presence of hydrogen peroxide. Besides, they proved that molecular oxygen was one of the products of the catalytic reactions. Other than the scavenging capabilities of H_2O_2 and O_2^-, several other catalytic potentials were also recently confirmed. NO can react with O_2^- to form a more dangerous and lethal free radical, peroxynitrite ($ONOO^-$), under the facilitation of SOD. According to the study, nanoceria might inhibit the formation of NO or even directly act on $ONOO^-$[7]. Interestingly, Self supported this theory later by obtaining the proof that naoceria was able to exhibit their scavenging feature for nitric oxide radical, which was especially prominent in the presence of nanoparticles with a lower ratio of Ce^{3+}/Ce^{4+}[29]. Hydroxyl radical, if exceeds homeostatic threshold, could transform into one of the most deleterious free radicals, causing unpredictable injuries in the biological systems. Another study drew a conclusion that nanceria exhibited hydroxyl radical scavenging activity, which was likely influenced by the size and Ce^{3+}/Ce^{4+} ratio of the particles[30]. Asati *et al*[31] demonstrated that polymer-coated nanoceria possessed a unique intrinsic oxidase ability in a slightly acidic environment without assistance from any oxidizing agents like hydrogen peroxide or enzymes such as peroxidase and oxidases. Furthermore, although the precise mechanisms are not fully understandable, the possible self-regenerating activities make this nanoparticle an astonishingly precious and useful antioxidant to be potentially applied in biological systems. Therefore, taken the above features together, nanoceria has been studied in various disease models both *in vivo* and *vitro*.

2.2 Application of Nanoceria in Cerebral Ischemic

Models

Due to the multipotent enzyme-like activities, as we mentioned in the previous context, nanoceria displays the potential to scavenge almost all the uncontrolled ROS. Efforts aimed at eliminating ROS for the treatment of various disorders associated with oxidative stress injury are being explored. Given its biological tolerability and minimal systemic toxicity[32], nanoceria is receiving much more attention nowadays for the purpose of being studied as therapeutic antioxidants. Free oxygen species such as ROS and RNS play vital roles in the ischemia and post-ischemia processes of CIS. Therefore, nanoceria, with the ability of targeting these free radicals, has been proposed as a potential neuroprotective reagent in cerebral ischemic or other injury models *in vivo* and *vitro*.

Das and his colleagues[33] presented that nanoceria could successfully protect cells of adult rat spinal cords from H_2O_2 induced oxidative damage. Administration of nanoceria, prepared by microemulsion methods, to a serum-free cell culture model of the adult rat spinal cord showed good functional biocompatibility, obvious neuroprotection and retention of neuronal function, which is basically in agreement with a previous study, reporting that nanoceria had a protective effect against exogenous natural pro-oxidant, glutamate for rodent nervous system derived HT22 cells[34]. In conclusion, nanoceria may be proven to be a potential new therapeutic strategy for ischemic insults and other oxidative injuries in other neurodegenerative diseases.

A very classic experiment conducted by Estevez *et al*[7] demonstrated that nanoceria reduced the ischemic cell death in a hippocampal brain slice of mouse model of cerebral ischemia. They introduced nanoceria with doses ranging from 0.1 to 2 μg/mL into the slices at the beginning of ischemia, and a dose-dependent reduction in cell death was observed. In order to investigate the appropriate timing of nanoceria administration, 1 μg/mL nanoceria were added to the ischemic brain slices 0, 2 and 4 h after ischemia, and neuroprotective effects were decreased in a time-dependent manner. Moreover, they also proposed that these protective effects were due to the scavenging activities of ROS/NOS and reduction in peroxynitrite (formed from the reaction of NO and superoxide) might be a pivotal mechanism through which the nanoceria exert functions during the ischemic and post-ischemic damage periods.

Interestingly, a recent review[35] concluded that targeting RNS, especially peroxynitrite ($ONOO^-$) and NO might be a potent approach to ameliorate cerebral ischemia-reperfusion damage. Excessive generation of $ONOO^-$ from the reaction of superoxide and NO, were discovered in both middle cerebral artery occlusion (MCAO) animal models and blood samples obtained from CIS patients. In terms of the complicated and bizarre mechanisms, $ONOO^-$ not only exhibited cytotoxic effects through lipid peroxidation, tyrosine nitration, DNA modification, but induced disruption of BBB via MMPs modulation and FeTMPyP decomposition. Thus, development of therapeutic agents with $ONOO^-$ scavenging ability might be a promising and novel strategy for CIS. Actually, several peroxynitrite scavengers, including uric acid, resveratrol, curcumin, green tea catechins and caffeic acid, have been demonstrated to exert protective effects on ischemic stroke models either *in vivo* or *vitro*. However, there is still lack of convincing evidence to support the neuoprotective effect of nanoceria in cerebral ischemia-reperfusion injury, and therefore further studies are required.

Kim and his coworkers[8] proposed the protective effect of nanoceria against CIS in a rat model. They synthesized phospholipid-polyethylene glycol (PEG) encapsulated nanoceria to get better colloidal stability and lower nonspecific uptake. They found that intravenously introduced nanoceria with optimal doses (0.5 and 0.7 mg/kg) could significantly mitigate the brain damage *in vivo*, as represented by reduced infarct volumes and apoptotic cells, and penetrate ischemic cerebral tissues, accumulate in the peri-infarct area. *In vitro*, they also demonstrated nanoceria, when added into CHO-K1 cells which had been pre-processed with tert-butyl hydroperoxide (tBHP), exhibited protective effect against ROS-induced cell death. Taken together, this study elucidated the neuroprotective effect of nanoceria after ischemic injury and provided possibility of the application in future clinical practice.

3 THE POTENTIAL OF NANOCERIA: PROS AND CONS

In recent years, application of nanoparticles themselves or as therapeutic carriers in the treatment of CIS has provided endless possibilities for researchers. It was presented that pharmacokinetics of nanoparticles in biological circumstances were greatly influenced by their size, dosage, solubility, surface chemical modification[36]. Surface modifications with biocompatible and biodegradable polymers such as polyethylene glycol (PEG), polybutylcyanoacrylate (PBCA) and poly lactic acid-co-glycolic acid (PLGA) could remit nanoparticles from elimination by the reticuloendothelial system (RES), prolong systemic circulation time, increase permeability to BBB and maintain satisfactory, sustained drug release[37, 38].

Several studies suggested that nanoparticles with surface modifications could assist delivery of exogenous agents such as SOD to exert biological functions. For example, Reddy *et al*[39] demonstrated that PLGA nanoparticles encapsulated SOD, when introduced into rats of MCAO via the intracarotid route, dramatically ameliorated ischemic insult. On the contrary, the simple use of SOD did not show significant neuroprotective effects. In another literature[40], N-methyl-D-aspartate receptor 1 (NR1) targeted antibody was decorated on the surface of SOD-loaded PBCA nanoparticles and this composite, not only showed better permeability across BBB and localization into cerebral tissue, but also suggested efficient neuro-protection with evidence of obviously less infarct volume, reduced inflammatory mediators, decreased free radicals and improved neurological behavior.

Nanoceria, as a vital component of the nanoparticle family, has emerged as a novel therapeutic agent or vector for various intractable diseases, including cancer, neurodegenerative disorders, ischemic stroke, radiation associated injury[18], cardiovascular disorders, retinal disorders by itself or as a drug carrier (table 1). However, to date, nanoceria targeted delivery of antioxidants or other neuroprotectives to the brain in the context of

ischemia-reperfusion has not been elucidated yet.

Table 1 Examples of potential applications of nanoceria in other oxidative stress-associated diseases

		Subjects	Protocol of synthesis	Doses	Size	Method of entry	Duration of observation	Outcome	References
1	Retinal diseases	(1) SD rats	Simple wet chemistry methods	1 μmol/L or 0.344 ng to 1 mmol/L or 344 ng	3–5 nm	IV injection	1 h to 120 days post-injection	Preferentially taken up; non-toxic effects on retinal functions	[41, 42]
		(2) Vldlr knockout mice	Simple wet chemistry methods	172 ng	3–5 nm	IV injection	1 to 4 weeks	Inhibit the rise in ROS/VEGF/the formation of neovascular tufts	[43]
2	Neurodegenerative disease	(1) Aβ (25–35) incubated human neuroblastoma-SH-SY5Y cells (ATCC)	PEG-CNPs-Aβ Ab (CNPs-Ab)	200 nmol/L	3–5 nm	/	20 h	Non-toxic to neuron cells; selectively delivered to Aβ plaques	[44]
		(2) Murine model of EAE	Citrate/EDTA coated CeNP	10, 20 and 30 mg/kg	2.9 nm	IV via tail vein	/	Able to penetrate brain, reduce ROS, alleviate clinical symptoms	[9]
3	Cardiomyopathy	(1) MCP-1 transgenic mice	NanoActive™ cerium oxide nanoparticles (NanoScale materials, Inc, USA)	15 nmol	7 nm	IV via tail vein	Twice a week for 2 weeks	Protect against the progression of cardiac dysfunction and remodeling	[10]
		(2) CPC	/	10, 25, and 50 μg/mL	5–8 nm	/	1, 3 and 7 days	Protect CPC from H2O2 induced cytotoxicity	[45]
4	Cancer	(1) Human bronchoalveolar carcinoma-derived cell line (A549)	Homogenous nucleation method	3.5, 10.5, and 23.3 μg/mL	20 nm	/	24, 48 and 72 h	Decrease cell viability significantly	[46]
		(2) Squamous tumor cells SCL-1/ human dermal fibroblasts/ myofibroblastic cells	Dextran-coated nanoparticles	50, 150, 300 μmol/L	5 nm	/	24/48 h	Increase ROS levels; lower the invasive capacity of tumor cells	[47, 48]
5	Chronic inflammation	J774A.1 murine macrophages	Simple wet chemistry methods	0 to 10 μmol/L	5 nm	/	24 h nanoceria pretreated; various time sets for observation	Protect against ROS damage; suppress iNOS protein levels	[6]

IV: intravitreal; SD: Sprague-Dawley; EAE: experimental autoimmune encephalomyelitis; CPC: cardiac progenitor cells; MCP: monocyte chemoattractant protein

Many studies reported that nanoceria were biocompatible and able to penetrate brain tissues. For example, to investigate whether nanoceria could cross the BBB and further exhibit the free radicals scavenging abilities, Portioli *et al*[49] injected in-house synthesized nanoceria into living animal models and 24 h later, they found the presence of fluorescein isothiocyanate conjugated nanoceria, also in limited amount, in the cerebral tissues under confocal microscopy and electron microscopy. Heckman *et al*[9] demonstrated that custom-synthesized nanoceria, when introduced intravenously to mice with autoimmune encephalomyelitis of multiple sclerosis, was able to pass through the brain to lower the level of ROS and ameliorate clinical symptoms. Another report presented by Kim[8] also analyzed the biodistribution of nanoceria in a rat ischemic stroke model and confirmed that intravitreal administered PEG-encapsulated particles were increased markedly in the penumbra area of the ischemic hemisphere.

Many pilot studies have proven that nanoceria is non-toxic. Hirst and colleagues[6] introduced nanoceria into mice and tracked them for one month; the result revealed that nanoceria were well tolerated and no obvious toxicity was found. Additionally, a recent published paper also suggested that nanoceria only selectively targeted cancerous cells, but normal structures in the organism were exempted from them[32]. However, in contrast to the above positive evidence, some researchers re-

vealed relatively unsatisfactory aspects of nanoceria. Ma *et al*[50] concluded that exposure of nanceria to rats induced pulmonary inflammation and damage, possibly via alveolar macrophages involved cellular signaling pathways. Later, the same laboratory conducted another experiment aiming at clarifying the mechanisms underlying the pulmonary injury caused by intra-tracheal instillation of nanoceria; the results showed that nanoceria contributed to the over-expression of a variety of pulmonary fibrosis-associated mediators, including hydroxyproline, TGF-β1, OPN, MMPs, some of which were increased in a time- and/or dose-dependent manner[51]. Furthermore, it is worth noticing that numerous parameters, such as surface charge, pH, particle size, surface coating, and ratio of Ce^{3+}/Ce^{4+} might endow naoceria with distinct toxicity, stability and even catalytic abilities[4, 52–55]. Given the possible application of nanoceria in central nervous system (CNS), concerns regarding neurotoxicity have arisen, although no overt adverse effect on neuronal cells or cerebral tissues in neurodegenerative or ischemic models has been reported so far.

Das *et al*[33] reported the possible autoregenerative mechanism of nanoceria. As has been stated before, nanoceria exhibited both SOD-mimetic and CAT-mimetic activities. Therefore, when we combine them together, a possible non-stop recycle reaction might occur, which may render it to become a valuable antioxidant with self-cycling potential. This new antioxidant candidate would be different from other antioxidants in that no repetitive dose is needed. Furthermore, nanoceria could also scavenge other free radicals, such as nitric oxide radical, peroxynitrite anion and even hydroxyl radical, all of which are well-known to exacerbate neuronal insult and cytotoxicity in cerebral ischemia-reperfusion period (fig. 2).

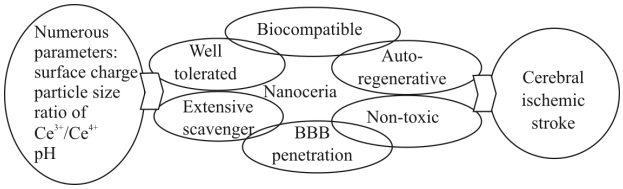

Fig. 2 Potential advantages for application of nanoceria in cerebral ischemic stroke

Similar to other nanoparticles, novel development of nanoceria-based drug delivery or targeting systems are also appearing in recent years. A research group proposed to use nanoceria as a carrier for carboxybenzenesulfonamide, a type of human carbonic anhydrase, to investigate the potential application in glaucoma therapy; successful derivatization and therapeutic effects were later observed both *in vivo* and *vitro*[56]. In another study, transferrin-conjugated nanoceria were demonstrated to improve selective uptake by cancer cells[57]. Furthermore, in terms of neurodegenerative disorders, Li *et al*[58] described the synergistic protective effects were obtained when nanoceria capped H_2O_2-responsive controlled drug release system was applied for the treatment of Alzheimer's disease. Taken together, it is easy to get nanoceria modified due to the ultra-small particle size and unique chemical structure. Nanoceria exhibit better biocompatibility, longer survival time in systemic circulation and better permeability across microcirculation after surface coating/modification with hydrophilic or amphiphilic biodegradable polymers such as PEG and PLGA. Additionally, when combined or incorporated with other targeting agents that pose affinity to specific protein, cell or tissue, nanoceria might exert more striking synergistic effects on enormous disease models. Unfortunately, there are only a handful studies concerning nanoceria application in ischemic stroke, and more investigations are anticipated.

4 CONCLUSIONS AND PROSPECTS

CIS has been demonstrated to be associated with multiple bizarre, poorly understandable pathological mechanisms such as excitotoxic injury, inflammation, oxidative stress, nitrative stress and apoptosis. Many of these mechanisms are influenced by free radicals including H_2O_2, O_2^-, HO^-, NO and $ONOO^-$. Overwhelming generation and accumulation of these free radicals during the ischemia-reperfusion periods may further exacerbate ischemic damage to cerebral tissues. Given that, targeting the deleterious free radicals may provide promising advances in ameliorating insults and improving outcomes in clinical research and therapy of CIS. Nanoceria possess unique antioxidant features owing to coexistence of Ce^{3+} (reduced) and Ce^{4+} (oxidized) ions, rapid shifting between these two states and oxygen-vacancy defects. Previous studies have also confirmed that nanoceria have multiple free racial scavenging enzyme-like properties. Furthermore, good biocompatibility, longer systemic circulation, better BBB permeability are all vital factors for any administered therapeutic agents to enter brain and exert efficient functions in cerebral ischemia, which could be easily achieved through surface modification with biodegradable and non-toxic polymers, such as PEG, PLGA. Utilizing polymermodified nanoparticles as drug carriers has indicated enormous potential in the treatment of CIS, whereas evidence concerning nanoceria is still lacking.

Notwithstanding, a broad range of issues concerning toxicological, biological and pharmacological profiles still needs to be addressed. Contrary to the majority of published literature, some presented toxicity and poor biocompatibility of nanoceria *in vivo* or *vitro*, which could possibly be explained by different synthetic protocols, thus with distinct particle sizes, chemical or physical structures, surface modifications; all of these features might exert undefined, equivocal effects on its pharmacokinetics including absorption, distribution, metabolism, excretion and catalytic properties in biological systems. In addition, methodological differences in administered doses, routes or even individual skills might alter its behavior and therapeutic effect. It has been suggested that nanoceria could exert pro-oxidant effects, thus increasing the generation of ROS, or even inducing apoptosis. Although explanation for this discrepancy from well-known antioxidant features has not been fully elucidated yet, environmental pH, especially an acidic milieu, is likely to transform nanoceria into oxidants[4, 26]. Moreover, pro-oxidants might be provoked due to a series of signal transduction cascades induced by possible interaction between nanoceria and different cells. Therefore, more detailed and systematic investigations based on

standardized protocols of synthesis and utility, toxicology and biocompatibility in experimental models which mimic biological internal environment are required, especially if our definitive purpose is to put nanoceria onto clinical application, such as in treatment of ischemic stroke and other neurodegenerative diseases.

Preclinical evidence has clarified that some peptides possess pivotal roles in the pathological cascades during ischemia-reperfusion periods; and therefore inhibition or promotion of one or more peptides might exert notable efficacy in the therapy of cerebral ischemia, particularly in the context of inflammation, edema and apoptosis. Up-regulation of integrin alpha v beta 3 has been reported in the context of cerebral ischemia, and selective inhibition of this integrin showed significant improvement after ischemic damage *in vivo*[59, 60]. Kim *et al*[8] also concluded that PEG-coated nanoceria conferred protection to the cerebral tissues. Therefore, continued efforts will be needed in future research, intending to test and analyze the neuroprotective effects of alpha v beta 3 inhibitor and nanoceria separately in acute ischemic stroke models. Furthermore, we propose utilizing possible biological composite carrier alpha v beta 3 inhibitor-CeO_2-NPs to investigate its biocompatibility, biodistribution, stability, and the synergistic effect against ischemic stroke *in vivo*, hoping that it could serve as a potential new type of highly efficient, targeted control-released carrier to deliver neural protective drugs for the treatment of CIS.

Conflict of Interest Statement

The authors declare that there is no conflict of interest with any financial organization or corporation or individual that can inappropriately influence this work.

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