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Cerium interfering effect on iron intestinal absorption in rats using the Everted Gut Sac (EGS) method

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Abstract

Background: Certain trace elements, like cerium, have the potential to disrupt iron metabolism. This study explored the impact of cerium on intestinal iron absorption, focusing on the initial stage of iron metabolism. We employed the rat everted gut sac (EGS) segments to assess the interference caused by cerium. The primary objectives of this study were to examine the absorption of cerium in the intestines and to compare iron absorption in the presence and absence of cerium.

Methods: For the EGS experiment, segments of the rat's duodenum, ileum, or jejunum were promptly excised, cut into 5-6 cm segments, and rinsed with a physiological solution. These freshly prepared rat EGS segments were then incubated in Earle's medium containing iron (III) and/or cerium (III). We examined the impact of ascorbic acid, glucose, and different time intervals on the intestinal absorption of cerium and iron. Specifically, we investigated how glucose (5 mM) and ascorbic acid (2.8 mM) affected the absorption of cerium and iron at various concentrations (ranging from 0 to 200 mg/L). Additionally, we assessed the interfering effect of cerium on iron absorption.

Results: The results indicated that the maximum intestinal absorption of Fe (III) and Ce (III) occurred at a concentration of 200 mg/L. Furthermore, it was observed that their uptake increased following the reduction by ascorbic acid. The absorption of these elements also rose in the presence of glucose, suggesting energy-dependent transport. Additionally, a consistent cerium concentration was found to decrease iron absorption by 24.3% ($P \leqslant 0.05$).

Conclusion: Based on the results, cerium likely reduces iron uptake by competing with iron. Cerium can also disrupt iron metabolism and lead to iron-related metabolic disorders. However, further studies at the molecular and intracellular levels are needed to gain a better understanding of this mechanism.

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Introduction

Trace and major elements play critical roles in animal health. Excess or deficiency of these elements can lead to a wide variety of clinical effects, making it essential to monitor their presence (1). One group of particularly important trace elements is the lanthanides, also known as the rare earth elements (REEs), which include elements 57 to 71 on the periodic table. Members of this group, including cerium, have been used for their anti-emetic and anti-coagulant properties for over 80 years. Additionally, the toxic properties of these elements have been extensively investigated and documented in the literature (2,3).

The initial stage of iron (Fe) absorption, a crucial element for cell function, takes place in the mucosal cells of the intestinal lumen. Although the mechanisms of iron uptake are not fully understood, it has been demonstrated that iron can either bind to a carrier protein or enter the ferritin molecule (4,5). In previous studies, we have explored some of the elements that interfere with the iron absorption process (6-8).

Various factors, including glucose, ascorbic acid, and even trace elements, have been found to influence iron absorption. Ascorbic acid stands out as the most potent enhancer of iron absorption. It is believed that ascorbic acid enhances iron absorption within the intestinal lumen. This substance maintains iron in a more soluble and absorbable form while preventing it from binding to inhibitory ligands. Additionally, it has been demonstrated that energy is required to transport certain elements across the intestinal membrane, relying on carriers powered by energy derived from glucose-ATPase. Furthermore, while both ferrous (II) and ferric (III) forms of iron can be absorbed into intestinal mucosal cells, the absorption of the former is higher. Consequently, ascorbic acid has been utilized as a reducing agent for Fe (III) (9-12).

Upon exposure to air and combustion at 150 °C, cerium forms a compound known as ceria (cerium oxide). Studies have demonstrated that ceria exhibits anti-oxidant and neuroprotective properties when formulated as nanoparticles (13-15).

Cerium has gained increasing significance in various domains, including animal husbandry, agriculture, daily life, and the medical and pharmaceutical sectors. Given its utilization across diverse industries, particularly in glass manufacturing, and the associated risk of toxicity, it has become imperative to investigate its absorption process and its potential interaction with iron absorption (16).

Currently, there is limited information available concerning the uptake of cerium within organisms. Additionally, the intestinal absorption mechanism of cerium and its interference with iron absorption remains unclear. This study aimed to explore intestinal cerium uptake and compare iron uptake in the presence and absence of this element.

Methods

In this study, the everted gut sac (EGS) method was employed to investigate the process of intestinal absorption and the interference of elements. This method serves as an efficient tool for studying in vitro drug absorption mechanisms, the role of transporters in drug absorption, intestinal drug metabolism, and the function of intestinal enzymes in drug transport through the intestine (17) (The average weight of EGS segments is 1 gram).

All materials used in this study were obtained from Sigma Chemical Company, UK. Male Wistar rats were purchased from the Falavarjan Animal Laboratory of the Islamic Azad University, Isfahan, Iran, and kept under standard conditions until their weights reached 200-250 g. For the EGS model, rats were fasted 1 day prior to the experiments and anesthetized with ether. The duodenum, ileum, or jejunum of the intestine was rapidly removed and divided into 5-6 cm segments. Each segment was washed with a physiological solution (pH 6.5, 4 °C). It should be noted that the glassware used in the experiment was soaked overnight in 10% nitric acid to deionize it. The washed intestine pieces were gently everted over a glass rod. Thereafter, one end of the everted intestine was clamped and tied with a silk braided suture and washed with 2 mL of the Krebs-Ringer Bicarbonate (KRB) solution at 37 °C. The other end of the segment was then tied with thread. The segments were then ready for examination of the effect of various factors and the absorption of substances. At least 3 EGS segments were used for each experiment.

The optimum concentration for the intestinal absorption of iron and cerium was determined as follows: The EGS segments were initially placed in separate test tubes containing 5 mL of the KRB buffer (pH 7.4), a mixture of oxygen-carbon dioxide (95:5), and various concentrations of iron and cerium complexed with citrate (0-200 mg/L). These elements, iron (III) chloride and cerium (III) chloride, were prepared in bi-distilled water and mixed with equal volumes of citric acid at a ratio of 1:20. After 60 minutes of incubation at 37 °C, the contents of the test tubes containing the EGS segments were removed, and their iron and cerium concentrations were measured using a UV2600 spectrophotometer (Shimadzu, Japan). For cerium concentration determination, 100 µL of the EGS segment content was diluted with 100 µL of 0.2% nitric acid, and then its value was obtained at a wavelength of 240 nm using the inductively coupled plasma (ICP) method.

Before placing the EGS segments into test tubes containing 5 mL of Earle's buffer and different concentrations of iron and cerium, 2 mL of Earle's buffer (pH 7.4) was added to the EGS segments. Each test tube, in addition to these substances, was examined for the effects of glucose (5 mM) and ascorbic acid (2.8 mM) on the absorption of cerium and iron at different concentrations (0-200 mg/L). Similar to the previous steps, the concentration of iron and cerium in the

intestinal segments was measured after incubation at 37 °C.

The same steps as before were followed to examine the interfering effect of cerium on intestinal iron absorption. After adding 2 mL of Earle's buffer (pH 7.4) into the EGS segments, they were inserted into the test tube containing 5 mL of Earle's buffer. This test was performed in 2 stages:

(i) Investigation of the intestinal absorption process for different concentrations of iron (III) (0-200 mg/L) in the presence of cerium (100 mg/L) and in its absence

(ii) Investigation of the intestinal iron (III) absorption process in the presence of different concentrations of cerium (0-200 mg/L) and a constant iron (III) concentration (100 mg/L) or in its absence

Similar to the previous stages, the amounts of iron and cerium were measured after 60 minutes of incubation at 37 °C.

The absorption of elements (starting concentrations of 100 mg/L for both iron and cerium) was examined at different experiment times (0-75 minutes with 15-minute intervals) to investigate the effect of time on their intestinal absorption. After each interval, the EGS segments were removed from the medium, and the concentrations of iron and cerium were measured.

Data were expressed as the mean ± standard error of the mean (SEM) and were analyzed by 2-way analysis of variance (ANOVA) using GraphPad Prism v. 9 (GraphPad Software, Inc., La Jolla, CA, USA). A significance level of $P<0.05$ was considered to indicate a statistically significant difference between group means.

Results

Figure 1 illustrates the results for the optimal intestinal absorption concentration for iron and cerium. It shows that with an increase in the concentration of iron and cerium in the incubation medium, the intestinal uptake of these elements also increases. The results reveal that the maximum intestinal absorption for both elements occur at a concentration of 200 mg/L.

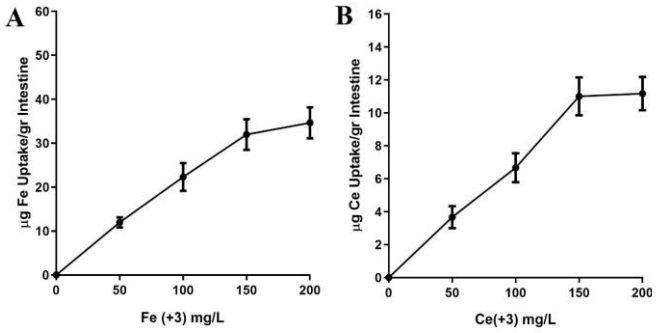

Figure 1. Determination of optimal intestinal absorption concentrations for Fe (III) (A) and Ce (III) (B) using the everted gut sac (EGS) method. Each data point represents the average of 3 independent experiments and is presented as the mean ± standard deviation. Cerium was complexed with citrate (1:20). (Incubation temperature: 37 °C, Incubation time: 60 minutes, Average weight of EGS segments: 1 gram)

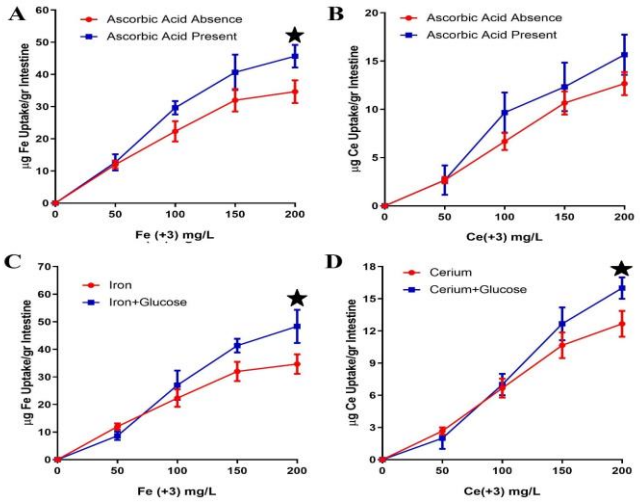

Figure 2. Comparison of intestinal uptake of iron and cerium in the presence and absence of ascorbic acid and glucose. The concentrations of ascorbic acid and glucose added to the everted gut sac (EGS) external medium were 2.8 mM and 5 mM, respectively. Each data point represents the average of 3 independent experiments and is presented as mean ± standard deviation. Cerium was complexed with citrate (1:20). (Incubation temperature: 37 °C, Incubation time: 60 minutes, Average weight of EGS segments: 1 gram). Each data point represents the group mean (mean ± standard error of the mean). *P<0.05, **P<0.01

Figure 2 shows the effects of ascorbic acid and glucose on the intestinal absorption of iron and cerium (*P<0.05, **P<0.01). The presence of these 2 substances at 200 mg/L in the incubation medium leads to an increase in the intestinal absorption of iron and cerium. The intestinal absorption of iron (200 mg/L) increased by 29.0% in the presence of ascorbic acid compared to when no ascorbic acid was introduced into the system. The intestinal absorption of the highest concentration of cerium also increased by 23.6% in the presence of ascorbic acid. Increased absorption of iron and cerium was also observed in the presence of glucose (37.4% and 26.3%, respectively).

In another experiment, the effect of constant and variable cerium concentrations on the intestinal uptake of iron was examined (Figure 3). Our results showed that cerium interferes with intestinal iron absorption, resulting in a reduction in the uptake of iron into EGS segments. In the presence of a constant concentration of cerium and a maximum concentration of iron, intestinal iron uptake is reduced by 24.3%. Figure 3 also illustrates that iron uptake at high cerium concentrations gradually decreases, confirming the interfering effect of cerium on iron uptake (a 44.1% reduction in the highest cerium concentration compared to when it is absent).

The effect of time intervals on the intestinal absorption of iron and cerium is shown in Figure 4. The results indicate that the highest uptake occurs at 30 minutes. Within 30 minutes, the average uptake for iron and cerium is 36.6 and 11.6, respectively.

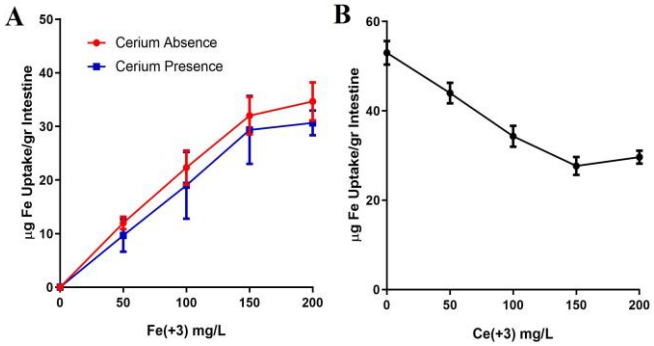

Figure 3. (A) Investigation of the effect of cerium presence and absence on intestinal uptake of iron. (B) Examination of the effect of different cerium concentrations on intestinal uptake of iron with a constant iron concentration of 100 mg/L. Each data point represents the average of 3 independent experiments and is presented as mean ± standard deviation. Cerium was complexed with citrate (1:20). (Incubation temperature: 37 °C, Incubation time: 60 minutes, Average weight of everted gut sac (EGS) segments: 1 gram)

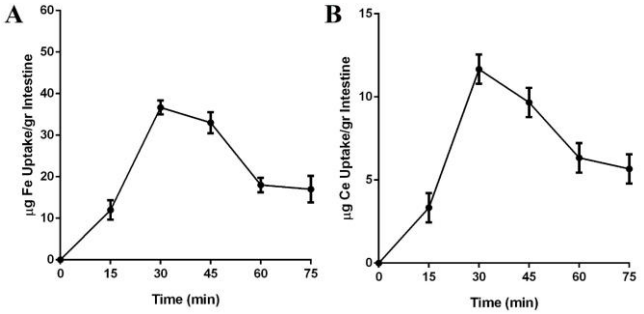

Figure 4. (A) The time effect on the intestinal absorption of iron. (B) The time effect on the intestinal absorption of cerium. The everted gut sac (EGS) segments were removed from the medium at intervals, and the concentrations of iron and cerium were measured. The concentration of iron and cerium in each experiment was 100 mg/L. Each data point represents the average of 3 independent experiments and is presented as mean ± standard deviation. Cerium was complexed with citrate (1:20). (Incubation temperature: 37 °C, Incubation time: 60 minutes, Average weight of EGS segments: 1 gram)

Discussion

Trace elements are essential for life and are necessary for the proper functioning of the human body. However, exceeding the concentrations required for biological functions in the body can be toxic and dangerous (18). The effects of certain trace elements on various diseases, such as bone disease, neurological diseases, kidney disease, and anemia, have been observed (19-21). Many studies have reported the competition of trace elements with iron and the risk of disrupting metabolic pathways of essential elements by either binding to vital molecules or substituting for such elements (22-24). Iron is an essential element for the life of animals, plants, and microbes. An adult human body contains about 3 to 5 grams of this element, with 75% found in myoglobin, hemoglobin, and iron-containing enzymes such as peroxidase enzymes and catalase (25).

Cerium is widely used in various industries, including nanomedicine (e.g., its use in ceria nanoparticles), and its usage is increasing. However, there is also evidence of its toxicity (26). In this study, we investigated intestinal iron and cerium absorption and the interference of cerium with iron absorption using EGS segments. Increasing the concentration of iron and cerium elevated the permeability of these 2 elements in EGS segments. The results of intestinal absorption for iron and cerium showed that the highest absorption of these elements occurs at 200 mg/L. In the case of cerium, however, there is not much difference in absorption between 150 and 200 mg/L. Based on the results, it can be concluded that cerium probably enters EGS segments through carriers with saturation properties.

Furthermore, although iron can be absorbed into intestinal mucosal cells in both ferrous (II) and ferric (III) forms, the absorption of the former is higher. Thus, ascorbic acid was used as a reducing agent for iron (III) (27,28). The results demonstrate that the presence of ascorbic acid increases the absorption of both iron (by 29.0%) and cerium. Additionally, reducing these elements results in an increase in their intestinal absorption.

According to previous experiments, carriers facilitate the transfer of these elements through the energy they receive from glucose (29). Iron uptake by EGS cells has also been reported to be energy-dependent (30). Therefore, to investigate the effect of glucose on the absorption of these elements, glucose was added to the incubation medium, leading to an increase in the absorption of both elements. However, the increase in the absorption of iron was higher than that of cerium. This result supports the possibility of active transfer of iron and cerium.

The study also examined the interference of cerium with iron absorption. The results showed that iron absorption decreases with increasing cerium concentration. However, there is no decrease in the absorption of iron when the cerium concentration is increased from 150 to 200 mg/L. This indicates that higher cerium concentrations do not play a role in preventing iron uptake.

The data from this study indicates that iron uptake by EGS depends on various factors, including incubation time. Accordingly, the maximum absorption was found to occur within 30 minutes. This may be due to mucosal cell death or depletion of the energy source after the initial consumption of energy.

According to the presented data, it is possible that both iron and cerium are transported into mucosal cells through active transport by a similar carrier. Additionally, it is concluded that despite the larger ion radius of cerium (1.14 Å) compared to that of iron (0.65 Å), cerium may compete with iron for absorption into mucosal cells. It is noteworthy that only a limited number of studies have been conducted on cerium in mammals (31). In contrast, cerium has been extensively studied in the form of dioxide nanoparticles. For example, cerium dioxide nanoparticles have been reported to exhibit neuroprotective effects in hippocampal brain slices, reducing ischemic cell death. This suggests that cerium dioxide nanoparticles could be potentially beneficial as therapeutic agents for strokes (32). Effective uptake of cerium in nanoparticle form has also been demonstrated in various articles. However, another study revealed the toxicity of CeO2 to macrophage cell lines and human alveolar epithelial cells (33).

Conclusion

In conclusion, this study demonstrates that cerium not only influences the initial stage of intestinal iron absorption but also interferes with iron metabolism. Iron absorption decreases with increasing cerium concentration. These findings may have implications for future nanomedical research, particularly in the field of drug delivery. However, to gain a deeper understanding of the mechanism underlying cerium's interference with iron uptake and metabolism, further investigations at the molecular and intracellular levels are warranted.

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Ethical statement

All protocols involving animals were approved by the university Ethics Committee with the ethics code IR.IAU.NAJAFABAD.REC.1399.024.

Conflicts of interest

The authors declare that there is no conflict of interest.

Author contributions

FHT, AAM, AAR: Research concept and design; FHT, AAM, AAR, HN: Collection and/or assembly of data; FHT, AAM, AAR, HN: Data analysis and interpretation; FHT, AAM, AAR, HN: Writing the manuscript; FHT, AAM: Critical revision of the manuscript. All the authors read and approved the final version of the manuscript.

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