CELLBOOST
Zinc Ionophore Activity
of
Quercetin *and* Epigallocatechin-gallate

REFERENCE:

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ABSTRACT: Labile zinc, a tiny fraction of total intracellular zinc that is loosely bound to proteins and easily interchangeable, modulates the activity of numerous signaling and metabolic pathways. Dietary plant polyphenols such as the flavonoids quercetin (QCT) and epigallocatechin-gallate act as antioxidants and as signaling molecules. Remarkably, the activities of numerous enzymes that are targeted by polyphenols are dependent on zinc. We have previously shown that these polyphenols chelate zinc cations and hypothesized that these flavonoids might be also acting as zinc ionophores, transporting zinc cations through the plasma membrane. To prove this hypothesis, herein, we have demonstrated the capacity of QCT and epigallocatechin-gallate to rapidly increase labile zinc in mouse hepatocarcinoma Hepa 1-6 cells as well as, for the first time, in liposomes. In order to confirm that the polyphenols transport zinc cations across the plasma membrane independently of plasma membrane zinc transporters, QCT, epigallocatechin-gallate, or clioquinol (CQ), alone and combined with zinc, were added to unilamellar dipalmitoyl phosphocholine/cholesterol liposomes loaded with membrane-impermeant FluoZin-3. Only the combinations of the chelators with zinc triggered a rapid increase of FluoZin-3 fluorescence within the liposomes, thus demonstrating the ionophore action of QCT, epigallocatechin-gallate, and CQ on lipid membrane systems. The ionophore activity of dietary polyphenols may underlay the raising of labile zinc levels triggered in cells by polyphenols and thus many of their biological actions.

KEYWORDS: clioquinol, epigallocatechin-gallate, flavonoids, liposomes, quercetin, zinc ionophores

1. INTRODUCTION

Quercetin (QCT), a water-insoluble flavonoid present in onions, nuts, and many other vegetables, and epigallocate-chin-3-gallate (EGCG), a water-soluble flavonoid present in green tea, are among the most consumed and most studied polyphenols present in the human diet.1 Flavonoids are considered bioactive micronutrients whose regular' consumption, either as food components, or as dietary supplements and nutraceuticals,[2] entails benefits for human health, including prevention and amelioration of cancers,[3] diabetes, and cardiovascular[4] and neurodegenerative[5] diseases. Many of the health benefits of flavonoids have historically been ascribed to their antioxidant activity, which they exert directly by scavenging reactive oxygen species (ROS) and by chelating the redox-active transition metals iron and copper, which may act as ROS generators in biological systems.[6] Flavonoids also act as antioxidants indirectly by inhibiting redox-sensitive transcription factors and pro-oxidant enzymes as well as through induction of phase II and antioxidant enzymes.[7]

Diverse polyphenols have been shown able to form complexes with the redox-inactive transition metal zinc.[10] Zinc is an essential micronutrient for humans, the deficiency of which causes multiple dysfunctions, including alterations of glucidic and lipidic metabolisms.[11] Within cells, the vast majority of zinc cations (in concentrations usually ranging from 100 to 300 μM for most cells) are tightly bound to proteins, functioning as a catalytic or structural component of an estimated 3000 mammalian proteins involved in virtually all cellular processes.[12]

Zinc from the extracellular milieu and from intracellular compartments enters the cytoplasm through 14 specialized transmembrane proteins of the ZIP/SLC39 family, whereas cytoplasmic extrusion of zinc toward organelles or the extracellular' environment is performed by 10 transporters of the ZnT/SLC30 family, being ZnT1, located at the plasma membrane, the main regulator of cellular zinc efflux and export of excess zinc in most cells.[17] Within the cytoplasm, zinc may bind to metal free apo-metallothionein (apo-MT) to generate Zn-MT complexes.

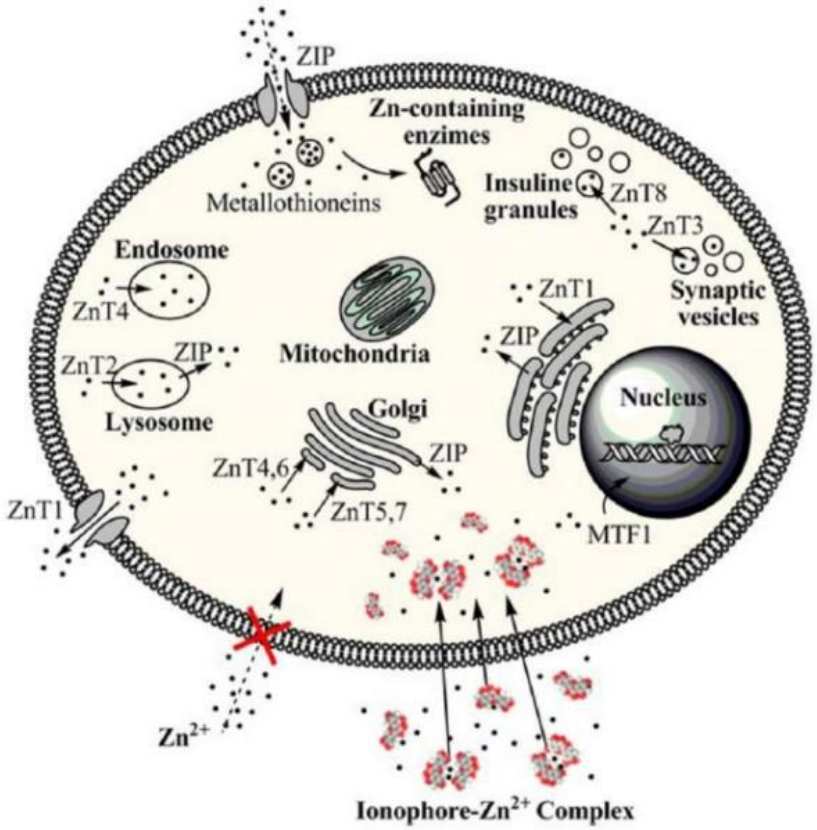

Figure 1.

Schematic representation of zinc homeostasis. Intracellular labile zinc is modulated by the coordinated activity of a large family of zinc transporters (ZnT and ZIP) and zinc-binding proteins, such as metallothionein or ionophore molecules.

RESULTS

3.1. QCT, EGCG, and CQ increase the Cytoplasmic Labile Zinc in Hepa 1-6 Cells. The increase of cytoplasmic labile zinc is modulated by the cellular zinc transporters, where the zinc ions are transported to the cytoplasm through specific channels of the ZIP family, bound to ionophore molecules that independently cross the lipid bilayer, or liberated from zinc-binding proteins such as metallothioneins (Figure 1).

3.2. Zinc Ionophore Activity of QCT, EGCG, and CQ using Liposomes as Membrane Models. Increases of cytoplasmic labile zinc levels triggered by CQ and pyrithione in a variety of cell lines have been attributed to their ionophore activity, that is, to the capacity of CQ-zinc and pyrithione-zinc complexes to cross the plasma membrane.

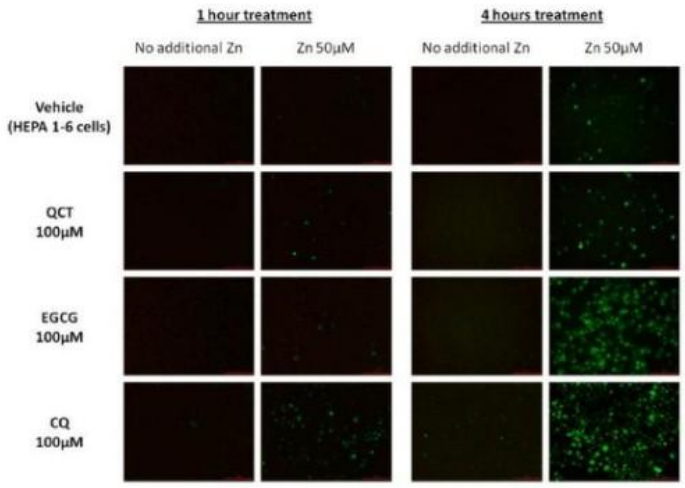

Figure 2.

Effect of QCT, EGCG, and CQ on the cytoplasmic pool of labile zinc in Hepa 1-6 cells. Hepa 1-6 cells were first treated with 100 μM QCT, EGCG, or CQ in the presence or absence of 50 μM $ZnCl_2$ for 1 and 4 h. The medium was then removed, and 3 μM FluoZin-3 (AM, cell permeant) was added. After 30 min incubation, cells were washed and examined using a confocal fluorescence microscope. Control cells were treated with vehicle (final 0.05% ethanol, 0.1% DMSO). Scale bars are 50 μM.

CONCLUSION

In conclusion, we have demonstrated that QCT, EGCG, and CQ rapidly increase intracellular labile zinc in Hepa 1-6 cells and that they function as ionophores for zinc in a liposomal system. Thus, natural flavonoids can be added to an arsenal of drugs that may be used to modulate zinc homeostasis and regulate zinc-dependent biological pathways.