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Regulation of SLC11A3 by inflammation

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Abstract

Acute and chronic inflammatory states are characterized by changes in body iron metabolism. These changes include a drop in serum iron, an increase in the rate of plasma iron disappearance, a decline in the rate of plasma iron turnover, reticuloendothelial system (RES) cell iron sequestration and a decline in intestinal iron absorption. This response is elicited by a variety of metabolic conditions and acute bacterial infections, especially gram-negative bacteria, and by experimental mediators of inflammation such as endotoxin and turpentine. These changes in iron metabolism contribute to the development of the anemia of chronic diseases. SLC11A3 (aka MTP1, ferroportin 1, IREG1) is a metal transporter that exports iron from the cytosol of cells and was initially identified as the duodenal epithelial basolateral iron transporter. Recent identification of a MTP1 mutation leading to hemochromatosis in man adds further weight to the hypothesis that MTP1 is involved in iron homeostasis. RES cells are responsible for the recycling of iron from the breakdown of heme from senescent erythrocytes and MTP1 has been hypothesized to be the key iron exporter in these cells. Supporting this hypothesis is the observation that MTP1 is expressed in the RES macrophages of the spleen, Kupffer cells, bone marrow and lymph node histiocytes, mesangial cells, brain microglial cells. In a mouse (C57/Bl6) model of lipopolysaccharide (LPS) induced acute inflammation, MTP1 expression in the cells of the RES is regulated by acute inflammation. Immunohistochemical staining of tissues, using an anti-MTP1 antibody, of mice given parenteral injections of LPS demonstrated down-regulation of MTP1 expression in the RES cells of the spleen and liver and also in the duodenal epithelial cells compared to control animals. Western blotting of total liver and spleen lysates confirmed the decline in MTP1 protein expression induced by LPS. In addition, RT-PCR analysis showed that LPS treatment also resulted in a decline in MTP1 mRNA in spleen, liver and duodenum compared to controls. One clue to the molecular signaling mechanism for MTP1 down-regulation by LPS comes from the study of the C3H/HeJ mouse, which lacks a functional LPS receptor, toll-like receptor 4 (TLR4). C3H/HeJ mice are resistant to the toxic and hypoferraemic effects of LPS. Similarly, a down-regulation of MTP1 in response to LPS in the C3H/HeJ mice was not observed. This finding indicates that the down-regulation of MTP1 by LPS requires signaling through TLR4. Despite resistance to LPS, treatment of C3H/HeJ mice with turpentine, an inducer of sterile inflammation, for a period of 24 hours resulted in down-regulation of MTP1 expression in the spleen. These data indicate that LPS mediated down-regulation of MTP1 requires a functional TLR4, but that there are non-TLR4 dependent mechanisms for the down-regulation of MTP1 by inflammatory stimuli. In vitro treatment of mouse adherent splenocytes with 5 ug ml of LPS also resulted in down-regulation of MTP1 mRNA. This in vitro down-regulation was not abrogated by co-treatment of cells with pyrrolidinedithiocarbamate (PDTC), a well-characterized inhibitor of NF-KB activation or anti-tumor necrosis factor-a antibodies. In addition, in vitro treatment of mouse splenocytes with recombinant TNF-α did not result in down-regulation of MTP1 mRNA. The lack of antagonism between LPS and PDTC and the lack of an effect of TNF-α in vitro indicates that NF-κB activation may not be required for MTP1 mRNA down-regulation. This inflammation-mediated down-regulation of MTP1 expression in the RES may be a component responsible for iron sequestration in the RES in both acute and chronic inflammatory states.

Metallothionein in homeostasis and oxidative damage of copper

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Abstract

Metallothionein (MT) plays a major role in the intracellular sequestration of metals such as Zn and Cu. In biological systems, metals are not found in the free ionic forms but are bound to certain specific metal binding proteins such as MT. These proteins play a major role in the storage of metals and also protecting cells from toxicity. During gestation and neonatal period, the high amounts of Cu and Zn are bound to MT in livers of mice. Increased amounts of MT have been detected in livers of Wilson's Disease patients, and in certain mutations of copper efflux pumps, ATP7A/B such as in LEC rats and Toxic Milk Mutant mice, and the excess Cu is mainly bound to MT. The high hepatic levels of Cu saturated MT in Wilson's Disease patients is localized both in cytoplasm and nuclei. Although MT can provide protection against Cu toxicity in initial stage, Cu-MT itself can cause toxicity unlike Zn-MT in increased accumulation. Thus the metal species associated with MT should be taken into account in deciding the antioxidant or pre-oxidant role of MT. It has been shown that Cu-MT does not initiate lipid peroxidation directly but it can enhance the lipid peroxidation reaction with hydroperoxide and iron. The mechanism of this effect is most likely due to the release of a chelatable form of Cu ion from Cu-MT, and this can participate in Fe initiated lipid peroxidation reactions. This results in morphological and histopathological changes in the liver. Thus the high cellular levels of Cu-MT as in the case of Wilson's Disease patients, may pose a threat to enhance lipid peroxidation in certain cells.

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The molecular control of human iron metabolism

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Abstract

Messenger RNAs encoding important proteins for human iron metabolism are regulated by iron-responsive elements (IREs) and iron regulatory proteins (IRPs). Binding of IRP-1 or IRP-2 to IREs either represses translation (when binding occurs to an IRE in the 5′ untranslated region, e.g. ferritin) or stabilizes transferrin receptor mRNA against degradation (by binding to IREs in the 3′UTR). While IRP-2 activity is regulated at the level of protein stability/degradation, IRP-1 is a constitutively stable protein. Its IRE-binding activity is controlled by reversible incorporation/loss of a 4Fe-4S cluster, the 'iron sulfur switch'. Only the cluster-less apoIRP-1 binds to IREs with high affinity. Importantly, IRP-1 is activated by oxidative stress in the form of H_2O_2 in cultured cells and intact organs. DMT1 (DCT1) and Ireg1 (ferroportin) appear to mediate intestinal iron absorption as apical and basolateral iron transporters, respectively. DMT1 and Ireg1 mRNAs both bear IREs in their untranslated regions, implicating the IRE/IRP system also in the regulation of duodenal iron absorption. Whether and how HFE-mediated regulation and the IRE/IRP system interface is currently unknown. To analyze gene regulation and the crosstalk between different iron regulatory systems in a more comprehensive way, we have recently developed DNA microarrays that allow the simultaneous assessment of some 200 genes from patient samples as well as from tissues of genetically altered mouse strains. We have begun to collect and compare the 'signature profiles' from cultured cells exposed to defined pharmacological conditions, from HFE-knock out mice (in collaboration with Prof. Stremmel's department) and from patients who suffer from HFE or non-HFE hemochromatosis. First results, future promises and current limitations of these genome-wide approaches will be discussed.

Iron chelators-radical scavengers 3,3-epigallocatechin-3-gallate (EGCG) from tea extract and apomorphine attenuate neuronal cell death in 6-hydroxydopamine and MPTP models of Parkinson's Disease: Possible gene targets employing CDNA microarray

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Abstract

The increase of iron at neurodegenerative sites in Parkinson's and Alzheimer's brains is a contributing factor to the on set of oxidative stress and pathogenesis of aging brain and these neurodegenerative disorders. It has been demonstrated that age-related decline in neuronal signal transduction is reversed by the consumption of polyphenol-rich foods, implicating dietary polyphenols as potential neuroprotective agents. Recently we have shown that EGCG, tea extracts and apomorphine inhibit iron-induced lipid peroxidation of brain mitochondrial fraction as well as 6-hydroxydopamine-induced cell damage in neuronal cell lines and MPTP-induced dopaminergic neurons loss *in vivo*. In an attempt to elucidate the neuroprotective actions of these iron chelating compounds and possible gene targets for their action, human neuroblastoma SH-SY5Y cells were treated with 6-hydroxydopamine (20 μM) and MPP^+ (400 μM). Pretreatment for 45 min with EGCG (0.1–5 μM) prevented toxin-induced cell damage, as it has been shown by MTT test. For *in vivo* studies, the MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model of Parkinson's disease was employed. C57-BL mice were treated with EGCG (4–20 μmols kg day 14 days, orally). MPTP (24 mg kg day, i.p.) was given for the last 5 days. 3 days after last injection, the mice were decapitated and brains were used for further investigations. Striatal Dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) content were determined by HPLC. MPTP caused a marked reduction in DA levels (40% of control). However, EGCG (4 μmols kg day 14 days) conferred a significant protection against MPTP induced DA loss, as indicated by striatal dopamine and its metabolites content (80% of control). Gene expression profile induced by the neurotoxin MPTP *in vivo* and their response to pretreatment with EGCG and apomorphine were analyzed using Clontech Atlas mouse cDNA expression array. This study is the first to demonstrate neuroprotective activities of EGCG and apomorphine as well as a detailed profile of simultaneous changes in 51 gene expressions in brain as a result of EGCG consumption and apomorphine treatment in the absence or presence of MPTP. However, our recent cell culture studies with EGCG and apomorphine , employing human neurobalstoma cell line, SH-SY5Y, have shown that neuroprotection and neurodegeneration induced by EGCG and apomorphine on gene expression in customized microarray chips, are concentration dependent. While at low concentrations (1–10 μM) they are antioxidant and neuroprotective, at higher concentrations (> 20 μM) they are pro-oxidant and induce apoptosis and expression of apoptotic genes.

Iron-dependent oxidative damage and iron homeostasis in hippocampal neurons

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Abstract

Iron is necessary for the adequate development and function of the Central Nervous System (CNS). However, intracellular reactive iron is a source of free radicals and a possible cause of cell damage. Little is known about how neurons regulate intracellular iron levels. In this study we analyzed the characteristics of iron homeostasis and the oxidative damages generated by iron accumulation in cultured hippocampal neurons. This neuron model had an active iron responsive element/iron regulatory proteins (IRE/IRP) system for the control of cellular iron levels, as determined by IRP activity, DMT-1 levels, and iron uptake regulation. Despite an active IRE/IRP system iron uptake did not shut-off, and cells accumulated iron in a time and concentration-dependent way. Culturing cells with increasing concentrations of iron resulted in increased levels of reactive oxygen species (ROS) and protein damage, as assessed by de immunoreactivity for 4-hydroxy-2-nonenal-modified proteins. Iron also induced DNA modifications, as determinated by the formation of 8-hydroxy-2′-deoxyguanidine. In summary hippocampal neurons accumulated iron as a function of time and extracellular iron concentration. Iron accumulation resulted in oxidative damage to proteins and the chromatin committing the viability and cellular function. This iron-induced oxidative damage may be relevant to understanding of the cascade of events associated with neuronal degeneration and death.

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Development and analysis of micromethods for measuring the non ceruplasmin copper fraction

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Abstract

This study is part of a project that aims at defining bio-markers of changes of copper status, in humans, and within the homeostatic range of copper intake. Studies previously carried out at INTA showed that chronic administration of copper to healthy volunteers (0,2, 4,6 or 8 mg Cu/l in water for two months) resulted in a positive correlation between serum/plasma copper concentration and plasma non-ceruloplasmin copper fraction. This latter fraction was calculated on the basis of ceruloplasmin molecular weight and the assumption of a ceruloplasmin:copper molar ration of 1 to 6. This was done so because methods to measure this fraction, or that provide more reliable estimates that the arithmetical calculation mentions are not available. This protocol was aimed at assessing potential methods for measuring the non-ceruloplasmin copper fraction by means of methods that eventually could be applied to population studies and to clinical practice. Plasma samples obtained from apparently healthy volunteers were used for the assessments. Methods used were microfiltration with Millipore, Ultrafree-MC filters (100000 NMWL) microdialysis with Spectra/Por CE (cellulose ester) membranes of 3500 and 10000 MWCO, minicolums (Sphadex G-15) and treatment with Chelex-100. Experiments were carried out at different copper and ceruloplasmin plasma concentrations and different experimental conditions, including micro methods for each technique used. Results show that none of the micro methods tested yielded a reproducible and direct measurement of the non-ceruloplasmin copper fraction. Several reasons may explain these results, mainly that the delicate balance characteristically maintains by plasma components was altered by the different experimental conditions required by the methods used. The loss of such plasma balance would imply a possible release of copper atoms from the ceruloplasmin molecule. Since the ceruloplasmin copper fraction represent the largest fraction (up to 90%) of total plasma copper concentration, any change in the copper ceruloplasmin molar ration may significantly alter the non-ceruloplasmin copper fraction. If this is correct, no measurement of this fraction would be reliable. Given the importance of the non-ceruloplasmin fraction in copper homeostasis, and the need for new markers of copper status, ongoing studies are evaluating an inmunoprecipitation technique that will support/not support the arithmetical calculation of the non-ceruloplasmin copper fractions.

Iron and copper transportes by DMT1 in CACO-2 cells

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Abstract

Copper and iron homeostasis are critical for mammalian cells not only because their requirement by metalloproteins and redox enzymes but also because of their toxicity. The regulated transport of iron from the foods through the intestinal epithelial cells determines body iron content. There is compelling evidence indicating that DMT1 is also active in endosomes and lisosomes, thus, DMT1 is responsible not only for the initial phase of intestinal iron absorption but, more generally, for iron entrance to mammalian cells. Little is known about the processes that underline intestinal copper absorption. Electrophysiological experiments indicate that DMT1 can an transport other divalent cations, including copper. Under the hypothesis that DMT1 could be a relevant intestinal copper transporter, we characterized iron and copper uptake and transport in Caco-2 cells cultured in media with varied concentrations of iron or copper. We found that the intracellular iron content was related with the activity of iron regulatory protein 1 (IRP1) and with DMT1 protein. Surprisingly, the activity of IRP1 was regulated by Cu^{2+} in a way indistinguishable from that of iron regulation. Caco-2 cells cultured in media with low (1–5 μM) iron showed a marked increased in the apical uptake of both iron and copper. Culturing cells in low iron or copper media resulted in increased IRP1 activity and DMT1 mass, an indication that DMT1 activity is up-regulated by iron or by copper deficiency. When we incubated the Caco-2 cells with Fe and Cu (1 μM and 10 μM, respectively) we found that copper competed for iron uptake inhibiting it in 60%. Moreover, treatment of cells with a DMT1 antisense oligonucleotide inhibited both Fe and Cu uptake, although to different extents. We conclude that: (i) Caco-2 cells IRP1 activity is regulated by both iron and copper availability, (ii) DMT1 mass and activity respond to both iron and copper availability, and (iii) DMT1 is an intestinal copper transporter.

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Effect of iron overload on liver nitric oxide (NO) generation in hyperthyroid rats

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Abstract

Iron overload and experimental hyperthyroidism induce liver oxidative stress in the rat through different mechanisms. As a catalyst of free radical generation by Fenton or Haber-Weiss reactions, iron promotes secondary chain reactions in the oxidation of proteins and lipids whereas thyroid hormone administration accelerates energy metabolism with increases in the generation of reactive oxygen and nitrogen pro-oxidant species. As in both conditions there is a higher consumption of cellular antioxidants liver oxidative stress is elicited with molecular and cellular deterioration. Our group previously reported an enhanced susceptibility of the liver of hyperthyroid rats to the toxic effects of iron, with increments in the oxidative status. As this experimental condition led to liver injury and the development of an inflammatory response, this study was aimed to investigate the involvement of NO as a mechanism triggering liver oxidative stress and hepatotoxicity in the interaction of acute mild iron overload and L-3,3;5-triodothyronine (T^3) administration. Studies were conducted in which L-3.3;5-triothyronine (T^3, 0.1 mg kg for two consecutive days) treated. (male Sprague-Dawley rats) were subjected to iron overload (iron-dextran, 200 mg kg). Assessments included NO generation in liver cytosolic samples (oxidation of oxyhemoglobin to methemoglobin) and hepatic protein oxidation (carbonyl groups/mg protein), as indicator of oxidative stress. Iron overload enhanced NO generation (109%) eliciting a synergistic effect in the liver of hyperthyroid rats (263%) as compared controls. Iron overload in hyperthyroid rats led to oxidative stress as shown by the enhancement in protein oxidation (116%). This effect may be conditioned by the enhanced NO generation elicited by the combined treatment (T_3 plus iron). In fact the net increase observed in this experimental group (0.172 nmol NO/mg protein) exceeds the sum of the effects produced by the separate treatments (T_3 0.042 nmol NO/mg protein; iron, 0.067: sum 0.109). *In vivo* effects of iron on NO generation were reproduced by *in vitro* addition of iron (1 μmol iron/mg protein) liver cytosolic samples of euthyroid and hyperthyroid rats with 97% and 173% increments, respectively. It is concluded that NO generation is a major factor associated with liver oxidative stress induced by iron overload in expression of the inducible NO synthase gene.

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Identification by transcription profiling in human colon carcinoma cells of genes regulated by tumor suppressor Caveolin-1

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Abstract

Caveolin-1 was recently shown to function as a tumor suppressor protein upon expression in human colorectal cells. Here we analyzed by transcriptional profiling, genes that were altered by the presence of Caveolin-1 in such cells. A very limited set of genes including Nuclear Factor and Activator of Transcription (NF-AT) and Cathepsin L were enhanced by the presence of Caveolin-1. The majority of genes displaying significant changes were suppressed by the presence of Caveolin-1. These included Survivin, Galectin-3, Galectin 4 and Carcinoembryonic antigen (CEA), all of which have been linked to tumor development, progression or metastasis. Survivin was of particular interest, since increased expression of this protein has been correlated with inhibition of apoptosis in human cancer cells. Indeed, human colon carcinoma cells expressing Caveolin-1, and hence lacking Survivin, were shown to be more susceptible to a variety of apoptotic stimuli, including serum withdrawal and cell permeable ceramides. Thus, the tumor suppressor activity of Caveolin-1 in human colon cancer cells is linked to transcriptional suppression of target proteins like Survivin that promote cell survival.

Effects of copper ions on the free radical-scavenging properties of homocysteine: Implications of a complex formation

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Abstract

The interaction between homocysteine (HC) and copper ions was addressed *in vitro* in terms of the consequences that such interaction may have on the free radical-scavenging properties of this thiol. For such purpose, the bleaching of a colored solution containing the stable free radical cation $ABTS^+$ was employed as an *in vitro* indication of the ability of the homocysteine to scavenge free radicals. While HC concentration-dependently (1.0–10 μM bleached the $ABTS^+$ containing solution, its pre-incubation (8 μM) in the presence of Cu^2 Ions led to a metal concentration-dependent (0.1–2.5 μM) decrease of its bleaching/capacity. At a ratio of about 1:3 (2.5 μM Cu plus 8 μM HC), the bleaching capacity of the preformed Cu-HC mixture was lowered to only one third of that seen for HC alone. However, further additions of copper (as to reach a ratio of 1) did not result in greater decrease of the HC-bleaching capacity. The mixtures of Cu plus HC (0.2–8 μM plus 8 μM) presented bleaching capacities that were proportional to their thio-reactivity. Mixing increasing concentration of copper plus HC at a fixed ratio of 1:1 result in a linear and concentration-dependent increase in their bleaching-capacities. However, when TRIEN, EDTA or histidine were added to a pre-incubated (1:1) mixture of Cu plus HC, the bleaching capacity of the CU-HC mixture remained altered. Yet, the incubation of Cu with TRIEN or EDTA (but not with histidine) prior to HC addition, totally prevented the loss of the original HC-bleaching capacity. EPR studies are being conducted to investigate changes in the paramagnetic properties of copper which resulted from its interaction with HC. Data supports the contention that: (i) at concentrations found plasma, Cu and homocysteine readily interact forming in vitro a Cu-HC complex; (ii) such interaction implies lowering to one-third the original free radical scavenging capacity of HC; (iii) the thio-reactivity of the complex proportional to its bleaching capacity; (iv) the complex formed is stable to the presence of some copper-chelators. It is suggested that, of occurring *in vivo*, the interaction between Cu and HC may lead to stabilizing copper ions under a redox-inactive form which partially retains the free radical scavenging properties of homocysteine.

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Characterization of a ferric reductase from brush border membranes

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Abstract

In earlier studies, it was found that Fe^2 is the preferred substrate of the divalent cation transporter DCT1 of brush border membranes (Knöpfel M, Schulthess G, Funk F, Hauser H. 2000 *Biophys J* **79**, 874–884). This suggested that an iron reductase is also present in these membranes. We here describe the characterization of a heme containing protein fraction with NADH dependent iron and copper reductase activity from brush border membranes. The fraction contained a major protein of about 3 kDa with a b_{558} type heme. Several other proteins were repeatedly associated with this fraction, suggesting that they are part of a membrane bound protein complex. The isolated protein showed increased reductase activity only in presence of ascorbic acid. The rate constant found for the reduction of iron was $k_1 = 1.38 \pm 0.12$ min^{-1} (half time $t_{1/2} = 0.5$ min) at 25 °C and is similar to transport rates measured for DCT1. Conceivably, this protein is a ferric reductase responsible for the reduction of Fe (III) to Fe (II) prior to transport by the divalent-cation transporter DCT1 (Patent Nr. 2000 0597/00, 29.03.2000, pending).

Metallothioneins regulate intracellular copper traffic and storage

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Abstract

Our studies about the homeostatic adaptation of copper cellular metabolism including a set of metabolic parameters (uptake, storage an efflux) have been focussed in relation to duration of exposure to copper. This work have been performed using: (1) Intestinal epithelial cells (Caco-2) preloaded with copper under acute and chronic conditions and (2) hepatic cells exposed to sub-, iso- and supra-physiological copper concentrations. Our results indicated that under these different physiological contexts the cells move from one metabolic state to the other, involving changes mainly in storage and efflux, as well as changes in iron and zinc cellular content due to the imbalance of copper metabolism. The role of metallothionein in this process is under analysis. We found that mutant fibroblast null for metallothionein exhibited a lower rate of copper accumulation, relative to wild type but not significant change in ^{64}Cu efflux process. Interestingly the viability in mutant cells was affected to lower intracellular copper concentration, which was associated to changes of the subcellular distribution of this metal. We conclude that copper homeostasis in MT null fibroblasts is preserved despite lack of cytosolic MT storage
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Experimental approaches towards perturbing copper homeostasis during zebrafish embryogenesis

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Abstract

Living organisms require copper for their growth and development. Due to its high redox potential, copper is an essencial cofactor for a variety of metabolic proteins involved in a substantial number of biological processes. Because little is known about the role of copper in early embryonic development in vertebrates, we have begun to analyze the morphological, physiological and molecular effects of copper ion concentrations on the development of the freshwater teleost *Danio rerio*, commonly known as the zebrafish. As a first approach, we have exposed fish embryos to increasing copper ion concentrations from 1 to 10 μM and we have analyzed the morphological effects on embryonic development, between the stages of fertilization and organogenesis. The embryos are sensitive to concentrations as low as 2 μM copper ion and the observed effects are retardation in growth and failure of the swim bladder to inflate. At higher concentrations (10 μM) the effects are severe and include extensive necrosis and death. In parallel studies, we have taken advantage of the availability of a Heat Shock 70-GFP transgenic fish line to observe the activation of this promoter in exposed embryos. We find that the intensity of expression of GFP is correlated with the amount of copper in the growth medium and can reach levels as high as those achieved with heat shock as an inducer. These results correlate well with studies that show that HSP70 is one of the proteins induced when intracellular copper concentrations are high. Finally, we have initiated studies aimed at interfering at the genetic level with copper homeostatic mechanisms. Towards this end, we intend to misexpress the zebra fish Ctr-1 high affinity copper transporter during early embryogenesis. We have a partial cDNA for zCtr-1 and we are cloning a full length gene for these studies.

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Copper promotes the formation of short neurotic β-amyloid fibrils

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Abstract

The formation of proteinaceous aggregates, called amyloid plaques (AP), is a common feature of Alzheimer's disease (AD). The amyloid β-peptide (Aβ), the most abundant component of AP, has been characterized as a metalloprotein that could form either a redox-active copper complex or an inactive zinc complex. This suggests the potential role of these metals on the biological function of the Aβ peptide and the neurodegenerative process associated with AD. We and other reported earlier that Aβ reduces copper(II) to copper (I), probably by coordinating the metal with its histidine residues. In the present work, we evaluated the effect of copper on Aβ aggregation and neurotoxicity. First, we observed that in a slightly basic environment (pH 7.4), copper accelerated aggregation of Aβ_{1-40} with a concomitant formation of insoluble Aβ_{1-40} copper complexes. These complexes showed amyloid characteristics such as green apple birrefringence under polarized light and protease resistance to trypsin. Second, we observed that copper decreased the size of amyloid fibrils in a concentration dependent manner. Third, these short amyloid fibrils that contained copper coordinated to its structure displayed neurotoxic properties, which were manifested as inhibition of mitochondrial activity, disturbance of neuronal morphology and axonal transport disruption of mitochondria. Finally, we propose a structural model that shows hypthetical changes on the Aβ structure when it coordinates copper forming a bioinorganic complex.

Regulation of brain tau phosphorylation by the CDK5/P35 complex under oxidative stress conditions

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Abstract

Alzheimer's disease, one of the major types of dementia in the elderly, is characterized by formation in the human brain of two protein aggregates: paired helical filaments (PHF) composed mainly of hyperphosphorylated tau, and senile plaques of the Aβ amyloid. An important feature of the Alzheimer's pathogenesis is the increment in oxidative stress markers including the stress resulting from iron colocalized with amyloid plaques and PHFs, and activation of brain protein kinases that respond to oxidative stress. We have focused on the links between tau hyperphosphorylation and the oxidative stress involved in deregulation of cdk5/p35 and cdk5/p25 complexes, by using rat hippocampal cells exposed to H_2O_2, N-Acetyl-L-cysteine (NAC), Fe^{++} and deferroxamine as a biological model for these studies. The H_2O_2 treatment of hippocampal cells for two hours resulted in tau desphophorylation as revealed by the increase in Tau-1 epitopes that corresponds to those of unphosphorylated tau. On the other hand, reactivity for AT-8 and PHF-1 antibodies decreased in the Western-blot assays. p35 levels were not affected by peroxide incubation, even though the truncated form p25 decreased two-fold with respect to the controls. The NAC incubation did not induce changes in tau phosphorylation or in the p25 and p35 levels. The levels of ROS were monitored by 2,7-dichlorodihydrofluorescein-diacetate. Oxidative stress markers 4-hidroxynonenal adducts and 3-nitrotyrosine were also analyzed. This set of studies suggest that peroxide effects in inducing neuronal degeneration does not appear to be mediated by tau hyperphosphorylations. However, the levels of cdk5 a major tau phosphorylating system increased in the H_2O_2-treated cells respect to the control, while NAC did not produce any changes. These results are of importance to elucidate the mechanisms involved in the cdk5 deregulation and tau phosphorylation linked to neurodegeneration.

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Antioxidant activity of selected Chilean medicinal plants on human low density lipoprotein oxidation

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Abstract

To demonstrate the antioxidant activity of Chilean plants *Ugni molinae*, *Geum quellyon, Cheilanthes glauca* and *Amomyrthus meli*, measuring the inhibition of low-density lipoprotein (LDL) oxidation. Human LDL (d 1.019–1.063 g ml), was isolated from fasting plasma of normalipidaemic volunteers. LDL was pre-incubated with the extracts for 10 min. before adding Cu^{++}. Afterwards aliquots of reaction mixture were incubated with $CuSO_4$ at 37°C to induce lipid peroxidation. Incubation was carried out at 37 °C for different time periods (0, 2, 4 and 24 h). Incubation was determined by measuring fluorescence emission, conjugated dienes, and reactive substance to thiobarbituric acid (TBARS). Besides, oxidative modification of LDL was confirmed through electrophoresis on agarose. Ethyl acetate extract of *U. molinae* (0.0023 μM GAE) protected the LDL during 24 h from copper and temperature oxidation. The same result was obtained with methanolic extract (1.13 μM GAE and 1.32 μM GAE). The *Ch. glauca* extracts (20 μM GAE) protected from copper oxidation until 12 h, and 24 h for temperature effect. A similar effect was obtained with *G. quellyon* extracts (20 μM GAE). While *A. meli* extract (20 μM GAE) protected during 24 h from temperature and copper oxidation. These results demonstrate a positive effect of the extracts, protecting the LDL from the oxidative damage. Preliminary phytochemical analysis of these extracts related the antioxidant activity with its phenolic composition. Therefore, these plants are considered an important natural antioxidant resource and it regular intake could be a prophylactic help against coronary heart disease and atherosclerosis.

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DMT1 and Apo- transferrin are internalized to the same compartment in CACO2 cells. Evidence for iron transport by transcytosis in intestinal cell

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Abstract

Caco-2 cells grown on porous membranes in bicameral chambers are used to study the transport of ^{59}Fe from the apical (luminal) chamber to the basal (serosal) chamber. We have previously shown that transport is stimulated by apo-transferrin (apo-Tf) in the basal chamber and that apo-Tf is handled differently from Fe-Tf with the apo-Tf being transported from the basal surface to an apical, perinuclear compartment. The divalent metal transporter, DMT1, is the transporter of Fe(II) across the apical surface of intestinal cells. We have demonstrated that with exposure of the intestinal mucosa to iron, DMT1 is internalized from the brush border membrane into cytoplasmic vesicles. In the current study we use confocal microscopy to examine the intracellular traffiking of DMT1 and apo-Tf in Caco2 cells. DMT1 was visualized with a rabbit antisera to the unique C-terminus of the IRE containing DMT1 isoform. Apo-Tf offered in basal chamber was labeled with Texas Red (TxR-apo-Tf). Upon the addition of Fe(II) to the apical chamber DMT1 was rapidly internalized from the brush border to a perinuclear compartment and then gradually returned to the surface of the cells. Simultaneously, TxR-apo-Tf was rapidly internalized from the basal surface. By 20 min a substantial compartment of colocalized DMT1 and TxR-apo-Tf was visualized. The half-life of ^{35}S-methionine labeled DMT1 was not altered by iron stimulated internalization. Using ultra small paramagnetic beads to separate endocytic vesicles, it was possible to demonstrate that endosomes contained both DMT1 and apo-Tf. When ^{59}Fe was in the apical chamber and ^{125}I-apo-Tf in the basal chamber, both moieties were present in isolated endosomes. Taken together–the colocalization of apo-transferrin with DMT1 observed by confocal microscopy and the results of vesicle isolation by paramagnetic beads–suggest that at least some of the brush border membrane DMT1 fuses with vesicles from the basolateral surface which may contain apo-transferrin. Further studies involving vesicle isolation will be required to quantitate the fraction of iron that is transported across the Caco-2 cells by transcytosis.