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Research Paper

Sodium selenite supplementation does not fully restore oxidative stress-induced deiodinase dysfunction: Implications for the nonthyroidal illness syndrome

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ABSTRACT

Nonthyroidal illness syndrome (NTIS) is marked by low T3 and high reverse T3 levels. The physiopathology is poorly understood but involves oxidative stress-induced disruption of the iodothyronine deiodinases, which activate or inactivate thyroid hormones. Selenium, an essential trace element, exerts antioxidant function mainly through the thioredoxin reductase (TRx) and glutathione peroxidase (GPx) redox-regulating systems. We evaluated the effect of sodium selenite on IL6-induced disruption on deiodinase function. Cell lines expressing endogenous deiodinases type 1(D1), 2(D2) or 3(D3) (HepG2, MSTO, and MCF-7 cells, respectively) were used in an intact cell model that mimics the deiodination process under physiological conditions of substrate and cofactor, in the presence or not of IL6, with or without selenite. Deiodinase activity was quantified by the amount of iodine-125 in the medium (D1 and D2) or by ion-exchange chromatography (D3). Oxidative stress was evaluated by measuring reactive species (RS), carbonyl content as well as enzymatic and non-enzymatic antioxidant defenses. *Results*: IL6 induced ROS and carbonyl content in all 3 cell lines (all $P<0.001$). Increased ROS was paralleled by D1 and D2-decreased T3-production ($P<0.01$) and increased D3-catalyzed T3-inactivation ($P<0.001$). Selenite decreases the IL6-induced ROS and carbonyl content, while enhances Gpx and Trx activities. Nevertheless, it failed on restoring D1 or D2 function and only attenuates D3 activation ($P<0.05$). In conclusion, although sodium selenite reduces IL6-induced redox imbalance it does not fully repair deiodinase function. These results shed light on NTIS physiopathology and might explain why low T3 levels are unaffected by selenium supplementation in sick patients.

1. Introduction

The conversion of peripheral thyroxine (T4) to 3,5,3′-triiodothyronine (T3) accounts for 80% of all T3 produced in euthyroid individuals. This critical step in thyroid hormone metabolism is catalyzed by two enzymes, the type 1 (D1) and type 2 (D2) iodothyronine deiodinases, via outer-ring deiodination of the prohormone T4. Type 3 iodothyronine deiodinase (D3) catalyzes the inner (5)-ring deiodination of T4 and T3, thus inactivating thyroid hormone activity [1]. The deiodination reactions are catalyzed by an as yet undefined cofactor, probably a thiol, which acts as a reducing agent, regenerating the active enzyme. All deiodinases contain the amino acid selenocysteine in their active site, an essential residue for efficient catalysis [2–4].

The nonthyroidal illness syndrome (NTIS) refers to changes in peripheral thyroid hormone levels, characterized by low T3 and high serum reverse T3 levels. This set of alterations is observed in $>70\%$ of critically ill patients in almost every form of illness and is correlated with increased mortality [5,6]. Changes in deiodinase activities have been postulated to play an important role in the altered circulating levels of thyroid hormones in NTIS [7]. Cytokines are elevated as a generalized response to illness and available data suggest that interleukin (IL)-1β, tumor necrosis factor-α (TNFα) and, particularly IL6, play a central role in in NTIS [7]. The increased levels of IL6 lead to increases in superoxide radical ($O_2^{\bullet -}$) production through the enzyme complex of the NAD(P)H oxidase system, a major pathway of increased reactive oxidative species (ROS) generation [8]. The resulting oxidative environment impairs D1 and D2 function while inducing the expression of D3 [9]. The decreases in D1 and D2 activities occur despite increases in D1 and D2 protein levels, indicating that IL6-induced oxidative stress decreases the catalytic activity of D1 and D2, possibly by interfering with the effects of the yet unknown endogenous cofactor(s). Indeed, N-acetylcysteine (NAC), an antioxidant that restores intracellular reduced gluthatione (GSH) levels, prevents the

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IL6-induced effects on intracellular redox state [9,10].

Selenium, an essential trace element, has biological functions vitally important to human health, exerting critical intracellular antioxidant function mainly as selenoproteins [11–13]. Sodium selenite, the most commonly used form of selenium supplementation, acts through the enzymatic gluthatione peroxidase (GPx) and thioredoxin (TRx) antioxidant defense system. The GPx isoforms (GPx1–GPx6), implicated in maintaining cellular redox homeostasis, reduce hydrogen and lipid peroxides, using GSH as cofactor. TRx enzyme isoforms (TRx1–TRx3) play a role on the cellular thiol-dependent redox mechanisms as a disulfide NAD(P)H-dependent reducer [12–14]. Several studies have investigated the effects of selenium supplementation in acutely ill patients; however, the results of these studies are conflicting. While selenium supplementation, most commonly with sodium selenite, did not improve the clinical outcome or decrease mortality in some studies [15,16], others reported an inverse correlation between plasma selenium levels and sepsis-related organ failure assessment [17]. Notably, decreases in selenium plasma levels have been inversely correlated with survival rate in patients with severe sepsis or septic shock [18,19].

The aim of the present study was to evaluate the antioxidative effect of sodium selenite on the deiodination process under IL6-induced oxidative stress in an intact cell system model that mimics the NTIS.

2. Methods

2.1. Reagents

Reagents were obtained from Invitrogen (Life Technologies Inc., NY, USA), Calbiochem-Novabiochem, or Sigma-Aldrich (St. Louis, MO, USA). Outer ring-labeled [^{125}I]T3 and [^{125}I]T4 (specific activity 4400 Ci/mmol) were obtained from PerkinElmer (Boston, MA, USA). Purification of [^{125}I]T4 or [^{125}I]T3 was performed on sephadex LH-20 columns just before it was used to reduce $^{125}I^-$ to $<1\%$.

2.2. Cell culture and condition studies

Human mesothelioma (MSTO-211H) and human embryonic kidney epithelial (HEK-293) cell lines were obtained from American Type Culture Collection (Manassas, VA, USA). Hepatocarcinoma (HepG2) and human breast carcinoma (MCF-7) cell lines, were obtained from Banco de Células do Rio de Janeiro (RJ, Brazil). MSTO-211 and MCF-7 cells were cultured in RPMI-1640 (Roswell Park Memorial Institute) medium supplemented with 10% fetal bovine serum, while HepG2 and HEK-293 cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air, and the culture medium was changed three times per week. The passage number of all cell lines was less then 10–15 at the time of experiments.

In all the experiments to evaluate the effect of IL6 and sodium selenite on intracellular oxidative parameters, enzyme activity and mRNA cells were cultured with IL6, in an attempt to reproduce the pathophysiological conditions as observed in NTIS patients [20], with or without 100 nM sodium selenite (Na(2)SeO(3)) per 24 h [21]. In all experiments 20 mM NAC was used as control, since it is able to completely restore deiodinase function in the presence of augmented reactive oxygen species [9].

2.3. Deiodinase activity for D1 and D2 in intact cells

Intact cell assay for D1 and D2 was performed as previously described [22]. Cells were cultured for 24 h in 1 mL serum-free 0.1% BSA plus 740 nM of total T4 (resulting in an FT4 concentration of 2.7%; [23]), including approximately 100,000 cpm/mL [^{125}I]T4 in the presence or not of IL6, sodium selenite or NAC. Experiments were performed in duplicate or triplicate for each condition and repeated at least three times. At the completion of the experiment, 300 μL medium were removed, added to 200 μL horse serum and protein precipitated by 100 μL 50% trichloroacetic acid (TCA). After vortexing, tubes were centrifuged at 12,000 *g* for 2 min. The $^{125}I^-$ generated was expressed as the fraction of the total T4 counts minus the nonspecific deiodination in untransfected HEK-293 cells, which does not express any deiodinase activity ($<5\%$ of total [^{125}I]T4 counts), and corrected for the 50% reduction in the specific activity relative to T4. Net T3 production was calculated by multiplying the fractional conversion by the T4 concentration in the media (740 pM) and expressed as total T3 production/mg protein per 24 h. Results were expressed as iodide production. Using high-performance liquid chromatography, the authors previously demonstrated that net iodide release in this particular system – although not in skeletal muscle – is specific and equivalent to T3 production [22,24].

2.4. Deiodinase activity for D3 in intact cells

For the studies with D3, cells in 6-well plates were cultured for 24 h in 1 mL serum-free 0.5% BSA (resulting in an FT3 fraction of 3.5%; [25]) in DMEM plus 195 pM T3 (FT3, ~7 pM), including approximately 200,000 cpm/mL [^{125}I]T3 in the presence or not of IL6, sodium selenite or NAC. Sephadex LH-20 chromatography was used to measure the D3 activity in intact cells [9]. Briefly, at completion of the experiment, 300 μL medium was collected, and the reaction was stopped with 200 μL horse serum and 100 μL 50% TCA, followed by centrifugation at 12,000 *g* for 2 min to precipitate the non-metabolized [^{125}I]T3. The supernatant was used for the determination of [^{125}I]T2 and [^{125}I]T1 produced. The LH-20 column (bed volume 2 mL) was equilibrated with 1:1 0.1 M HCl, an equal volume of 0.1 M HCl was added to 500 μL samples and the mixture was then applied. Stepwise elution was performed by successive application of 2×1 mL 0.1 M HCl (for $^{125}I^-$ release), 6×1 mL 20% ethanol ([^{125}I] for T1 release), and 4×1 mL 50% ethanol in 0.1 M NaOH (1:1 v/v [^{125}I] for T2 release). The 1 mL fractions were collected and counted for radioactivity. Results are presented as the mean values derived from at least two independent experiments. Nonspecific deiodination was $<1.5\%$. Net D3 activity was calculated by multiplying the fractional conversion by the T3 concentration in the media and expressed as T3 inactivation (fmol/mg protein per 24 h). The reaction was saturated by excess unlabeled T3.

2.5. Real-time PCR

Using the same incubation conditions above, after 24 h incubation total RNA was extracted from MSTO-211, HepG2 and MCF-7 cells with an RNeasy kit (Qiagen, Hilden, Germany) and used to synthesize complementary DNA (cDNA) (SuperScript First-Strand Synthesis System for RT-PCR; Invitrogen). The generated cDNAs were used in a real-time PCR with a SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) in ABI Prism 7500 Sequence Detection System (Applied Biosystems). Standard curves representing five-point serial dilutions of cDNA of the experimental and control groups were analyzed and used as calibrators of the relative quantification of product generated in the exponential phase of the amplification curve. The r^2 was >0.99, and the amplification efficiency varied between 80% and 100%. Samples were measured by relative quantification (change in expression in the experimental group vs. control; i.e., untreated vs. treated cells). The data generated by the ABI Prism 7500 system