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The effects and underlying mechanism of excessive iodide on excessive fluoride-induced thyroid cytotoxicity

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ABSTRACT

In many regions, excessive fluoride and excessive iodide coexist in groundwater, which may lead to biphasic hazards to human thyroid. To explore fluoride-induced thyroid cytotoxicity and the mechanism underlying the effects of excessive iodide on fluoride-induced cytotoxicity, a thyroid cell line (Nthy-ori 3-1) was exposed to excessive fluoride and/or excessive iodide. Cell viability, lactate dehydrogenase (LDH) leakage, reactive oxygen species (ROS) formation, apoptosis, and the expression levels of inositol-requiring enzyme 1 (IRE1) pathway-related molecules were detected. Fluoride and/or iodide decreased cell viability and increased LDH leakage and apoptosis. ROS, the expression levels of glucose-regulated protein 78 (GRP78), IRE1, C/EBP homologous protein (CHOP), and spliced X-box-binding protein-1 (sXBP-1) were enhanced by fluoride or the combination of the two elements. Collectively, excessive fluoride and excessive iodide have detrimental influences on human thyroid cells. Furthermore, an antagonistic interaction between fluoride and excessive iodide exists, and cytotoxicity may be related to IRE1 pathway-induced apoptosis.

1. Introduction

Fluorine is a trace element that plays an important role in human health. The health effects resulting from excessive fluoride is not only presented on tooth and skeletal tissue but may also spread to the soft tissues, such as the heart, liver, kidney, gastrointestinal mucosa, lung, and brain (Barbier et al., 2010). The thyroid is capable of accumulating fluoride (Liu et al., 2012), but the effects of fluoride on the thyroid remain controversial. Some epidemiological studies have found that fluoride exposure can generate endemic thyroid goiter and

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can affect thyroid hormone synthesis. Moreover, endemic fluorosis and endemic thyroid goiters usually occur in the same region (Liu et al., 2012; Doull et al., 2006); however, many epidemiological studies have failed to characterize the relationship between excessive fluoride intake and thyroid goiter or thyroid hormone synthesis (Ozsvath, 2009). Previous animal experiments have demonstrated that excessive fluoride can weaken the function of the thyroid by impairing the thyroid structure, interfering with thyroid hormone synthesis and causing oxidative stress (Cartona, 2006; Susheela et al., 2005). Excessive fluoride can even affect thyroid function and morphology in offspring (Bouaziz et al., 2005). Although the mechanism underlying fluoride-induced thyroid damage remains unclear, considerable research has revealed that excessive fluoride could induce apoptosis in various cell types, including human embryo hepatocytes, osteoblasts, and rat hippocampal neurons (Wang et al., 2004; Xu et al., 2010; Zhang et al., 2008). Therefore, we hypothesized that fluoride-induced apoptosis may be closely associated with the thyroid cytotoxicity of fluoride.

Endoplasmic reticulum (ER) stress-induced apoptosis is a vital apoptotic pathway in mammals (Kim et al., 2008). Multiple disturbances, such as oxidative stress, can result in the accumulation of unfolded or misfolded proteins in the ER and can trigger an evolutionarily conserved response termed the "unfolded protein response" (UPR). The UPR functions to reestablish homeostasis and normal ER function. If the UPR fails to compensate for the stimulus, ER stress-induced cell death will occur (Kim et al., 2008). The inositol-requiring enzyme 1 (IRE1) pathway of the UPR is one of the most evolutionarily conserved pathways and is involved in the activation of a variety of apoptotic pathways (Oyadomari and Mori, 2004). It is also the only one of the UPR pathways to exist in all eukaryotic cells (Parmar and Schroder, 2012). Glucose-regulated protein 78 (GRP78) is a monitor of ER stress. Moreover, IRE1 and spliced X-box-binding protein-1 (sXBP-1) are important molecules of the IRE1 pathway, and CHOP is an important apoptotic factor of ER stress. These molecules were employed to evaluate the IRE1 pathway (Jiang et al., 2012).

Some scholars believe that the prevalence of endemic goiter in high fluoride areas may be the result of the joint effects of excessive fluoride and another substance (Ozsvath, 2009). Other scholars have insisted that fluoride directly causes goiter (Liu et al., 2012). Iodine, another halogen element that is similar to fluorine, also causes toxic effects in the thyroid. The results of numerous studies have suggested that iodide can alter the morphology and function of the thyroid (Joanta et al., 2006; Teng et al., 2009), induce apoptosis in thyroid cells (Vitale et al., 2000), and trigger hypothyroidism, papillary thyroid cancer or other diseases (Burgi, 2010). Thus, in theory, the presence of both excessive fluoride and excessive iodide in water may pose a biphasic threat to thyroid function.

In China, excessive fluoride and excessive iodide coexist in the groundwater in many places, such as regions near the Bohai Sea, where the concentrations of iodide and fluoride reach 1.15 and 2.85 mg/L, respectively (Hong et al., 2008). Millions of inhabitants are exposed to these two elements due to geological environmental factors.

The human thyroid follicular epithelial cell line (Nthy-ori 3-1) is comprised of normal human thyroid epithelial cells that have been immortalized *in vitro via* simian virus 40 DNA transfection but retain most of the functionality of normal thyroid cells, making it useful as a normal thyroid cell model (Ock et al., 2013). In the present study, we used Nthy-ori 3-1 cells to elucidate the mechanism underlying the development of thyroid cytotoxicity induced by excessive fluoride and to characterize the effects of excessive iodide on fluoride-induced thyroid cytotoxicity.

2. Materials and methods

2.1. Reagents

The RevertAid First Strand cDNA Synthesis Kit and PCR mix were purchased from Fermentas Inc. (Vilnius, Lithuania). Fetal bovine serum (FBS) and TRIzol were obtained from Gibco/Invitrogen Corp. (Carlsbad, CA, USA). Antibodies specific for GRP78, IRE1, and CHOP were obtained from Santa Cruz Biotechnology (CA, USA). Propidium iodide (PI) was obtained from Sigma Inc. (St. Louis, MO, USA). Sodium fluoride (NaF) was obtained from Shanghai Chemical Reagent Corp. (Shanghai, China). Potassium iodide (KI) was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All of the other reagents that were used were of analytical grade and were obtained from standard commercial suppliers.

2.2. Cell culture and treatment

Nthy-ori 3-1 cell line was purchased from the Health Protection Agency Culture Collection (HPACC, Salisbury, Wiltshire, UK). Nthy-ori 3-1 cells were cultured in 25-cm^2 flasks at a density 2–4 × 10^4/cm^2 at 37 °C in a 5% CO_2 humidified incubator and were maintained in RPMI 1640 culture medium that had been supplemented with 10% FBS. When the Nthy-ori 3-1 cells were in the logarithmic growth period, the medium was discarded, and the cells were washed twice with PBS, digested with 0.25% trypsin, and then resuspended in new RPMI 1640 medium and plated onto 6- or 96-well plates. After 36 h of incubation, the cells were divided into six groups: triple-distilled water as a control, various concentrations of NaF (0.1, 1, or 3 mM), 50 mM KI, and 1 mM NaF + 50 mM KI. After a 24-h culture, the media or cells were used for the experiments described below.

2.3. Determination of cell viability

After a 24-h incubation of the cells in the presence of six different concentrations of the working solutions, the media was replaced with 200 μl of freshly prepared media and 20 μl of MTT (5 mg/ml). After another 4 h in the incubator, 150 μl of DMSO was added, and the plate was shaken for 10 min. The optical density (OD) of each well was measured at 490 nm using a microplate reader. The results were calculated according to the following formula: S = (OD treated well − OD blank)/(OD control well − OD blank) × 100%.

2.4. Assessment of LDH leakage

After the cells had been treated for 24 h, the incubation media was collected, and the cells were gathered and lysed by adding