

Propylthiouracil, Perchlorate, and Thyroid-Stimulating Hormone Modulate High Concentrations of Iodide Instigated Mitochondrial Superoxide Production in the Thyroids of Metallothionein I/II Knockout Mice

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Background: Increased oxidative stress has been suggested as one of the underlying mechanisms in iodide excess-induced thyroid disease. Metallothioneins (MTs) are regarded as scavengers of reactive oxygen species (ROS) in oxidative stress. Our aim is to investigate the effects of propylthiouracil (PTU), a thyroid peroxidase inhibitor, perchlorate (KClO₄), a competitive inhibitor of iodide transport, and thyroid stimulating hormone (TSH) on mitochondrial superoxide production instigated by high concentrations of iodide in the thyroids of MT-I/II knockout (MT-I/II KO) mice.

Methods: Eight-week-old 129S7/SvEvBrd-Mt1^{tm1Bri} Mt2^{tm1Bri}/J (MT-I/II KO) mice and background-matched wild type (WT) mice were used.

Results: By using a mitochondrial superoxide indicator (MitoSOX Red), lactate dehydrogenase (LDH) release, and methyl thiazolyl tetrazolium (MTT) assay, we demonstrated that the decreased relative viability and increased LDH release and mitochondrial superoxide production induced by potassium iodide (100 μM) can be relieved by 300 μM PTU, 30 μM KClO₄, or 10 U/L TSH in the thyroid cell suspensions of both MT-I/II KO and WT mice (*P*<0.05). Compared to the WT mice, a significant decrease in the relative viability along with a significant increase in LDH release and mitochondrial superoxide production were detected in MT-I/II KO mice (*P*<0.05).

Conclusion: We concluded that PTU, KClO₄, or TSH relieved the mitochondrial oxidative stress induced by high concentrations of iodide in the thyroids of both MT-I/II KO and WT mice. MT-I/II showed antioxidant effects against high concentrations of iodide-induced mitochondrial superoxide production in the thyroid.

Keywords: Propylthiouracil; Perchlorate; Thyrotropin; Metallothionein I/II knockout; Iodides

INTRODUCTION

Iodide is one of the main components in thyroid hormone synthesis. The biosynthesis of thyroid hormones involves the con-

centration of iodide in the thyroid, formation of hydrogen peroxide (H₂O₂), production of thyroglobulin, oxidation of iodide and tyrosine, and the reaction of enzymes and substrates within the cells. During the formation of H₂O₂, a process known to

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take part in oxidative stress, iodide possesses stimulatory properties [1,2]. Various iodine concentrations in the thyroid gland produce different results of oxidative stress, which may either be benign or malignant [3]. In order to maintain physiological adequacy for thyroid hormone synthesis, certain amounts of reactive oxygen species (ROS) made by the thyroid epithelial cells are required. However, toxicity may occur if ROS are excessively produced [3].

Serving as protection against injuries caused by ROS due to their antioxidant properties, metallothioneins (MTs) are from a group of intracellular, cysteine-rich, metal-binding proteins [4]. There are four isoforms of mammalian MT: MT-1, MT-2, MT-3, and MT-4. The MT-1 and MT-2 isoforms, which only differ by one negative charge, are the most extensively expressed isoforms in a variety of tissues [5,6]. They play an important role in heavy metal detoxification and metal homeostasis [7]. Additionally, MTs have a potent antioxidant function, which may act as an adaptive protein that shields cells and tissues from oxidative stress [8,9]. In light of these characteristics, MT-I/II knockout (MT-I/II KO) mice and background-matched wild type (WT) mice were used to investigate the effects of mitochondrial oxidative stress in the thyroid in our present study.

Propylthiouracil (PTU) is an anti-thyroid agent and an inhibitor of thyroid peroxidase (TPO), it plays a role in inhibiting iodide oxidation and monoiodotyrosine iodination, interferes with the synthesis of thyroxine (T₄) production, and blocks the peripheral conversion of T₄ to triiodothyronine [10]. Perchlorate (KClO₄) competitively inhibits iodide uptake into the thyroid at the sodium/iodine symporter (NIS), decreases intrathyroidal iodide content, and interrupts hypothalamic-pituitary-thyroid axis homeostasis [11]. Thyroid stimulating hormone (TSH) is the main regulator of iodide uptake [12]. In addition to regulating NIS transcription and translation, TSH modulates NIS activity by a post-transcriptional mechanism [13]. Moreover, TSH can distinctively enhance the expression of thiol-specific antioxidant (TSA), which protects the thyroid against excessive ROS production [14].

Based on the role of MTs and on our previous report that shows PTU, KClO₄, or TSH can modulate high concentrations of iodide-induced oxidative stress in Fischer rat thyroid cell line (FRTL) cells [15], we aim to investigate the effects of PTU, KClO₄, and TSH on mitochondrial superoxide production instigated by high concentrations of iodide in the thyroid with or without MT-I/II in our present study. We propose that thyrocytes with MT-I/II may have stronger antioxidant ability than those without MT-I/II. Furthermore, we aim to figure out

whether PTU, KClO₄, or TSH may aid the thyroid in times of high concentrations of iodide induced oxidative stress.

METHODS

Reagents

MitoSOX Red was purchased from Invitrogen (Invitrogen Life Technologies, Carlsbad, CA, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (methyl thiazolyl tetrazolium [MTT]), TSH, PTU, KClO₄ and Coon's F12 medium were purchased from Sigma (Sigma-Aldrich, MO, USA). Fetal bovine serum (FBS) were purchased from GE Healthcare Life Sciences (Hyclone, UT, USA). Lactate dehydrogenase (LDH) release was measured using a cytotoxicity detection kit (LDH; Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). All other chemicals that made in China were of analytical grade.

Animals and thyroid cell suspension preparation

Eight-week-old 129S7/SvEvBrd-Mt1^{tm1Bri} Mt2^{tm1Bri}/J (MT-I/II KO) mice were ordered from Jackson Lab (NO. 002211, Jackson Lab, Bar Harbor, ME, USA) and background-matched WT mice were used. The thyroid glands were obtained after the mice were sacrificed. The glands from 8 mice were pooled and used for the preparation of the thyroid cell suspensions for each treatment. The fascia and connective tissue were removed. The thyroid tissue was cut into small pieces under sterile conditions, digested with 1 mg/mL of trypsin, and were oscillated for 40 minutes in a 37°C water bath. The thyroid cell suspensions were collected in a centrifuge tube and centrifuged at 800 rpm/min for 10 minutes, after which the supernatant was removed. A cell suspension (5×10⁴ cells/well) was incubated in Coon's F12 medium with 10% FBS at 37°C in an atmosphere containing 5% CO₂ and 95% air while being exposed to 100 μM potassium iodide (KI), with or without 30 μM KClO₄, 300 μM PTU, and 10 U/L TSH for 2 hours. The group without KI was regarded as the control group. Animal procedures were approved by the Institutional Animal Care and Use Committee of Tianjin Medical University and in accordance with the National Institutes of Health Guide.

Cell viability assay

Cell viability was evaluated using the MTT assay. A thyroid cell suspension (5×10⁴ cells/well) was prepared first. The thyroid cell suspension was exposed to KI (100 μM), with or without 30 μM KClO₄, 300 μM PTU, and 10 U/L TSH for 2 hours. The group without KI served as the control group, the well

which contained solely medium was regarded as the blank group. Subsequently, 10 μ L MTT (5 mg/mL) was added in the dark and covered with aluminum foil, and incubated at 37°C in an atmosphere containing 5% CO₂ and 95% air for 4 hours. After the incubation, the supernatant was removed, and 100 μ L dimethyl sulfoxide was added to each well and was shaken for 10 minutes in order to dissolve the formazan crystals that were formed. The values of absorbance were measured by a spectrophotometer at 490 nm (Wallac 1420 VICTOR3, PerkinElmer, Waltham, MA, USA).

LDH release

LDH release in the supernatant following different treatments was measured using a cytotoxicity detection kit. LDH is an oxidoreductase that catalyzes the interconversion of lactate and pyruvate. LDH is a stable cytosolic enzyme found in all cells. It is released swiftly into the cell culture supernatant upon membrane damage. This is completed in order to evaluate the presences of damage or toxicity in the tissues and cells. The LDH assay was conducted by following the manufacturer's protocols. The assay is based on the reduction of the 2-p-iodophenyl-3-nitrophenyl tetrazolium chloride (tetrazolium INT) to a red formazan, which is specifically detected by colorimetric (450 nm) assay. The values of absorbance were measured using a spectrophotometer at 450 nm (Wallac 1420 VICTOR3).

Flow cytometry

Flow cytometry was carried out using a FACScalibur (BD Bioscience, San Jose, CA, USA). A mitochondrial superoxide indicator (MitoSOX Red) was used to measure mitochondrial superoxide production by flow cytometry. A thyroid cell suspension was prepared first. The thyroid cell suspension was then exposed to 100 μ M KI, with or without 30 μ M KClO₄, 300 μ M PTU, and 10 U/L TSH for 2 hours. Subsequently, 5 μ M MitoSOX was added and incubated for 10 minutes at 37°C in the dark. Next, the cells were centrifuged, washed with Hank's solution, and then suspended in Hank's solution with 1% bovine serum albumin. The fluorescence intensity of MitoSOX was detected using a FACScalibur, and the excitation/emission wavelength was 488 nm/575 nm. By collecting FL2 channel forward scattering and lateral scattering data, 10,000 cells were collected for each sample. The control group without MitoSOX was regarded as the blank zero group for standardization.

Statistics

The data was represented as mean \pm SD. One-way analysis of

variance with the least significant difference test was performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) to determine the differences between the groups. A *P* value of less than 0.05 was considered to be statistically significant.

RESULTS

PTU regulates KI-induced mitochondrial oxidative stress *Effects of PTU (300 μ M) on KI (100 μ M) induced mitochondrial superoxide production*

Compared to the control group, a significant increase in mitochondrial superoxide production was detected in the KI group in both MT-I/II KO and WT mice ($P < 0.05$); however, no significant differences were detected in the PTU and the KI+PTU groups. Compared to the KI group, a significant decrease in mitochondrial superoxide production was detected in both the PTU groups and the KI+PTU groups for both MT-I/II KO and WT mice ($P < 0.05$). Compared to the WT mice, a strong increase in mitochondrial superoxide production was observed in the KI, PTU, and KI+PTU groups of the MT-I/II KO mice ($P < 0.05$) (Fig. 1A, B).

Effects of PTU (300 μ M) on KI (100 μ M) induced LDH release

Compared to the control group, a significant increase in LDH release was detected in the KI group for both MT-I/II KO and WT mice ($P < 0.05$); however, no significant differences were observed in the PTU group and the KI+PTU group ($P > 0.05$). Compared to the KI treatment group, the PTU group and the KI+PTU group exhibited significantly decreased LDH release in both MT-I/II KO and WT mice ($P < 0.05$). A strong increase in LDH release was detected in the KI group for MT-I/II KO mice compared to the WT mice ($P < 0.05$). No significant differences were observed between the MT-I/II KO mice and WT mice in both the PTU group and KI+PTU group ($P > 0.05$) (Fig. 1C).

Effects of PTU (300 μ M) on KI (100 μ M) induced relative viability

Compared to the control group, the relative viability of the KI treatment group in both MT-I/II KO and WT mice was significantly decreased ($P < 0.05$), while the PTU and KI+PTU groups showed no significant differences. Compared to the KI group, a significant increase in relative viability was detected in both the PTU and KI+PTU groups for MT-I/II KO and WT mice ($P < 0.05$). Compared to the WT mice, there was a significant decrease in relative viability in the KI treatment group for MT-I/II KO mice ($P < 0.05$), while no significant changes were observed in both the PTU and KI+PTU groups between the

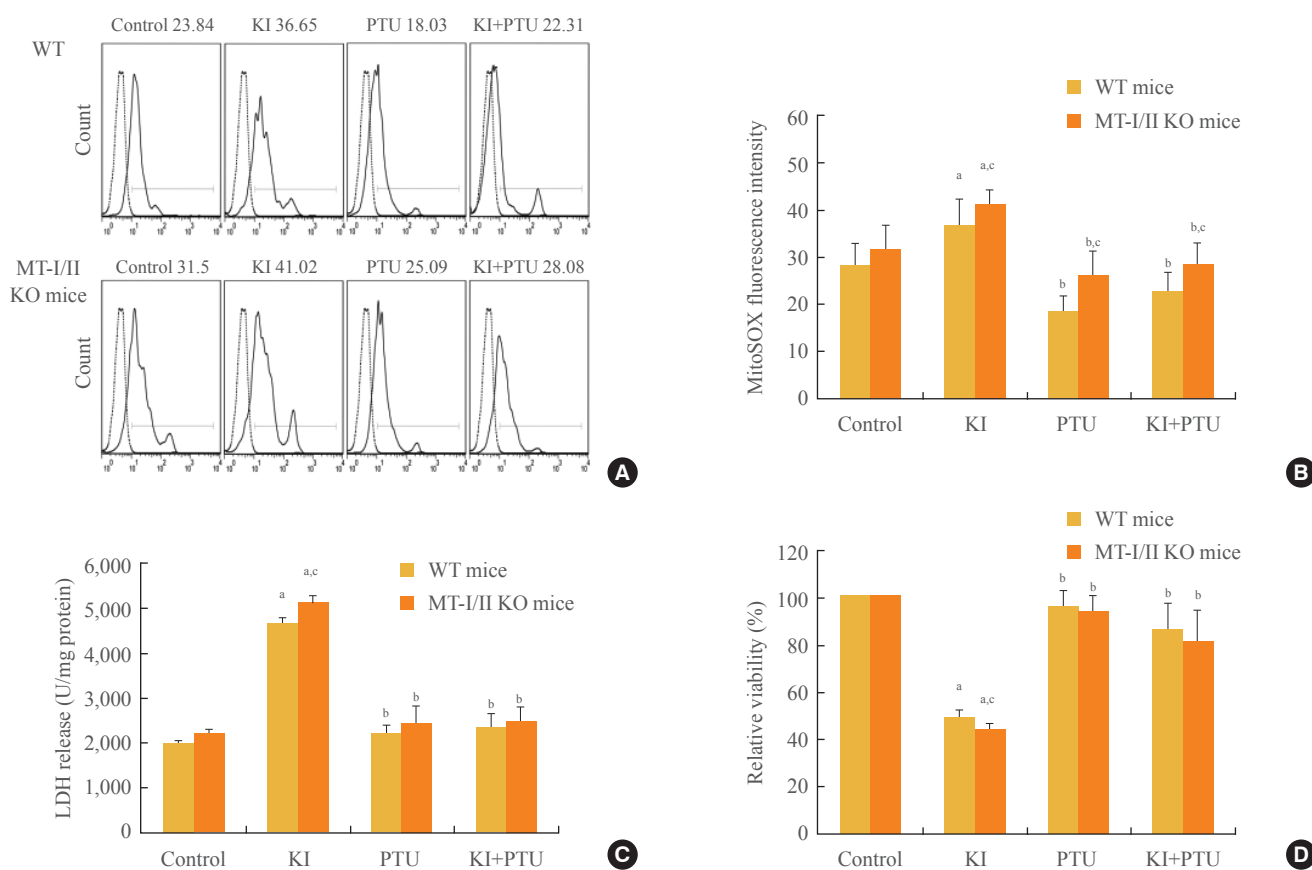


Fig. 1. Effect of propylthiouracil (PTU) on high concentrations of iodide induced relative viability, lactate dehydrogenase (LDH) release and mitochondrial superoxide production in metallothionein I/II knockout (MT-I/II KO) and wild type (WT) mice. (A, B) Effects of PTU on high concentrations of iodide induced mitochondrial superoxide production. Histogram analysis was performed on the mean fluorescence intensity of MitoSOX Red as measured by flow cytometry. Experiments were repeated 3 times with similar results. (C) Effects of PTU on high concentrations of iodide induced LDH release. (D) Effects of PTU on high concentrations of iodide induced relative viability. One-way analysis of variance with a least significant difference test was used. ^a $P < 0.05$ compared with the control group of WT or MT-I/II KO mice respectively; ^b $P < 0.05$ compared with the potassium iodide (KI) group of WT or MT-I/II KO mice respectively; ^c $P < 0.05$, WT mice compared with the MT-I/II KO mice under the same treatment.

MT-I/II KO and WT mice ($P > 0.05$) (Fig. 1D).

KClO₄ modulates KI induced oxidative stress

Effects of KClO₄ (30 μ M) on KI (100 μ M) induced mitochondrial superoxide production

Compared to the control group, a significant increase in mitochondrial superoxide production was detected in the KI group ($P < 0.05$), while no significant differences were observed in the KClO₄ and the KI+KClO₄ group for both MT-I/II KO and WT mice ($P > 0.05$). Compared to the KI group, a significant decrease in mitochondrial superoxide production was seen in both the KClO₄ and KI+KClO₄ groups for MT-I/II KO and WT mice ($P < 0.05$). Compared to the WT mice, MT-I/II KO mice exhibited a significant increase in mitochondrial superoxide

production in both KI and KI+KClO₄ groups ($P < 0.05$) (Fig. 2A, B).

Effects of KClO₄ (30 μ M) on KI (100 μ M) induced LDH release

Compared to the control group, the LDH release in the KI treatment group was significantly increased in both the MT-I/II KO and WT mice ($P < 0.05$), while no significant differences were observed in KClO₄ or KI+KClO₄ group for MT-I/II KO and WT mice ($P > 0.05$). Compared to the KI group, a significant decrease in LDH release was detected in both KClO₄ and KI+KClO₄ groups for MT-I/II KO and WT mice ($P < 0.05$). Compared to the WT mice, a significant increase in LDH release was detected in the KI group for MT-I/II KO mice ($P < 0.05$). No significant differences were detected in the

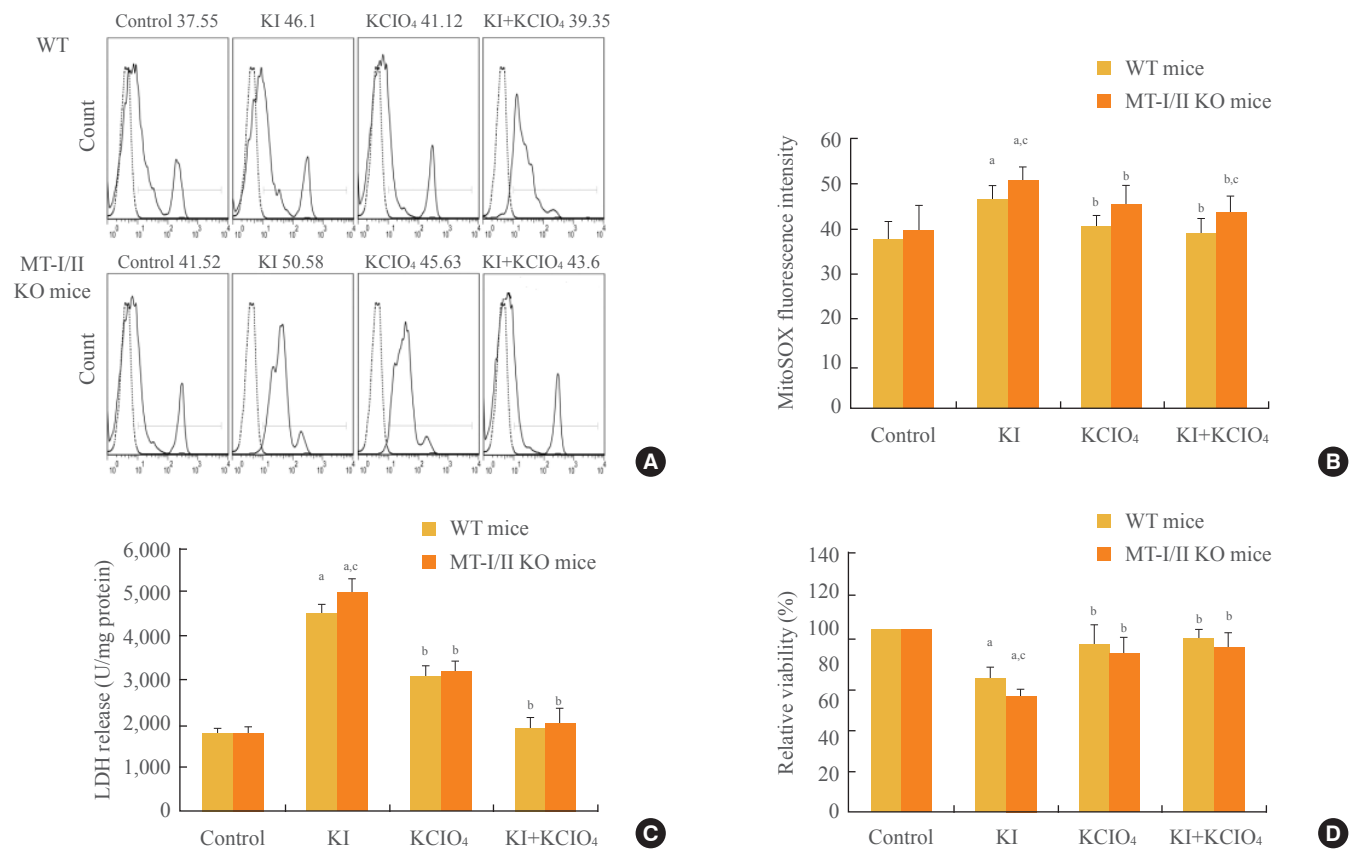


Fig. 2. Effect of perchlorate (KClO_4) on high concentrations of iodide induced relative viability, lactate dehydrogenase (LDH) release and mitochondrial superoxide production in metallothionein I/II knockout (MT-I/II KO) and wild type (WT) mice. (A, B) Effects of KClO_4 on high concentrations of iodide induced mitochondrial superoxide production. Histogram analysis was performed on the mean fluorescence intensity of MitoSOX Red as measured by flow cytometry. Experiments were repeated 3 times with similar results. (C) Effects of KClO_4 on high concentrations of iodide induced LDH release. (D) Effects of KClO_4 on high concentrations of iodide induced relative viability. One-way analysis of variance with a least significant difference test was used. ^a $P < 0.05$ compared with the control group of WT or MT-I/II KO mice respectively; ^b $P < 0.05$ compared with the potassium iodide (KI) group of WT or MT-I/II KO mice respectively; ^c $P < 0.05$ WT mice compared with the MT-I/II KO mice under the same treatment.

KClO_4 and KI+ KClO_4 groups between the MT-I/II KO and WT mice ($P > 0.05$) (Fig. 2C).

Effects of KClO_4 (30 μM) on KI (100 μM) induced relative viability

Compared to the control group, the relative viability of the KI group was significantly decreased for both the MT-I/II KO and WT mice ($P < 0.05$), while no significant differences were observed in the KClO_4 group or the KI+ KClO_4 group ($P > 0.05$). Compared to the KI group, a significant increase in relative viability was detected in the KClO_4 and the KI+ KClO_4 groups for both MT-I/II KO and WT mice ($P < 0.05$). Compared to the WT mice, there was a significant decrease in relative viability in the KI group for MT-I/II KO mice ($P < 0.05$). No significant differences were detected in the KClO_4 and KI+ KClO_4 groups

between the MT-I/II KO and WT mice ($P > 0.05$) (Fig. 2D).

TSH modulates KI induced oxidative stress

Effects of TSH (10 U/L) on KI (100 μM) induced mitochondrial superoxide production

Compared to the control group, a significant increase was detected in the KI group in mitochondrial superoxide production ($P < 0.05$), while no significant differences were detected in the TSH or the KI+TSH group for both MT-I/II KO and WT mice ($P > 0.05$). Compared to the KI group, a significant decrease can be observed in the TSH and KI+TSH group for both MT-I/II KO and WT mice ($P < 0.05$). Compared to the WT mice, significant increases in mitochondrial superoxide production can be detected in both the KI and TSH groups for MT-I/II KO

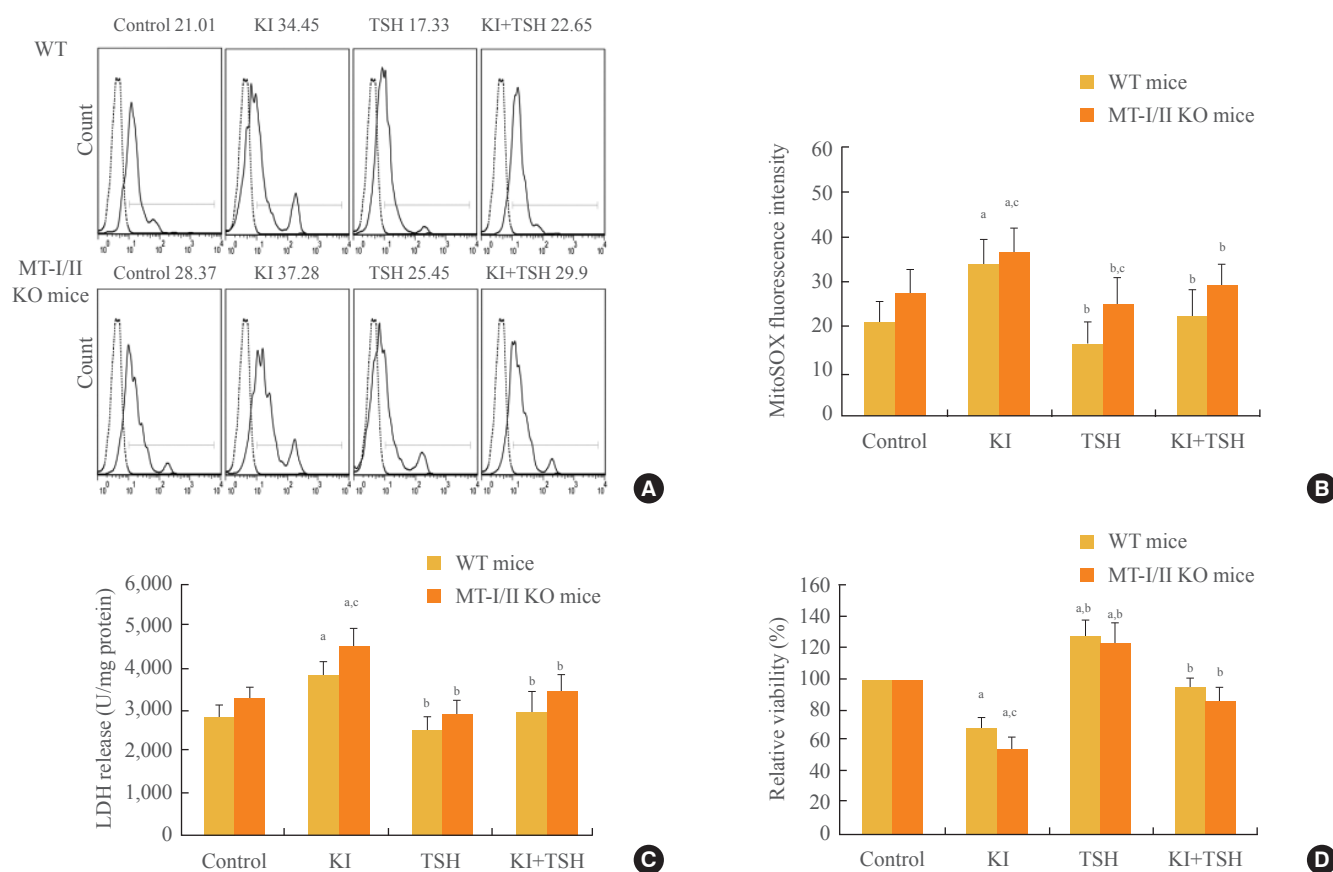


Fig. 3. Effect of thyroid stimulating hormone (TSH) on high concentrations of iodide induced relative viability, lactate dehydrogenase (LDH) release and mitochondrial superoxide production in metallothionein I/II knockout (MT-I/II KO) and wild type (WT) mice. (A, B) Effects of TSH on high concentrations of iodide induced mitochondrial superoxide production. Histogram analysis was performed on the mean fluorescence intensity of MitoSOX Red as measured by flow cytometry. Experiments were repeated 3 times with similar results. (C) Effects of TSH on high concentrations of iodide induced LDH release. (D) Effects of TSH on high concentrations of iodide induced relative viability. One-way analysis of variance with a least significant difference test was used. ^a $P < 0.05$ compared with the control group of WT or MT-I/II KO mice respectively; ^b $P < 0.05$ compared with the potassium iodide (KI) group of WT or MT-I/II KO mice respectively; ^c $P < 0.05$ WT mice compared with the MT-I/II KO mice under the same treatment.

mice ($P < 0.05$). No significant differences were detected between MT-I/II KO and WT mice in the KI+TSH treatment group ($P > 0.05$) (Fig. 3A, B).

Effects of TSH (10 U/L) on KI (100 μ M) induced LDH release

Compared to the control group, a significant increase in LDH release was detected in the KI group ($P < 0.05$), while no significant differences were observed in the TSH treatment group and the KI+TSH treatment group for MT-I/II KO and WT mice ($P > 0.05$). Compared to the KI group, a significant decrease in LDH release was detected in the TSH and KI+TSH groups in both MT-I/II KO and WT mice ($P < 0.05$). Compared to the WT mice, there was a significant increase in LDH release in the KI group for MT-I/II KO mice ($P < 0.05$), while no significant

changes were detected in the TSH and KI+TSH groups for MT-I/II KO mice ($P > 0.05$) (Fig. 3C).

Effects of TSH (10 U/L) on KI (100 μ M) induced changes of relative viability

Compared to the control group, a significant decrease in relative viability was detected in the KI group; however, a significant increase was detected in the TSH groups for both MT-I/II KO and WT mice ($P < 0.05$). Compared to the KI treatment group, an increase in relative viability was seen in both the TSH and KI+TSH groups ($P < 0.05$) for both MT-I/II KO and WT mice. Compared to the WT mice, MT-I/II KO mice showed a significant decrease in relative viability in the KI group ($P < 0.05$). MT-I/II KO mice showed no significant

changes in the TSH or the KI+TSH group when compared to the WT mice ($P>0.05$) (Fig. 3D).

DISCUSSION

We demonstrated in the present study that PTU, $KClO_4$, and TSH modulate high concentrations of iodide-induced mitochondrial superoxide production in the thyroid cells of MT-I/II KO mice. In addition, MT-I/II exhibited antioxidant effects on high concentrations of iodide-induced mitochondrial superoxide production in the thyroid.

Iodide excess is considered an environmental risk factor during thyroid disease development and has been linked to various associated disorders like autoimmune thyroiditis [16-23]. The imbalance caused by increased oxidative stress due to high concentrations of iodide is an underlying mechanism that is unable to be corrected by endogenous antioxidant systems [3,15]. In this study, we focused on the effects of PTU, $KClO_4$, and TSH on high concentrations of iodide-induced mitochondrial superoxide production in the thyroid with or without MT-I/II. We have previously demonstrated the concentration-response and time-course response of KI on FRTL cells [15]. A significant iodide stimulatory effect was detected in the 10^{-4} M KI and 10^{-3} M KI exposure groups following 2 hours of exposure [15,24]. Our results are in accord with the report that a maximum stimulatory effect on H_2O_2 production was achieved after 2 hours incubation with 10^{-4} M KI in bovine thyroid slices [1]. Accordingly, for our present study, we chose a concentration of 10^{-4} M KI in order to investigate the effects of PTU, $KClO_4$, and TSH on mitochondrial oxidative stress in the thyroids of MT-I/II KO mice.

We showed that PTU attenuates mitochondrial superoxide production induced by high concentrations of iodide in both MT-I/II KO and WT mice. After employing 300 μ M PTU in this study, there were no significant cytotoxic effects on cell viability in contrast to the control group. These findings are consistent with previous research that 300 μ M PTU completely inhibited the morphological change brought about by KI. These cells remained in their original morphology as flat, polygonal, and adherent cells in appearance [25]. TPO is a tissue-specific peroxidase involved in transforming ionic iodide into its molecular form (I_2) in the thyroid cell membrane through oxidation [24]. The inhibitory effect of PTU on TPO is evident through the inhibition of TPO and molecular iodine formation in the thyroid [26]. Inhibition of nicotinamide adenine dinucleotide phosphate (NADPH)-dependent H_2O_2 production is re-

garded as a probable method of action [27,28]. Another possible method of action is via inhibition of Ca^{2+} /NADPH-dependent H_2O_2 production in the thyroid [29]. Additionally, PTU is considered to be very adept at scavenging for ROS [30]. Certain cellular injuries such as radiation-induced damage require protection by PTU in the form of reduced apoptotic events and decreased ROS production [31]. As a reliable scavenger for ROS, PTU treatment is very effective for arsenic trioxide toxicity in the livers of rats [32]. Substantial inhibitory effects on superoxide induced reduction of cytochrome c reflected the antioxidant characteristics of PTU. These effects that protect against liver damage are brought about by certain scavenging reactions with hydroxyl radicals, which further demonstrate the antioxidant capabilities of PTU [30].

Additionally, we found that significantly increased mitochondrial superoxide production induced by high concentrations of iodide can be relieved by $KClO_4$ in both MT-I/II KO and WT mice. $KClO_4$ is a competitive inhibitor of iodide transport. It is also known to interfere with thyroid iodide accumulation and is able to block iodine uptake by the inhibition of NIS at the thyroid follicle [33,34]. Inhibition of iodide uptake at the NIS by $KClO_4$ was found to be 30 times more potent than that of iodide [35]. $KClO_4$ protects against high concentrations of iodide-induced oxidative stress by not allowing iodide to enter the thyroid. Due to various indications of greater environmental distribution of $KClO_4$ in water, food, and other media, $KClO_4$ is under specific regulation [36]. To prevent goitrogenic effects of $KClO_4$ exposure among persons with insufficient iodine, a modest iodine dietary intake is recommended for those who consume $KClO_4$ in drinking water [37].

We demonstrated that TSH (10 U/L) decreases KI-mediated mitochondrial superoxide production in both MT-I/II KO and WT mice. This result is in accord with our report regarding FRTL cells [23]. It is known that TSH augments the function of NIS and leads to increased ^{131}I uptake [38], which subsequently increases ROS production and reduces cellular viability. In addition, TSH regulates NIS transcription and translation, and modulates NIS activity by a post-transcriptional process [13]. In response to the physiological function of TSH, thyroid cells are continually subject to ROS activity as they are engaged in the production of elevated amounts of H_2O_2 [14]. However, thyrocytes utilize multiple defense mechanisms specific to ROS, such as TSA and superoxide anion dismutase. TSH is known to activate antioxidant systems and control the TSA gene [14,23]. Regulation of the transcriptional expression of TSA in thyrocytes is completed by TSH due to its antioxidant

features [14]. Usually, an elevated concentration of H₂O₂ induces cellular apoptosis; however, this phenomenon was diminished by the overexpression of TSA. This suggests that constitutive TSA expression is involved in this regulation and that TSH-induced TSA expression plays a pivotal role in removing excess H₂O₂ during thyroid cellular homeostasis [14]. Moreover, TSH can prevent apoptosis and sustain cell viability by strengthening the cell-matrix adhesion and by assisting cell cycle progression to a certain degree via the cyclic adenosine monophosphate pathway in FRTL-5 cells. Therefore, this suggests that in addition to regulating growth and differentiation, TSH may also function as a mechanism for survival in thyroid cells [39].

Furthermore, we demonstrated that significant increases in mitochondrial superoxide production and LDH release along with the decrease in relative cell viability of the MT-I/II KO mice displays the protective action of MT-I/II against mitochondrial superoxide production induced by high concentrations of iodide in thyrocytes. We have previously demonstrated that iodide excess in thyroid glands without MT-I/II protection may result in strong mitochondrial oxidative stress, which further leads to the damage of thyrocytes *in vivo* [40]. This may be explained by the protective effect of MTs against oxidative stress. MTs are derived from a group of proteins that have intracellular, cysteine-rich, and metal-binding characteristics [4,41], and they are found in almost every kind of tissue and cell [42-45]. MTs possess high antioxidant qualities that protect against damage due to ROS production, including exogenous sources such as ionizing radiation, as well as various chemotherapy drugs [42]. Moreover, the upregulation of antioxidant enzymes improves plant stress tolerance by maintaining redox balance and lessens injuries as a result of the damaging effects of ROS production [46]. MTs can be beneficial [39] in a surplus of oxidative events, such as doxorubicin cardiotoxicity, ischemia/reperfusion injury, diabetes, and alcohol administration [47-51].

In regard to MTs expression during states of excess iodide, since iodide excess increases oxidative stress [52,53], similar degrees of MT expression may be observed in various types of cells, especially thyroid cells, during oxidative stress. Previous studies have exhibited the link between excess iodide exposure and its role in increasing oxidative stress in the rat model [52]. Specifically, high concentrations of KI damaged rat thyroid follicular cells and increased oxidative stress indicators, which demonstrated the effects of oxidative damage caused by varying concentrations of KI [52]. In mice, the MT-I and MT-II genes are highly upregulated in response to oxidative stress

[54]. Also, most studies have reported that cells lacking MT-I or MT-II are more sensitive to a wide range of stressors, such as oxidative stress and excess of heavy metals. Santon et al. [55] in their experiments on MT^{+/+} cells, have shown that MT biosynthesis is readily induced by Cd treatment, with a concomitant decrease in sensitivity to injury by ROS. Inoue et al. [56] suggested that MT demonstrates strong antioxidant properties. MT protein levels in rodent liver and mRNA levels in hepatic cell lines are increased following injection with compounds that result in free radical formation, such as carbon tetrachloride, menadione, or paraquat. An injection of ferric nitrilotriacetate, which produces ROS, induces transcription of MT in the liver and kidney. These findings suggest that MT plays a key role in oxidative stress. Consistent with this, MT is able to scavenge a wide range of ROS including superoxide, H₂O₂, hydroxyl radicals, and nitric oxide [56]. Higashimoto et al. [57] also showed that MT plays a preventive role by acting as an antioxidant along with a decrease in glutathione by repeated stress, and that MT may be an essential factor for inducing carnitine under stress. Hu et al. [58] reported that the cardiac-specific overexpression of MT rescues nicotine exposure-induced cardiac contractile dysfunction and fibrosis possibly through the inhibition of ROS accumulation and apoptosis. Ruiz-Riol et al. [59] also reported MTs are small proteins induced by tissue stress that can contribute to the restoration of homeostasis in tissue inflammation, and it was found to be increased in a transcriptomic analysis of Graves disease (GD) glands, which shows that overexpression of MT-I/II is a new feature of thyroid follicular cells in GD. Moreover, Zhou et al. [60] suggested that chronic intermittent hypoxia may lead to aortic damages characterized by oxidative stress and inflammation, and MTs may play a pivotal role in the above pathogenesis. Yang et al. [61] suggested that MTs protect against endoplasmic reticulum stress-induced cardiac anomalies possibly through the attenuation of cardiac autophagy. Similarly, in our previous study, we have reported that iodide excess in a thyroid without MT-I/II protection may result in strong mitochondrial oxidative stress, which further leads to the damage of thyrocytes [40].

In conclusion, our study provides evidence that MT-I/II display its antioxidant effects in high concentrations of iodide-induced oxidative stress in the thyroid. These 300 μM PTU, 30 μM KClO₄, or 10 U/L TSH relieve the oxidative stress induced by high concentrations of iodide (10⁻⁴ M KI) in both MT-I/II KO and WT mice.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Corvilain B, Collyn L, van Sande J, Dumont JE. Stimulation by iodide of H₂O₂ generation in thyroid slices from several species. *Am J Physiol Endocrinol Metab* 2000;278:E692-9.
2. Bürgi H. Iodine excess. *Best Pract Res Clin Endocrinol Metab* 2010;24:107-15.
3. Poncin S, Gerard AC, Boucquey M, Senou M, Calderon PB, Knoop B, et al. Oxidative stress in the thyroid gland: from harmlessness to hazard depending on the iodine content. *Endocrinology* 2008;149:424-33.
4. Chiaverini N, De Ley M. Protective effect of metallothionein on oxidative stress-induced DNA damage. *Free Radic Res* 2010;44:605-13.
5. Miles AT, Hawksworth GM, Beattie JH, Rodilla V. Induction, regulation, degradation, and biological significance of mammalian metallothioneins. *Crit Rev Biochem Mol Biol* 2000;35:35-70.
6. Vasak M. Advances in metallothionein structure and functions. *J Trace Elem Med Biol* 2005;19:13-7.
7. Palmiter RD. The elusive function of metallothioneins. *Proc Natl Acad Sci U S A* 1998;95:8428-30.
8. Cai L, Klein JB, Kang YJ. Metallothionein inhibits peroxynitrite-induced DNA and lipoprotein damage. *J Biol Chem* 2000;275:38957-60.
9. Kang YJ, Chen Y, Yu A, Voss-McCowan M, Epstein PN. Overexpression of metallothionein in the heart of transgenic mice suppresses doxorubicin cardiotoxicity. *J Clin Invest* 1997;100:1501-6.
10. Garcia-Mayor RV, Larranaga A. Treatment of Graves' hyperthyroidism with thionamides-derived drugs: review. *Med Chem* 2010;6:239-46.
11. Martino E, Mariotti S, Aghini-Lombardi F, Lenziardi M, Morabito S, Baschieri L, et al. Short term administration of potassium perchlorate restores euthyroidism in amiodarone iodine-induced hypothyroidism. *J Clin Endocrinol Metab* 1986;63:1233-6.
12. Suzuki K, Mori A, Saito J, Moriyama E, Ullianich L, Kohn LD. Follicular thyroglobulin suppresses iodide uptake by suppressing expression of the sodium/iodide symporter gene. *Endocrinology* 1999;140:5422-30.
13. Sue M, Akama T, Kawashima A, Nakamura H, Hara T, Tanigawa K, et al. Propylthiouracil increases sodium/iodide symporter gene expression and iodide uptake in rat thyroid cells in the absence of TSH. *Thyroid* 2012;22:844-52.
14. Kim H, Park S, Suh JM, Chung HK, Shong M, Kwon OY. Thyroid-stimulating hormone transcriptionally regulates the thiol-specific antioxidant gene. *Cell Physiol Biochem* 2001;11:247-52.
15. Yao X, Li M, He J, Zhang G, Wang M, Ma J, et al. Effect of early acute high concentrations of iodide exposure on mitochondrial superoxide production in FRTL cells. *Free Radic Biol Med* 2012;52:1343-52.
16. Luo Y, Kawashima A, Ishido Y, Yoshihara A, Oda K, Hiroi N, et al. Iodine excess as an environmental risk factor for autoimmune thyroid disease. *Int J Mol Sci* 2014;15:12895-912.
17. Du Q, Zhu H, Yao L. Thyroid function: comparison of women in late pregnancy with control women of reproductive age in regions of dietary iodine excess. *Asia Pac J Public Health* 2013;25(4 Suppl):36S-42S.
18. Pearce EN, Andersson M, Zimmermann MB. Global iodine nutrition: where do we stand in 2013? *Thyroid* 2013;23:523-8.
19. Ong CB, Herdt TH, Fitzgerald SD. Hyperplastic goiter in two adult dairy cows. *J Vet Diagn Invest* 2014;26:810-4.
20. Roti E, Uberti ED. Iodine excess and hyperthyroidism. *Thyroid* 2001;11:493-500.
21. Teng W, Shan Z, Teng X, Guan H, Li Y, Teng D, et al. Effect of iodine intake on thyroid diseases in China. *N Engl J Med* 2006;354:2783-93.
22. Tan L, Sang Z, Shen J, Liu H, Chen W, Zhao N, et al. Prevalence of thyroid dysfunction with adequate and excessive iodine intake in Hebei Province, People's Republic of China. *Public Health Nutr* 2015;18:1692-7.
23. Laurberg P, Cerqueira C, Ovesen L, Rasmussen LB, Perrild H, Andersen S, et al. Iodine intake as a determinant of thyroid disorders in populations. *Best Pract Res Clin Endocrinol Metab* 2010;24:13-27.
24. Wang L, Duan Q, Wang T, Ahmed M, Zhang N, Li Y, et al. Mitochondrial respiratory chain inhibitors involved in ROS production induced by acute high concentrations of iodide and the effects of SOD as a protective factor. *Oxid Med Cell*

- Longev 2015;2015:217670.
25. Vitale M, Di Matola T, D'Ascoli F, Salzano S, Bogazzi F, Fenzi G, et al. Iodide excess induces apoptosis in thyroid cells through a p53-independent mechanism involving oxidative stress. *Endocrinology* 2000;141:598-605.
 26. Pereira A, Braekman JC, Dumont JE, Boeynaems JM. Identification of a major iodolipid from the horse thyroid gland as 2-iodohexadecanal. *J Biol Chem* 1990;265:17018-25.
 27. Ferreira AC, de Carvalho Cardoso L, Rosenthal D, de Carvalho DP. Thyroid Ca²⁺/NADPH-dependent H₂O₂ generation is partially inhibited by propylthiouracil and methimazole. *Eur J Biochem* 2003;270:2363-8.
 28. Engler H, Taurog A, Nakashima T. Mechanism of inactivation of thyroid peroxidase by thioureylenes. *Biochem Pharmacol* 1982;31:3801-6.
 29. Smyth PP. Role of iodine in antioxidant defence in thyroid and breast disease. *Biofactors* 2003;19(3-4):121-30.
 30. Hicks M, Wong LS, Day RO. Antioxidant activity of propylthiouracil. *Biochem Pharmacol* 1992;43:439-44.
 31. Perona M, Dagrosa MA, Pagotto R, Casal M, Pignataro OP, Pisarev MA, et al. Protection against radiation-induced damage of 6-propyl-2-thiouracil (PTU) in thyroid cells. *Radiat Res* 2013;179:352-60.
 32. Allen T, Rana SV. Effect of n-propylthiouracil or thyroxine on arsenic trioxide toxicity in the liver of rat. *J Trace Elem Med Biol* 2007;21:194-203.
 33. Wolff J. Perchlorate and the thyroid gland. *Pharmacol Rev* 1998;50:89-105.
 34. Van Sande J, Massart C, Beauwens R, Schoutens A, Costagliola S, Dumont JE, et al. Anion selectivity by the sodium iodide symporter. *Endocrinology* 2003;144:247-52.
 35. Tonacchera M, Pinchera A, Dimida A, Ferrarini E, Agretti P, Vitti P, et al. Relative potencies and additivity of perchlorate, thiocyanate, nitrate, and iodide on the inhibition of radioactive iodide uptake by the human sodium iodide symporter. *Thyroid* 2004;14:1012-9.
 36. Kucharzyk KH, Crawford RL, Cosens B, Hess TF. Development of drinking water standards for perchlorate in the United States. *J Environ Manage* 2009;91:303-10.
 37. Lewandowski TA, Peterson MK, Charnley G. Iodine supplementation and drinking-water perchlorate mitigation. *Food Chem Toxicol* 2015;80:261-70.
 38. Saito T, Endo T, Kawaguchi A, Ikeda M, Nakazato M, Kogai T, et al. Increased expression of the Na⁺/I⁻ symporter in cultured human thyroid cells exposed to thyrotropin and in Graves' thyroid tissue. *J Clin Endocrinol Metab* 1997;82:3331-6.
 39. Li X, Lu S, Miyagi E, Katoh R, Kawaoi A. Thyrotropin prevents apoptosis by promoting cell adhesion and cell cycle progression in FRTL-5 cells. *Endocrinology* 1999;140:5962-70.
 40. Zhang N, Wang L, Duan Q, Lin L, Ahmed M, Wang T, et al. Metallothionein-I/II knockout mice aggravate mitochondrial superoxide production and peroxiredoxin 3 expression in thyroid after excessive iodide exposure. *Oxid Med Cell Longev* 2015;2015:267027.
 41. Lee JD, Lee MH. Metallothionein overexpression of bladder biopsies associated with tissue hypoxia in patients with interstitial cystitis/painful bladder syndrome. *Int J Urol* 2014;21:719-23.
 42. Yang L, Wang J, Yang J, Schamber R, Hu N, Nair S, et al. Antioxidant metallothionein alleviates endoplasmic reticulum stress-induced myocardial apoptosis and contractile dysfunction. *Free Radic Res* 2015;49:1187-98.
 43. Bieniek A, Pula B, Piotrowska A, Podhorska-Okolow M, Salwa A, Koziol M, et al. Expression of metallothionein I/II and Ki-67 antigen in various histological types of basal cell carcinoma. *Folia Histochem Cytobiol* 2012;50:352-7.
 44. Dziegiel P. Expression of metallothioneins in tumor cells. *Pol J Pathol* 2004;55:3-12.
 45. Pedersen MO, Larsen A, Stoltenberg M, Penkowa M. The role of metallothionein in oncogenesis and cancer prognosis. *Prog Histochem Cytochem* 2009;44:29-64.
 46. Liu J, Shi X, Qian M, Zheng L, Lian C, Xia Y, et al. Copper-induced hydrogen peroxide upregulation of a metallothionein gene, OsMT2c, from *Oryza sativa* L. confers copper tolerance in *Arabidopsis thaliana*. *J Hazard Mater* 2015;294:99-108.
 47. Sun X, Zhou Z, Kang YJ. Attenuation of doxorubicin chronic toxicity in metallothionein-overexpressing transgenic mouse heart. *Cancer Res* 2001;61:3382-7.
 48. Kang YJ, Li Y, Sun X, Sun X. Antiapoptotic effect and inhibition of ischemia/reperfusion-induced myocardial injury in metallothionein-overexpressing transgenic mice. *Am J Pathol* 2003;163:1579-86.
 49. Cai L, Wang J, Li Y, Sun X, Wang L, Zhou Z, et al. Inhibition of superoxide generation and associated nitrosative damage is involved in metallothionein prevention of diabetic cardiomyopathy. *Diabetes* 2005;54:1829-37.
 50. Wang L, Zhou Z, Saari JT, Kang YJ. Alcohol-induced myocardial fibrosis in metallothionein-null mice: prevention by zinc supplementation. *Am J Pathol* 2005;167:337-44.

51. Merten KE, Feng W, Zhang L, Pierce W, Cai J, Klein JB, et al. Modulation of cytochrome C oxidase-va is possibly involved in metallothionein protection from doxorubicin cardiotoxicity. *J Pharmacol Exp Ther* 2005;315:1314-9.
52. Liu D, Lin X, Yu F, Zhang M, Chen H, Bao W, et al. Effects of 3,5-Diiodotyrosine and potassium iodide on thyroid function and oxidative stress in iodine-excess wistar rats. *Biol Trace Elem Res* 2015;168:447-52.
53. Zhang M, Zou X, Lin X, Bian J, Meng H, Liu D. Effect of excessive potassium iodide on rat aorta endothelial cells. *Biol Trace Elem Res* 2015;166:201-9.
54. Andrews GK. Regulation of metallothionein gene expression by oxidative stress and metal ions. *Biochem Pharmacol* 2000;59:95-104.
55. Santon A, Formigari A, Albergoni V, Irato P. Effect of Zn treatment on wild type and MT-null cell lines in relation to apoptotic and/or necrotic processes and on MT isoform gene expression. *Biochim Biophys Acta* 2006;1763:305-12.
56. Inoue K, Takano H, Shimada A, Satoh M. Metallothionein as an anti-inflammatory mediator. *Mediators Inflamm* 2009;2009:101659.
57. Higashimoto M, Isoyama N, Ishibashi S, Ogawa N, Takiguchi M, Suzuki S, et al. Preventive effects of metallothionein against DNA and lipid metabolic damages in dyslipidemic mice under repeated mild stress. *J Med Invest* 2013;60(3-4):240-8.
58. Hu N, Guo R, Han X, Zhu B, Ren J. Cardiac-specific overexpression of metallothionein rescues nicotine-induced cardiac contractile dysfunction and interstitial fibrosis. *Toxicol Lett* 2011;202:8-14.
59. Ruiz-Riol M, Martínez-Arconada MJ, Alonso N, Soldevila B, Marchena D, Armengol MP, et al. Overexpression of metallothionein I/II: a new feature of thyroid follicular cells in Graves' disease. *J Clin Endocrinol Metab* 2012;97:446-54.
60. Zhou S, Wang Y, Tan Y, Cai X, Cai L, Cai J, et al. Deletion of metallothionein exacerbates intermittent hypoxia-induced oxidative and inflammatory injury in aorta. *Oxid Med Cell Longev* 2014;2014:141053.
61. Yang L, Hu N, Jiang S, Zou Y, Yang J, Xiong L, et al. Heavy metal scavenger metallothionein attenuates ER stress-induced myocardial contractile anomalies: role of autophagy. *Toxicol Lett* 2014;225:333-41.