Journal of Trace Elements in Medicine and Biology 26 (2012) 36–41

Contents lists available at SciVerse ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.de/jtemb

Nutrition

Selenium and/or iodine deficiency alters hepatic xenobiotic metabolizing enzyme activities in rats

Pinar Erkekoglu, Belma Kocer Giray, Aydan Caglayan, Filiz Hincal*

Hacettepe University, Faculty of Pharmacy, Department of Toxicology, Ankara 06100, Turkey

ARTICLE INFO

Article history:
Received 17 January 2011
Accepted 11 December 2011

Keywords:
Iodine deficiency
Selenium deficiency
Combined iodine and selenium deficiency
Thyroid hormones
Xenobiotic metabolizing enzymes
CYP450

ABSTRACT

The objective of this study was to investigate the effects of iodine (I_2) and/or selenium (Se) deficiency on thyroid hormones and hepatic xenobiotic metabolizing enzyme systems using a triple animal model. Three-week-old male Wistar rats were fed for seven weeks. Se deficiency was introduced by a diet containing <0.005 mg/kg Se, and I_2 deficiency was produced by sodium perchlorate containing drinking water. The levels of plasma thyroid hormones [total T_4 (TT_4), total T_3 (TT_3)], thyroid stimulating hormone (TSH); total microsomal cytochrome P450 (CYP450) and cytochrome b5 (CYP b5) levels; activities of microsomal NADPH-cytochrome P450 reductase (P450R), microsomal aniline hydroxylase (CYP2E1), microsomal 7-ethoxyresorufin *O*-deethylase (EROD), microsomal 7-pentoxyresorufin *O*-dependentylase (PROD) and cytosolic glutathione S-transferase (GST) were determined. In I_2 deficiency total CYP450 levels, activities of CYP2E1, EROD and GST decreased, and CYP b5 content increased significantly. In Se-deficient rats, total CYP450 level and CYP2E1 activity increased, and EROD and GST activities and CYP b5 level decreased significantly. In combined I_2 and Se deficiency, except for CYP450 content and CYP2E1 activity, all enzyme activities and CYP b5 content decreased significantly compared to control group. Overall results of this study have suggested that metabolism of xenobiotics as well as endogenous compounds is affected by Se and I_2 status.

Introduction

Thyroid hormones regulate multiple metabolic processes and play an essential role in normal growth and development. Physiological actions of thyroid hormones are produced predominantly by the active hormone 3,5,3′-triiodothyronine (T_3), and the majority of circulating T_3 is derived by the deiodination of thyroxine (T_4) with the isozymes of iodothyronine 5′-deiodinase (DIOs) [1]. Structure and function of thyroid hormones require adequate availability of I_2 and Se; thereby these two essential trace elements affect homeostasis of thyroid hormone-dependent metabolic pathways [1]. As a structural component of thyroid hormones, I_2 is the primary requirement for the thyroid function. Biosynthesis of thyroid hormones involves thyroidal trapping of serum iodide by the thyroid gland, incorporation of I_2 into thyrosine, coupling of iodinated thyrosyl residues of thyroglobulin, and proteolytic cleavage of follicular thyroglobulin to release the iodothyronines [2]. I_2 deficiency is known to induce a variety of disorders of thyroid function including endemic goiter, considered as the greatest cause of preventable brain damage and mental retardation, and more than two billions of people are reported to be at the risk of I_2 deficiency disorders throughout the world [3].

Selenium (Se) is the integral component of several major metabolic pathways of cellular antioxidant defence and cellular redox control [4]. Besides the fact that DIOs are selenoenzymes [1,5], Se, as a component of the glutathione peroxidase (GPx) and thioredoxine reductase (TRxR) enzyme families, is involved in the protection of the thyroid gland from excess hydrogen peroxide and reactive oxygen species (ROS) that are produced in thyrocytes for the biosynthesis of thyroid hormones [5]. Therefore, Se is essential for thyroid function. In fact, human thyroid gland is among the human tissues with the highest Se content per mass unit similar to other endocrine organs and the brain. Like I_2, Se is inadequately available for man and livestock in many parts of the world, thus, the deficiencies of I_2 and Se may have important impacts on the health of large populations. Furthermore, occurrence of a combined I_2 and Se deficiency is possible and in fact severe cases were reported as a cause of endemic myxedematous cretinism which resulted in mental retardation [6].

Cytochrome P450 (CYP450) enzymes, the super family of hemproteins, are present in diverse forms of life from bacteria to humans [7]. They are responsible for the metabolism of xenobiotics and play important roles in the activation of chemical carcinogens, detoxification of numerous xenobiotics, as well as oxidative metabolism of endogenous compounds. Their functions

* Corresponding author. Tel.: +90 312 309 29 58; fax: +90 312 311 47 77.
E-mail address: fhincal@tr.net (F. Hincal).

0946-672X/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jtemb.2011.12.002

are under the control of several liver-enriched transcription factors and nuclear receptors, including nuclear thyroid hormone receptors [8]. The results of several clinical studies have suggested that oxidative metabolism of drugs and steroids is perturbed in disease states in association with altered concentrations of circulating iodothyronines, and with therapeutic use of hormones [9,10]. Experiments in rodents have shown that most constitutive hepatic P450 enzymes are subject to hormonal regulation. Among other hormones, particularly iodothyronines contribute to the regulation of constitutive P450s in rats and modulate xenobiotic-induced expression of P450s [11,12]. It has been demonstrated that iodothyronines act directly on human hepatocytes to regulate the expression of several CYPs including CYP3A4, and this effect appears to be exerted at a pre-translational level [13]. Whereas Se supplementation does not seem to affect the CYP3A4 activity [14], and there is no data available for Se deficiency. In fact, the studies on the alterations of xenobiotic metabolizing enzyme systems in Se deficiency are limited and their results are controversial [15]. More importantly, there is no data regarding the effects of combined I_2 and Se deficiency on CYP450 enzymes.

Taking together those available knowledge and data, present study was undertaken to investigate the effects of I_2 and/or Se deficiency on thyroid hormone levels, hepatic CYP450 and cytochrome b5 (CYP b5) contents and on the activities of hepatic enzymes responsible for detoxification or bioactivation of xenobiotics on a triple animal model. Considering the frequency of inadequate Se and/or I_2 intake among human populations, it was expected to obtain data contributing to envisage the alteration of drug metabolism in humans with such nutritional deficiencies.

Methods and materials

Chemicals

Rat thyroid stimulating hormone (TSH) kit was obtained from Biocode (Liège, Belgium) and total T_4 (TT_4) and total T_3 (TT_3) radioimmunoassay kits were supplied by Abbott Laboratories (Illinois, USA). All other chemicals were from Sigma–Aldrich (St. Louis, MO, USA) or Merck (Darmstad, Germany). Se deficient diet (<0.005 mg Se/kg) was supplied by Scientific Animal Food and Engineering (SAFE) Laboratories (Augy, France).

Animals and diets

Three-week-old, male Wistar rats, obtained from Hacettepe University Experimental Animals Laboratory were used in the experiments. The animals were divided randomly in six groups and each group was housed in plastic cages with stainless-steel grid tops. The cages were placed in a room with controlled temperature (23 °C), humidity (50%) and a 12-h light–dark cycle. Body weights (bws) were monitored weekly. Feeding period was 7 weeks. Eight animals were used for each group and received *ad libitum* diet and water. The animals were treated humanely and with regard for alleviation of suffering, and the study was approved by Hacettepe University Ethical Committee.

Experimental groups

(1) Control group (C) was fed regular rat chow (~0.15 mg/kg Se) and drinking water; (2) I_2 deficient group (ID) was fed the same regular rat chow and received 1% sodium perchlorate containing drinking water; (3) Se deficient group (SeD) was fed Se-deficient diet containing <0.005 mg of Se/kg and received normal drinking water; (4) I_2 and Se-deficient group (ISeD) received Se-deficient diet (<0.005 mg Se/kg) and 1% sodium perchlorate containing drinking water.

Preparation of cytosolic and microsomal fractions

At the end of feeding period, overnight fasted animals were weighed and then decapitated under i.p. thiopental anesthesia. Blood samples were collected into heparinized tubes and the plasma was separated after centrifugation at $800 \times g$ for 15 min. Livers were rapidly removed, weighed and frozen at −80 °C until the preparation of cytosolic and microsomal fractions.

The homogenization of liver was carried out in a Teflon-glass homogenizer in a volume of potassium chloride–Tris buffer (0.154 M potassium chloride and 50 mM Tris–HCl, pH 7.4) to obtain a 3 g/mL liver homogenate. Following centrifugation at $2500 \times g$ for 10 min, separated supernatant was further centrifuged at $10{,}000 \times g$ for 10 min. The latter supernatant was centrifuged at $105{,}000 \times g$ for 60 min and cytosolic supernatant was collected and used for the measurement of GST activity.

The microsomal pellet was re-suspended in a buffer containing Tris–EDTA–sucrose (20 mM Tris, 5 mM EDTA and 0.25 M sucrose, pH 7.4; 1 g liver tissue/mL). All samples, including plasma, microsomal and cytosolic fractions were aliquoted and stored in a freezer at −80 °C until analysis.

Measured parameters and methods

Thyroid hormones

Plasma TSH, TT_4 and TT_3 levels were determined by radioimmunoassay using commercial kits.

CYP450 and CYP b5 levels

CYP450 and CYP b5 contents in hepatic microsomes were determined according to the method of Omura and Sato [16] with extinction coefficients of 91 mM^{-1} cm^{-1} (between 490 and 450 nm) and 185 mM^{-1} cm^{-1} (between 424 and 409 nm), respectively.

NADPH-cytochrome reductase activity

Microsomal NADPH-cytochrome reductase (P450R) activity was measured at 550 nm and 37 °C by monitoring the reduction of cytochrome c in the presence of NADPH [17].

Xenobiotic metabolizing enzyme activities

Microsomal aniline hydroxylase (CYP2E1) activity was determined by measuring p-aminophenol production according to the method of Imai et al. [18].

Microsomal 7-ethoxyresorufin *O*-deethylase (EROD) as a measure of CYP1A1, and 7-pentoxyresorufin *O*-depentylase (PROD) as a measure of CYP2B1/2 activities were determined spectrofluorometrically from the amount of resorufin produced using ethoxyresorufin and pentoxyresorufin as substrates, respectively, at excitation and emission wavelengths of 530 and 585 nm [19].

Cytosolic glutathione-S-transferase (GST) activity was determined using 1-chloro-2,4-dinitrobenzene as a substrate, according to the method of Habig et al. [20].

Protein concentrations were determined by the standard method of Lowry et al. [21].

Statistical analysis

Experimental data were analyzed with one-way analysis of variance (ANOVA) followed by the Student's *t*-test using a Statistical Package for Social Sciences Program (SPSS) for windows packed program. The p values <0.05 were considered significant. All the values are given as mean ± SEM. For TSH, values were also given

as median, minimum and maximum. And for TSH median values, Mann–Whitney's *U* test with Bonferroni correction was performed.

Results

Body and liver weights

As shown in Table 1, after a 7-week treatment period, I_2 deficiency caused a slight (~10%, $p < 0.05$) decrease in bw of the animals, while Se deficiency, and combined I_2 and Se deficiency caused marked decreases (~40% in SeD and ~25% in ISeD, $p < 0.05$) compared to control group. Liver weights were also significantly low in all treatment groups and followed the order of SeD < ISeD < ID < C. However, the relative liver weight (liver weight/100 g bw) was significantly low only in SeD group compared to controls. The weight, but not relative weight of thyroid gland decreased in SeD rats, whereas I_2 deficiency, and combined I_2 and Se deficiency produced significant increases in absolute and relative thyroid weights.

Thyroid hormone parameters

I_2 deficiency increased TSH (~130%), decreased TT_4 (~70%) and TT_3 (~60%) levels significantly, thus, caused hypothyroidism. Se deficiency did not alter TSH and TT_3 levels, but increased TT_4 significantly (~30%). Highest increases in TSH were observed in ISeD group (~170%) along with a marked decrease in TT_4 (~75%), but TT_3 levels did not differ from those of control and SeD groups (Table 2).

Hepatic xenobiotic-metabolizing enzyme assessments

The xenobiotic enzyme levels/activities measured in experimental groups are shown in Fig. 1. I_2 deficiency caused significant changes in all parameters measured except for the P450R and PROD activities. Total CYP450 content (~25%), activities of 204 microsomal CYP2E1 (~30%) EROD (~30%), and cytosolic GST 205 (~80%) decreased significantly ($p < 0.05$) compared to those of control group, showing a general depression of xenobiotic-metabolizing enzyme system in ID animals. However, the level of CYP b5 was found to be enhanced significantly.

The total CYP450 content (~80%) and the activity of CYP2E1 (~75%) significantly increased in SeD rats. The activities of P450R and PROD did not change; but significant reductions were observed in the level of CYP b5 and in the activities of EROD and GST (~60%, ~75% and ~25%, respectively, $p < 0.05$).

In contrast to ID group, combined I_2 and Se deficiency did not cause significant alterations in total CYP450 content and CYP2E1 activity; but decreased markedly CYP b5 levels (~60%), and P450R, EROD, PROD, and GST activities (~75%, ~60%, ~40% and ~80%, respectively) compared to control. Thus, while both I_2 deficiency and Se deficiency states did not alter P450R, PROD activities, in combined I_2 and Se deficiency significant decreases were observed. On the other hand, except for GST and EROD activities all the activities or contents of the measured enzymes in ISeD were significantly lower than those of SeD.

Discussion

Prolonged I_2 deficiency results in hypothyroidism, elevates TSH secretion, disrupts thyroid function and eventually promotes goiter formation. In the present study, I_2 deficiency and resulting hypothyroidism was evident by high TSH and low plasma TT_4 and TT_3 levels along with increased thyroid weights. We used perchlorate to induce I_2 deficiency, since it blocks effectively the uptake of I_2 by thyroid gland [22,23], by competing with iodide at the sodium-iodide symporter (NIS), the transport system that transports I_2 into the thyroid [24,25].

Highly elevated TT_4 levels we observed in SeD rats are the characteristics of Se deficiency which results in reduction of Se-dependent enzyme activities (DIOs). The profile of thyroid hormones in combined I_2 and Se deficiency was similar to I_2 deficiency with its very high TSH and low TT_4 levels, while it was similar to Se deficiency with regard to the unchanged TT_3 concentrations. These results were in agreement with the data of our previous study which was designed similarly except for 5 weeks of feeding period [26].

Effects of iodine deficiency on hepatic enzymes

Thyroid hormones regulate the basal rate of all cells, including hepatocytes and thereby modulate hepatic function; the liver in turn metabolizes the thyroid hormones and regulates their systemic endocrine effects [27]. Thyroid effect on metabolic pathways can be exemplified as follows: several forms of CYP450 proteins including CYP2E1 are elevated in hypophysectomized rats [28]; microsomal GST protein levels in testis decrease in hypothyroidic rats [28]; P450R expression is regulated by thyroid hormones by pretranslational mechanisms [29]; thus, thyroid hormones are involved in the regulation of CYP450 isozymes covering both Phase I and Phase II enzymes [30,31].

In the present study we observed marked decreases in total CYP450 content, CYP2E1 and EROD activities in ID rats. These results were in line with earlier reports and suggested that I_2 deficiency might lead to decreases in the metabolism of several drugs and other xenobiotics that undergo biotransformation by these enzymes. CYP2E1 is responsible for the metabolism of several flurane anesthetics, as well as ethanol, acetaminophen, dapsone, and theophylline [32]; and in the metabolism of latter drug, EROD is also involved. It can be foreseen that the plasma levels of such xenobiotics might increase in I_2 deficiency and high level of pharmacological activity as well as unwanted effects can be observed as a consequence. Therefore, in clinically hypothyroid individuals dose adjustments might be needed for drugs which are metabolized by CYP2E1 and CYP1A1, especially for those like theophylline that have a narrow therapeutic index [33]. On the other hand, the marked decrease (~80%) observed in cytosolic GST activity of ID rats is of importance as the metabolic pathways through GSTs are crucial steps in the biotransformation of several environmental xenobiotics like benzo[*a*]pyrene, as well as anticancer drugs, so that the decrease in cytosolic GST activity may lead to a decrease in their detoxification processes. The elevation in CYP b5 levels in our study is also in accordance with previous literature data that shows both hypothyroidism and hyperthyroidism induce hepatic microsomal CYP b5 levels [34].

Effects of selenium deficiency on hepatic enzymes

Selenium with its several forms of cellular selenoproteins is primarily involved in the modulation of intracellular redox equilibrium and has a critical importance for the cellular antioxidant defence [4]. Low dietary Se intakes are associated with health disorders including oxidative stress-related pathologies and Se supplementation was shown to modify the detoxification of several carcinogens [4,13]. Se deficiency causes changes in the activity of glutathione requiring enzymes [35], and despite the conflictions existing data indicate significant alterations in xenobiotic metabolism in Se deficient animals [14,36–38]. Although there was no effect of Se status on the contents of P450 and CYP b5 in earlier studies [14,37], a significant induction (~50%) of CYP450 was reported recently [39], and oxidative stress caused by Se deficiency was suggested to be a possible trigger of CYP450 induction. These

Table 1
Effects of iodine and/or selenium deficiency on body weight, liver weight and relative liver weight.

	Body weight (g)	Liver weight (g)	Relative liver weight (g/100 g bw)	Thyroid weight (mg)	Relative thyroid weight (mg/100 g bw)
Control	239.8 ± 5.7[a]	9.6 ± 0.3[a]	4.0 ± 0.1[ac]	26 ± 0.5[a]	11 ± 0.1[a]
ID	215.4 ± 8.3[b]	8.3 ± 0.2[b]	3.9 ± 0.1[a]	51 ± 1.2[b]	24 ± 0.1[b]
SeD	141.7 ± 4.8[c]	5.0 ± 0.2[c]	3.5 ± 0.1[b]	14 ± 0.5[c]	9.9 ± 0.1[a]
ISeD	179.3 ± 4.7[d]	7.4 ± 0.2[d]	4.2 ± 0.1[c]	53 ± 1.0[b]	29.4 ± 0.1[b]

Experimental groups for 7 weeks were on: control group (C), regular rat chow (~0.15 mg/kg Se) and drinking water; iodine deficient group (ID), regular rat chow (~0.15 mg/kg Se) and 1% sodium perchlorate containing drinking water; selenium deficient group (SeD), selenium-deficient diet (<0.005 mg/kg Se) and normal drinking water; iodine and selenium-deficient group (ISeD), selenium-deficient diet (<0.005 mg/kg Se) and 1% sodium perchlorate. Values are given as mean ± SEM of triplicate measurements. Means within each row that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

results are in line with our current results, as well as those of a previous study in which we reported increased level of lipid peroxidation in SeD rats [26].

Among the alterations of metabolizing enzymes, the highest increase we observed was the activity of CYP2E1 (~75%). The effects of Se deficiency on CYP2E1, as well as on CYP450 and CYP b5 were in contrast to the effect of ID, whereas EROD and GST activities decreased in both SeD and ID rats. CYP2E1 is induced by chronic alcohol consumption 10–20-fold. Alcohol induced activity of CYP2E1 and P450R is suggested to be associated with enhanced production of ROS and enhanced activation of various pro-carcinogens present in alcoholic beverages [40]. While higher Se status was shown to reduce the risk of esophageal and gastric cancers in Se deficient populations, alcohol consumption was also linked to these particular cancer types. This pathology might thus be associated with the induction of CYP2E1 in Se deficiency, particularly in alcoholics, however, further research is needed for better understanding of the issue [41,42].

Effects of combined iodine and selenium deficiency on hepatic enzymes

The prominent results of this study were the alterations of hepatic enzymes observed in ISeD group. To our knowledge, there is no study or published data concerning the effects of this combined deficiency state on xenobiotic metabolizing enzymes and their electron donors. In the present study, the profile we observed in ISeD rats seemed to be based on and, thus, reflecting the effects of Se deficiency rather than the effects of I_2 deficiency. However, the effect on individual enzymes was either more pronounced or compensated with the opposite effects of the two deficiency states. The total CYP450 content in ISeD animals was not different than the control, but higher than that of ID, and lower than that of SeD rats. In fact, all the enzyme levels or activities in ISeD rats were significantly lower than those of SeD group, except for the activities of EROD and GST which were not different than SeD, and these two enzyme activities were decreased by all the three types of deficiency states compared to control. While neither ID nor SeD altered the activities of PROD and P450R, they decreased significantly in combined deficiency. The effects of ID and SeD on CYP b5 were in opposite direction and the effect of ISeD was found to be similar to SeD, but the lowering effect of ISeD was significantly higher. The effects of ID and SeD were also in opposite directions on CYP2E1, so that in combined deficiency no alteration was observed compared to both control and ID.

The decreases of electron donors of CYP450 enzymes, the CYP b5 content and P450R activities may result in a decreased reaction rate in CYP-catalyzed reactions. P450R is obligatory for catalysis of many reactions of P450, particularly of those catalyzed by CYP3A4. In the endoplasmic reticulum membrane, P450R transfers the electrons from NADPH into the P450 catalytic cycle [43]. Antibodies against P450R were shown to significantly impair CYP3A-mediated drug metabolism in a concentration-dependent manner [44]; and in recombinant enzyme systems, CYP3A reactions cannot proceed without P450R. While increasing concentrations of P450R enhance the metabolism of testosterone in a concentration-dependent manner [45,46], a reduction in cholesterol metabolism was shown in the hepatic deletion of P450R gene in mice [47]. Similarly, CYP b5 participates in drug metabolism augmenting the reactions mediated by some P450 isoforms, including CYP3A4. In recombinant systems, the metabolism of testosterone, nifedipine, and midazolam, the three typical index substrates for CYP3A, is enhanced by the addition of CYP b5 [45,46,48]. In human liver microsomes, the CYP b5 antibody inhibited the metabolism of testosterone and nifedipine, suggesting that CYP b5 is an essential component in the modulation of CYP3A activity [44]. Therefore, the alterations in these critical components of CYP450 system in combined I_2 and Se deficiency are of importance for xenobiotic metabolism as well as for endogenous substances.

The decreases observed in both EROD and PROD activities might also have important impacts on xenobiotic metabolism. PROD as an indicator of CYP2B1/2 activity, is responsible for the metabolism of environmental chemicals such as nicotine, arenes, arylamines and nitrosamines [32]. The significant decrease observed in the activity of PROD in ISeD might lead to decreased metabolism of those substances. As indicated before, GSTs play a major role in detoxication of carcinogens [49] and the decrease observed in GST activity in all deficiency states may make those individuals more prone to the effect of such chemicals.

Table 2
Plasma TT_4, TT_3, and TSH levels in the experimental groups.

	T_3 (nmol/L)	T_4 (nmol/L)	TSH (ng/mL)
C ($n = 8$)	0.66 ± 0.07[a]	47.34 ± 2.14[a]	5.40 ± 0.60[a] (median: 6.30; min: 4.00; max: 8.00)
ID ($n = 8$)	0.27 ± 0.03[b]	14.15 ± 0.47[b]	12.44 ± 1.50[b] (median: 10.45; min: 6.7; max 14.00)
SeD ($n = 8$)	0.63 ± 0.03[a]	61.91 ± 0.52[c]	4.08 ± 1.18[a] (median: 4.35; min: 2.9; max: 5.00)
ISeD ($n = 8$)	0.56 ± 0.05[a]	11.74 ± 2.94[b]	14.99 ± 1.48[b] (median: 14.00; min: 8.6; max: 20.40)

Experimental groups for 7 weeks were on: control group (C), regular rat chow (~0.15 mg/kg Se) and drinking water; iodine deficient group (ID), regular rat chow (~0.15 mg/kg Se) and 1% sodium perchlorate containing drinking water; selenium deficient group (SeD), selenium-deficient diet (<0.005 mg/kg Se) and normal drinking water; iodine and selenium-deficient group (ISeD), selenium-deficient diet (<0.005 mg/kg Se) and 1% sodium perchlorate. Values are given as mean ± SEM of triplicate measurements. Besides, in parentheses, TSH median were also given with minimum (min) and maximum (max) values. Means within each row that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

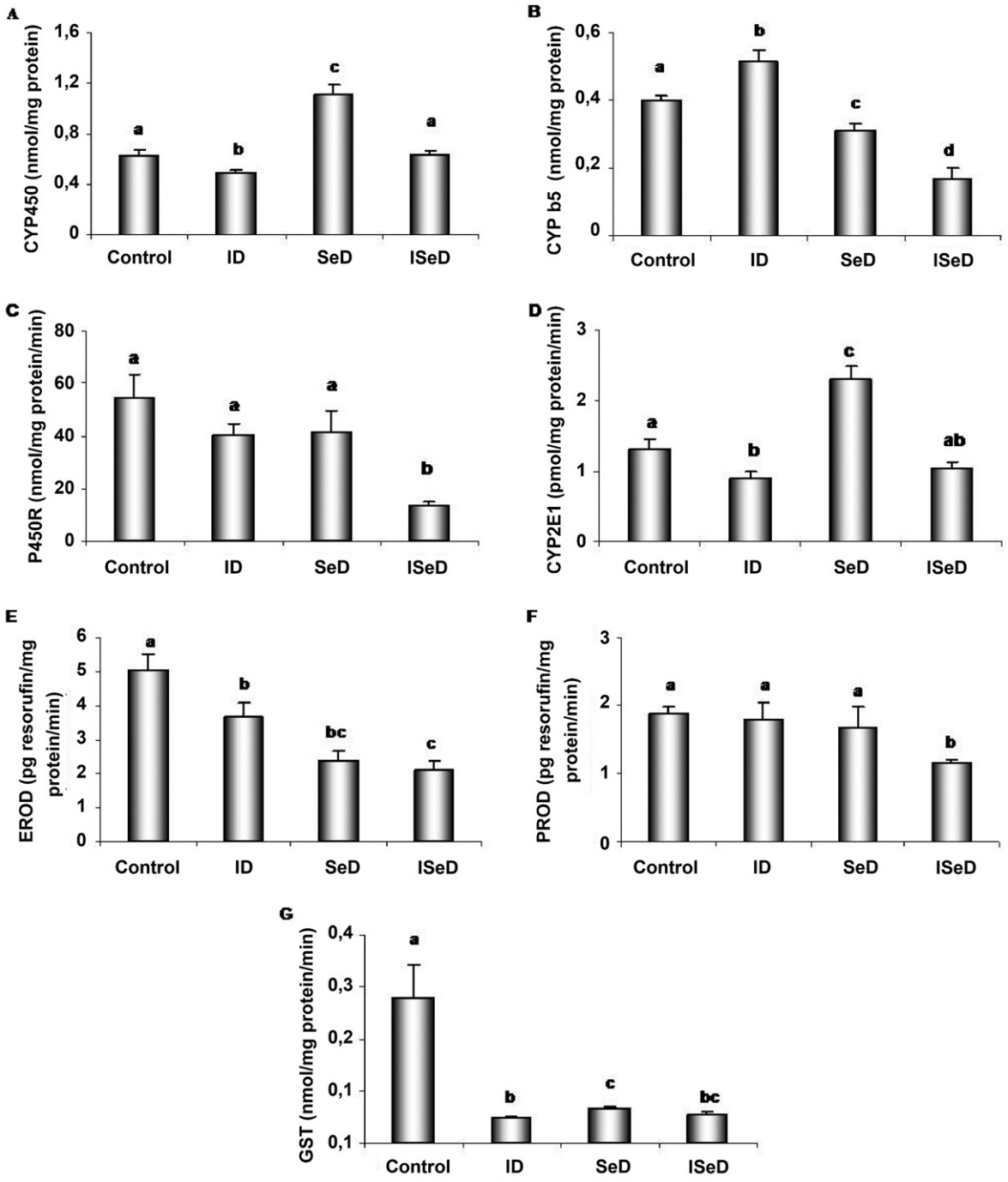

Fig. 1. Hepatic CYP450 and CYP b5 contents, activities of drug-metabolizing enzymes in the experimental groups. (A) CYP450 levels; (B) CYP b5 levels; (C) P450R activity; (D) CYP2E1 activity; (E) EROD activity; (F) PROD activity; (G) GST (cytosolic) activity. Experimental groups for 7 weeks were on: control group (C), regular rat chow (~0.15 mg/kg Se) and drinking water; iodine deficient group (ID), regular rat chow (~0.15 mg/kg Se) and 1% sodium perchlorate containing drinking water; selenium deficient group (SeD), selenium-deficient diet (<0.005 mg/kg Se) and normal drinking water; iodine and selenium-deficient group (ISeD), selenium-deficient diet (<0.005 mg/kg Se) and 1% sodium perchlorate. Values are given as mean ± SEM of triplicate measurements. Means within each bar that do not share same letters (superscripts) are significantly different from each other ($p < 0.05$).

In conclusion, our findings indicate that the levels and/or activities of hepatic xenobiotic metabolizing enzymes and their electron donors are significantly altered by I_2 and/or Se deficiency in rats. As the complexity of the function of thyroid hormones is a drawback in the interpretation of such enzyme studies, and as the roles of Se in biological systems are not fully elucidated, the explanation of the results is not straightforward. Further studies on a wider spectrum that may provide more specific information and comprehension on the effects of I_2 and/or Se deficiency upon hepatic xenobiotic metabolism, both in experimental animals and in humans are, therefore, needed. Nevertheless, the results of the present study emphasize the importance of I_2 and Se status in hepatic xenobiotic metabolism.

Acknowledgements

This study was supported by Hacettepe University Research Fund, 01.01.301.001. The authors would like to thank Düzen

Laboratories (Ankara/Turkey) for their technical assistance in the measurement of TSH levels.

References

[1] Köhrle J, Jakob F, Contempré B, Dumont JE. Selenium, the thyroid, and the endocrine system. Endocr Rev 2005;26:944–84.
[2] Gentile F, Di Lauro R, Salvatore G. Biosynthesis and secretion of thyroid hormones. In: DeGroot LJ, editor. Endocrinology. Philadelphia, PA: Saunders; 1995. p. 517–42.
[3] Delange F, de Benoist B, Pretell E, Dunn JT. Iodine deficiency in the world: where do we stand at the turn of the century? Thyroid 2001;11:437–47.
[4] Steinbrenner H, Sies H. Protection against reactive oxygen species by selenoproteins. Biochim Biophys Acta 2009;1790:1478–85.
[5] Köhrle J, Gärtner R. Selenium and thyroid. Best Pract Res Clin Endocrinol Metab 2009;23:815–27.
[6] Vanderpas JB, Contempré B, Duale NL, Goossens W, Bebe N, Thorpe R, et al. Iodine and selenium deficiency associated with cretinism in northern Zaire. Am J Clin Nutr 1990;52:1087–93.
[7] Danielson PB. The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. Curr Drug Metab 2002;3:561–97.
[8] Akiyama TE, Gonzalez FJ. Regulation of P450 genes by liver-enriched transcription factors and nuclear receptors. Biochim Biophys Acta 2003;1619:223–34.
[9] Fishman J, Hellman L, Zumoff B, Gallagher TF. Effect of thyroid on hydroxylation of estrogen in man. J Clin Endocrinol Metab 1965;25:365–8.
[10] Cheung NW, Liddle C, Coverdale S, Lou JC, Boyages SC. Growth hormone treatment increases cytochrome P450-mediated antipyrine clearance in man. J Clin Endocrinol Metab 1995;81:1999–2001.
[11] Ram PA, Waxman DJ. Pretranslational control by thyroid hormone of rat liver steroid 5α-reductase and comparison to the thyroid dependence of two growth hormone-regulated CYP2C mRNAs. J Biol Chem 1990;265:19223–9.
[12] Waxman DJ, Morrissey JJ, Naik S, Jauregui HO. Phenobarbital induction of cytochromes P-450. High-level long-term responsiveness of primary rat hepatocyte cultures to drug induction, and glucocorticoid dependence of the phenobarbital response. Biochem J 1990;271:113–9.
[13] Ip C, Lisk DJ. Modulation of phase I and phase II xenobiotic-metabolizing enzymes by selenium-enriched garlic in rats. Nutr Cancer 1997;28:184–8.
[14] Liddle C, Goodwin BJ, George J, Tapner M, Farrell GC. Separate and interactive regulation of cytochrome P450 3A4 by triiodothyronine, dexamethasone, and growth hormone in cultured hepatocytes. J Clin Endocrinol Metab 1998;83:2411–6.
[15] Reiter R, Wendel A. Selenium and drug metabolism – I. Multiple modulations of mouse liver enzymes. Biochem Pharmacol 1983;32:3063–7.
[16] Omura T, Sato R. The carbonmonoxide binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J Biol Chem 1964;239:2370–8.
[17] Phillips AH, Langdon RG. Hepatic triphosphopyridine nucleotide-cytochrome c reductase: isolation, characterization and kinetic studies. J Biol Chem 1962;237:2652–60.
[18] Imai Y, Ito A, Sato R. Evidence for biochemically different types of vesicles in the hepatic microsomal fraction. J Biochem 1966;60:417–28.
[19] Prough RA, Burke MD, Mayer RT. Direct fluorometric methods for measuring mixed function oxidase activity. Methods Enzymol 1978;52:372–7.
[20] Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases, the first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130–9.
[21] Lowry OH, Rosebrough NJ, Faar AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
[22] Chow SY, Woodbury DM. Kinetics of distribution of radioactive perchlorate in rat and guinea-pig thyroid glands. J Endocrinol 1970;47:207–18.
[23] van Wijk N, Rijntjes E, van de Heijning BJ. Perinatal and chronic hypothyroidism impair behavioural development in male and female rats. Exp Physiol 2008;93:1199–209.
[24] Yamada T. Effects of perchlorate and other anions on thyroxine metabolism in the rat. Endocrinology 1967;81:1285–90.
[25] Tran N, Valentín-Blasini L, Blount BC, McCuistion CG, Fenton MS, Gin E, et al. Thyroid-stimulating hormone increases active transport of perchlorate into thyroid cells. Am J Physiol Endocrinol Metab 2008;294:E802–6.
[26] Giray B, Riondel J, Richard MJ, Favier A, Hincal F. Oxidant/antioxidant status in relation to thyroid hormone metabolism in selenium- and/or iodine-deficient rats. J Trace Elem Exp Med 2004;17:109–21.
[27] Malik R, Hodgson H. The relationship between the thyroid gland and the liver. QJM 2002;95:559–69.
[28] Mehrotra K, Morgenstern R, Lundqvist G, Becedas L, Bengtsson Ahlberg M, Georgellis A. Effects of peroxisome proliferators and/or hypothyroidism on xenobiotic-metabolizing enzymes in rat testis. Chem Biol Interact 1997;104:131–45.
[29] Ram PA, Waxman DJ. Thyroid hormone stimulation of NADPH P450 reductase expression in liver and extrahepatic tissues. Regulation by multiple mechanisms. J Biol Chem 1992;267:3294–301.
[30] Skett P, Weir A. Sex- and substrate-dependent effects of the thyroid gland on drug metabolism in the rat. Biochem Pharmacol 1983;32:3115–7.
[31] Coecke S, Vanhaecke T, Foriers A, Phillips IR, Vercruysse A, Shephard EA, et al. Hormonal regulation of glutathione S-transferase expression in co-cultured adult rat hepatocytes. J Endocrinol 2000;166:363–71.
[32] Parkinson A. Biotransformation of xenobiotics. In: Klaassen CD, editor. Casarett and Doull's toxicology. The basic science of poisons. North America: McGraw Comp; 2001. p. 133–224.
[33] Ha HR, Chen J, Freiburghaus AU, Follath F. Metabolism of theophylline by cDNA-expressed human cytochromes P-450. Br J Clin Pharmacol 1995;39:321–6.
[34] Hoch FL, Depierre JW, Ernster L. Thyroid control over biomembranes. Liver-microsomal cytochrome b5 in hypothyroidism. Eur J Biochem 1980;109:301–6.
[35] Hill KE, Burk RF. Effect of selenium deficiency and vitamin E deficiency on glutathione metabolism in isolated rat hepatocytes. J Biol Chem 1982;257:10668–72.
[36] Pascoe GA, Sakai-Wong J, Soliven E, Correia MA. Regulation of intestinal cytochrome P-450 and heme by dietary nutrients. Critical role of selenium. Biochem Pharmacol 1983;32:3027–35.
[37] Wrighton SA, Elswick B. Modulation of the induction of rat hepatic cytochromes P-450 by selenium deficiency. Biochem Pharmacol 1989;38:3767–71.
[38] Beckett GJ, Nicol F, Proudfoot D, Dyson K, Loucaides G, Arthur JR. The changes in hepatic enzyme expression caused by selenium deficiency and hypothyroidism in rats are produced by independent mechanisms. Biochem J 1990;266:743–7.
[39] Matsumoto K, Suzuki A, Washimi H, Hisamatsu A, Okajo A, Ui I, et al. Electron paramagnetic resonance decay constant and oxidative stresses in liver microsomes of the selenium-deficient rat. J Nutr Biochem 2006;17:677–81.
[40] Zimmerman HJ. Hepatotoxicity: the adverse effects of drugs and other chemicals on the liver. Philadelphia: Lippincott, Williams & Wilkins; 1999.
[41] Mark SD, Qiao YL, Dawsey SM, Wu YP, Katki H, Gunter EW, et al. Prospective study of serum selenium levels and incident esophageal and gastric cancers. J Natl Cancer Inst 2000;92:1753–63.
[42] Seitz HK, Stickel F, Homann N. Pathogenetic mechanisms of upper aerodigestive tract cancer in alcoholics. Int J Cancer 2004;108:483–7.
[43] Elmore CL, Porter TD. Modification of the nucleotide cofactor-binding site of cytochrome P-450 reductase to enhance turnover with NADH in vivo. J Biol Chem 2002;277:48960–4.
[44] Yamazaki H, Nakano M, Imai Y, Ueng YF, Guengerich FP, Shimada T. Roles of cytochrome b5 in the oxidation of testosterone and nifedipine by recombinant cytochrome P450 3A4 and by human liver microsomes. Arch Biochem Biophys 1996;325:174–82.
[45] Yamazaki H, Nakajima M, Nakamura M, Asahi S, Shimada N, Gillam EM, et al. Enhancement of cytochrome P-450 3A4 catalytic activities by cytochrome b(5) in bacterial membranes. Drug Metab Dispos 1999;27:999–1004.
[46] Yamazaki H, Nakamura M, Komatsu T, Ohyama K, Hatanaka N, Asahi S, et al. Roles of NADPH-P450 reductase and apo- and holo-cytochrome b5 on xenobiotic oxidations catalyzed by 12 recombinant human cytochrome P450s expressed in membranes of *Escherichia coli*. Protein Expr Purif 2002;24:329–37.
[47] Gu J, Weng Y, Zhang QY, Cui HD, Behr M, Wu L, et al. Liver-specific deletion of the NADPH-cytochrome P450 reductase gene: impact on plasma cholesterol homeostasis and the function and regulation of microsomal cytochrome P450 and heme oxygenase. J Biol Chem 2003;278:25895–901.
[48] Voice MW, Zhang Y, Wolf CR, Burchell B, Friedberg T. Effects of human cytochrome b5 on CYP3A4 activity and stability in vivo. Arch Biochem Biophys 1999;366:116–24.
[49] Perera FP, Mooney LA, Stampfer M, Phillips DH, Bell DA, Rundle A, et al. Associations between carcinogen-DNA damage, glutathione S-transferase genotypes, and risk of lung cancer in the prospective Physicians' Health Cohort Study. Carcinogenesis 2002;23:1641–6.