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Hypothyroidism minimizes the effects of acute hepatic failure caused by endoplasmic reticulum stress and redox environment alterations in rats

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ABSTRACT

The aim of this study was to investigate if a protective effect from hypothyroidism in acute liver failure resulted from reduced endoplasmic reticulum stress and changes to the redox environment. Twenty male Sprague-Dawley rats were divided in four groups: (1) euthyroid (sham surgery), (2) hypothyroid, (3) euthyroid (sham surgery) + thioacetamide and (4) hypothyroid + thioacetamide. Hypothyroidism was confirmed two weeks after thyroidectomy, and thioacetamide (TAA) (400 mg/kg, ip) was administrated to the appropriate groups for three days with supportive therapy. Grades of encephalopathy in all animals were determined using behavioral tests. Animals were decapitated and their blood was obtained to assess liver function. The liver was dissected: the left lobe was used for histology and the right lobe was frozen for biochemical assays. Body weight, rectal temperature and T_4 concentration were lower in hypothyroid groups. When measurements of oxidative stress markers, redox environment, γ-glutamylcysteine synthetase and glutathione-S-transferase were determined, we observed that hypothyroid animals with TAA compensated better with oxidative damage than euthyroid animals treated with TAA. Furthermore, we measured reduced expressions of GADD34, caspase-12 and GRP78 and subsequently less hypothyroidism-induced cellular damage in hypothyroid animals. We conclude that hypothyroidism protects against hepatic damage caused by TAA because it reduces endoplasmic reticulum stress and changes to the redox environment.

1. Introduction

Acute liver failure (ALF) is characterized by massive hepatic necrosis that causes jaundice, encephalopathy, coagulopathy and multi-organ failure. Viral hepatitis, hepatotoxic chemical-induced liver injury and drugs can cause ALF. Although a wide variety of medical therapies have been tested, emergency liver transplantation has proven the only successful treatment (Bruck et al., 1998). Experimentally, it has been proposed that the state of the thyroid modulates some hepatic diseases like ALF, cirrhosis and portal hypertension (Bruck et al., 1998; Oren et al., 1995, 1996). Moreover, hypothyroidism is considered a protective state against events that cause oxidative stress and cellular damage in organs with elevated metabolic rates in the heart, brain and kidney (Bobadilla et al., 2002; Tenorio-Velásquez et al., 2005; Rastogi et al., 2006; Estevez-Carmona et al., 2013). In addition, it has been shown that decreased thyroid function may be beneficial for a damaged liver (Bruck et al., 1998). Some mechanisms involved in hypothyroidism prevent cell damage like a reduced basal metabolic rate and production of oxidants (Bruck et al., 1998; Estevez-Carmona et al., 2013). Moreover, because thyroid hormones are pleiotropic, it is possible that they can modulate several signaling pathways that offer cells protection; for example, they could modulate endoplasmic reticulum stress.

The endoplasmic reticulum (ER) is an intracellular organelle responsible for the synthesis, folding, trafficking and maturation of proteins. The ER is also related with cholesterol-derived lipids, sterols and hormone synthesis as well as intracellular calcium regulation. Furthermore, when misfolded or unfolded proteins

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accumulate in the lumen of the ER (unfolded protein response or UPR), they sequester the chaperone-binding immunoglobulin protein/glucose-regulated protein 78 (GRP78) away from the luminal domain of ER sensors, causing their activation (Malhi and Kaufman, 2011). This state produces a pathological response called the ER stress response (Wu and Kaufman, 2006), which plays a main role in nonalcoholic steatohepatitis, alcoholic liver disease, ischemia/reperfusion injury, cholestasis models of liver disease and other hepatic diseases (Dara et al., 2011; Malhi and Kaufman, 2011). Under these conditions, activated events include lipogenesis, inflammation and apoptotic pathway stimulation due to oxidative processes or cellular damage (Guo and Li, 2014). Because hypothyroidism could minimize ALF symptomatology, our objective was to demonstrate that a protective effect from hypothyroidism in ALF is caused by a reduction in ER stress and reduced changes to the redox environment.

2. Materials and methods

2.1. Animals and housing

Twenty male Sprague-Dawley rats from our animal care facilities were singly housed (metallic cages, 20 cm × 30 cm × 18 cm, with food and water *ad libitum*) in cages, located together in racks (to preserve auditory and olfactory exposure) in a light (0800–2000 lights on) and temperature (21 ± 1 °C) controlled room. Rats were acclimatized to the colony/room conditions for at least 1 week prior to experiments. All experimental procedures described in this study were in accordance with the guidelines of the Laws and Codes of Mexico in the Seventh Title of the Regulations of the General Law of Health Regarding Health Research and the Mexican Official Standard NOM-062-ZOO-1999, which details the technical specifications for production, care and use of laboratory animals. The ENCB-IPN Bioethics Committee approved this protocol. We used the minimum number of animals required to attain the goals of this study.

2.2. Experimental design

First, rats were randomly divided into two groups: a control euthyroid group ($n = 10$) that underwent a surgical procedure without the removal of the thyroid gland and a hypothyroid group ($n = 10$) that had the thyroid gland removed, the parathyroid reimplanted and postoperative treatment. The thyroidectomy was performed in rats anesthetized with ketamine (PISA Laboratories, Mexico) (10 mg/kg, intramuscular *via*: im) and xylazine (PISA Laboratories, Mexico) (5 mg/kg, im) as previously described (Cano-Europa et al., 2010; Ortiz-Butron et al., 2011; Estevez-Carmona et al., 2013). In brief, for better observation, we used a stereomicroscope (Zeiss, Germany) to cut the stenothyroid muscle, exposing the trachea. After locating the parathyroid gland, the thyroid gland was carefully dissected to avoid injury to the laryngeal nerve and reimplanted into the surrounding neck muscle. After surgery, we injected ketorolac (Syntex-Mexico) (50 mg/kg im), gentamicin (ScheringPlough-Mexico) (10 mg/kg im) and mephenamic acid (1 mg/kg) (oral *via*: ov) over 5 d to alleviate pain and prevent infection. During treatment, body weight and rectal temperature were evaluated as an indirect measure of thyroid state. After decapitating the rats, we evaluated the serum concentration of thyroid hormone (T_4) in all groups.

2.3. Induction of acute liver failure

Two weeks after surgery, the animals were subdivided into four groups with five animals per group: (1) euthyroid, (2) hypothyroid, (3) euthyroid + thioacetamide (TAA) (400 mg/kg/d) (intraperitoneal *via*: ip) and (4) hypothyroid + TAA (400 mg/kg/d) (ip). TAA was administrated every 24 h for three consecutive days. A support therapy (5% glucose, 0.9% sodium chloride and 0.15% potassium chloride) was offered every 12 h. After 60 h of the first TAA administration, a behavioral assessment was performed to determine the grade of encephalopathy. The stages were evaluated as (1) lethargic, (2) mild ataxia, (3) lacking spontaneous movements and loss of reflexes and (4) loss of pain response loss and/or coma (Bruck et al., 1998). Animals were killed by decapitation. The blood and liver were removed immediately; the left lobe was stored in paraformaldehyde fixative 4% and the right lobe was stored at −70 °C for biochemical assays.

2.4. Histological

Liver parts were fixed with paraformaldehyde (4% in PBS) for 16 h and embedded in paraffin. 5-μm slices were obtained with a standard microtome (LEICA RM 2145, Germany). Each section was stained with hematoxylin–eosin (HE), dehydrated and mounted in resin. An unbiased observer defined the inflammation/necrosis scale for approximately ten sections per animal as (0) normal, (1) mild, (2) moderate or (3) severe (Bruck et al., 1999).

2.5. Functional thyroid and liver tests

Using an ELISA kit (Diagnostic System Laboratories, Inc.), we determined T_4 levels. Liver function was tested using serum samples by measuring concentrations of ammonia, glucose, cholesterol, indirect bilirubin, total bilirubin and direct bilirubin. In addition also we used serum samples for glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities were evaluated (Randox kit).

2.6. Oxidative stress, the redox environment and glutathione cycle markers

Sections of the right lobe of the liver were individually homogenized in 5 mL of 10-mM phosphate buffer (pH 7.4), and the homogenate was used to determine oxidative stress markers (lipid peroxidation and ROS quantification) and redox environmental markers (GSH, GSSG and the GSH^2/GSSG ratio). We also evaluated γ-glutamylcysteine ligase (γ-GCL) and gluthatione-S-transferase (GST) as glutathione cycle markers, as previously described (Ortiz-Butron et al., 2011).

2.7. Oxidative stress markers

We evaluated the oxidative stress markers, lipid peroxidation (LP) and quantified reactive oxygen species (ROS), as previously described (Cano-Europa et al., 2008).

2.8. Redox environment and glutathione cycle markers

To evaluate the redox environmental markers, we evaluated reduced glutathione (GSH), oxidized glutathione (GSSG) and the GSH^2/GSSG ratio, as previously described (Cano-Europa et al., 2008; Schafer and Buettner, 2013).

We assessed the activity of γ-glutamylcysteine ligase (γ-GCL) with 100 μL of cell homogenate added to 1 mL of 100-mM Tris-HCl at pH 7.5, containing 5-mM ATP, 20-mM $MgCl_2$, 10-mM L-glutamate, 10-mM l-α-aminobutyric acid, 2-mM EDTA and 0.005% bovine albumin. The mixture was incubated at 37 °C for 1 h. Next, we precipitated 500 μL of the mixture with 0.5-M trichloroacetic