Hypothyroidism induced by propylthiouracil decrease sirtuin1 content in rat heart

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Background: In this study, the changes in sirtuin1 (SIRT1) levels in the heart of rats with hypothyroidism (HT) were evaluated.

Methods: A total of 20 Wistar male rats were randomly divided into control groups (n=10) and HT groups (n=10). In the HT groups, we used 0.05% propylthiouracil (PTU) saline solution to prepare the HT model, while 0.9% sodium chloride solution was used as a placebo in the control groups. During the intervention period, the body weight of rats was measured weekly, and serum triiodothyronine (TT_3), tetraiodothyronine (TT_4), thyroid-stimulating hormone (TSH), body weight, heart weight, and SIRT1 content in the heart were measured after intervention. We compared the differences in SIRT1 content between the two groups and analyzed the correlation between SIRT1 and thyroid hormone concentrations.

Results: After 8 weeks of intervention, the HT rat model was successfully produced. The body weight of the HT group rats increased more slowly than that of the control groups. The heart weight of the HT groups was significantly lower than that of the control groups, and SIRT1 content decreased significantly compared to in control group rats. However, there were no significant differences between the two groups in heart weight index. The relative content of SIRT1 was significantly correlated with TT3 concentrations, both in the control group and HT group.

Conclusions: Our results suggest that HT induced by PTU significantly reduces myocardial SIRT1 content. Decreased TT3 was significantly associated with decreased SIRT1 content.

Keywords: Hypothyroidism (HT); heart; sirtuin; propylthiouracil (PTU)

Introduction

Silent information regulator 2 (SIR2) proteins are a family of class III histone deacetylases that are highly conserved in mammalian species. Seven subtypes (sirtuin1–7) have been identified (1-3). Among them, sirtuin1 (SIRT1) is highly expressed in mammalian hearts and can regulate a wide variety of cellular processes such as apoptosis, survival, DNA repair, and metabolism (4-6). Previous studies reported that SIRT1 can protect the myocardium through multiple pathways (7-9). The mitochondrial pathway has been widely examined. These studies indicated that SIRT1 can promote the anti-apoptotic effect of the mitochondria in myocardial cells by regulating the permeability, synthesis, and morphology of mitochondria (7-10). Decreased SIRT1 levels may have adverse effects on the myocardium such as

increased myocardial oxidative stress (11). Previous studies also showed that SIRT1 was significantly reduced in patients with heart failure (11,12). These studies indirectly suggest that SIRT1 has protective effects on the myocardium, which may affect thyroid hormones.

Thyroid hormone is secreted by the thyroid gland, has been confirmed to play a crucial role in regulating myocardial metabolism, and is related to the myocardium and SIRT1 (13). Thyroxine can inhibit cardiomyocyte apoptosis, particularly in patients with ischemia-reperfusion injury, and thyroid hormones exert their protection effects mainly through the mitochondria (14). Previous studies showed that the levels of circulating thyroid hormones are decreased in patients with acute myocardial infarction (15) and pretreatment with thyroid hormones significantly alleviates myocardial ischemia-reperfusion injury (14). Therefore, appropriate thyroxine levels may have protective effects on the myocardium, and low thyroxine levels do not contribute to myocardial viability. However, studies found that hypothyroidism (HT) levels are high in patients with clinical coronary heart disease (16) and can lead to various disorders such as cardiac dysfunction and heart failure (17). Recently published clinical studies have suggested that HT is associated with increased all-cause and cardiovascular mortality (18). In addition, decreased thyroxine and SIRT1 are harmful to the myocardium.

The effect of HT on SIRT1 content in myocardial cells remains unknown. Therefore, the purpose of this study was to determine the effects of HT on the content of SIRT1 in myocardial cells of rats.

Methods

Animals and treatments

We used 20 6-week-old (adults) male Wistar rats with body weights (BWs) of 180–200 g. All rats were obtained from the Animal Center of the Academy of Military Medical Sciences (Beijing, China). All studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH, publication number 85-23, revised 1996, http://grants.nih.gov/grants/olaw/olaw.htm). All protocols were approved by the Animal Subjects Committee of the Academy of Military Medical Sciences, Beijing, China, and the study was approved the Ethics committee of The Second People's Hospital of Yichang. Hypothyroid rats were prepared according to a previous study (19). Briefly, the rats were housed in cages and maintained at 22–24 °C with a normal 12-h/12-h light-dark cycle. The rats had free access to chow and water. The rats were randomly divided into control groups or HT groups to receive the diet and the following drug regimens for 8 weeks: (I) control groups (n=10): 0.9% sodium chloride solution was administered via gavage at 1 mL/day; (II) HT groups (n=10): 0.05% PTU saline solution was administered via gavage at 1 mL/day.

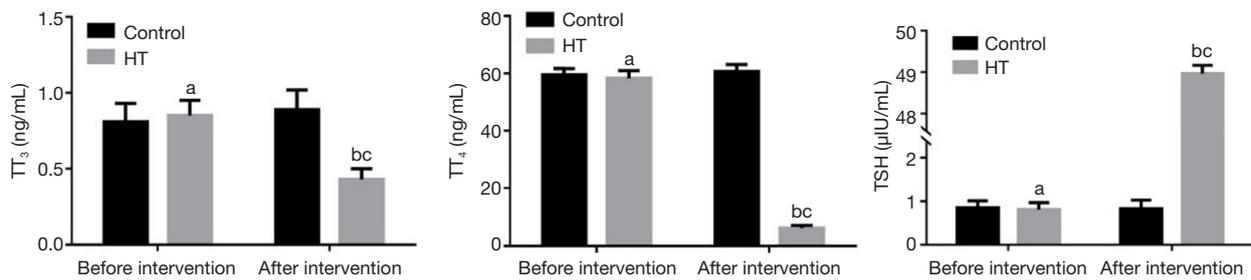### Measure of triiodothyronine (TT_3), tetraiodothyronine (TT_4) and thyroid-stimulating hormone (TSH)

We obtained blood samples (1 mL) from the femoral artery of rats before intervention to measure the baseline levels of TT_3, TT_4, and TSH. The rats were weighed using standard methods and then euthanized by cervical dislocation after receiving intervention for 8 weeks. We collected blood samples (1 mL) from each rat by cardiac puncture after opening the chest. These blood samples were centrifuged at 12,000 rpm and 4 °C for 10 min. The supernatant was collected to determine TT_3, TT_4, and TSH levels using commercial RIA kits (BNIBT, A01TFB for TT_3, A02TFB for TT_4, A05FZB for TSH, Beijing, China) in accordance with the manufacturer instructions.

Protein extract and measurement

After 8 weeks of intervention, blood samples were collected and the heart was excised immediately. The hearts were rinsed with cold (4 °C) normal saline, dried with filter paper, and measured to determine the heart weight (HW) index (HWI, HW/BW). Next, the left ventricular tissue was isolated, placed on ice immediately, and homogenized in radio-immunoprecipitation assay lysis buffer (CWbio, Beijing, China). Total protein in the supernatant was extracted from the homogenate by centrifugation (12,000 rpm, 4 °C, 10 min). Quantification of total protein was conducted using the BCA method with a BCA Kit (Takara, Shiga, Japan).

Western blot analysis to determine relative SIRT1 content

For direct immunoblotting, aliquots of the lysate were mixed with 5× SDS-PAGE sample loading buffer (containing 5% 2-mercaptoethanol) and boiled for 10 min. The same amounts of proteins (60 µg) were loaded into 10% acrylamide gels and then transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA,

Figure 1 Thyroid-related hormones levels of serum in different group and different time. a, P>0.05 *vs.* controls; b, P<0.05 *vs.* controls; c, P<0.05 *vs.* before intervention. HT, hypothyroidism; TT$_3$, total triiodothyronine; TT$_4$, total tetraiodothyronine; TSH, thyroid-stimulating hormone.

USA). The PVDF membranes were blocked with 8% nonfat milk in Tris-buffered saline containing 0.1% (v/v) Tween 20 (TBST) for 1 h at room temperature (about 25 ℃) and then incubated overnight with an anti-SIRT1 antibody (1:8,000, ab110304; Abcam, Cambridge, UK) and anti-β-actin (1:1,000, ab8226; Abcam) at 4 ℃. The PVDF membranes were washed extensively with TBST before incubation for 1 h with a secondary anti-mouse or anti-rabbit IgG (1:1,000, Cell Signaling Technology, Inc., Danvers, MA, USA) conjugated to horseradish peroxidase. Protein bands were detected by the standard enhanced chemiluminescence method, and images were digitized. Next, relative band intensities were measured by densitometry using Image Lab software version 4.1.0 (Bio-Rad, Hercules, CA, USA).

Statistical analysis

All data were analyzed with SPSS16.0 (SPSS, Inc., Chicago, IL, USA). Data from independent experiments were expressed as the mean ± SD of at least three experiments. Differences of continuous variables between the two groups were compared by analysis of variance with repeated measures and the paired *t*-test was used to compare differences before and after intervention. The Spearman approach was used to analyze the correlation. P<0.05 was considered statistically significant.

Results

Effects of PTU on thyroid function in rats

PTU has a significant effect on the thyroid function of rats. No statistical differences were found between the two groups in TT$_3$, TT$_4$, and TSH before intervention. After intervention for 8 weeks, serum TT$_3$ and TT$_4$

concentrations were significantly lower than those before intervention and in the control groups (P<0.05), while serum TSH levels were higher than those before intervention and in the control groups (P<0.05) (*Figure 1*).

Effects of PTU on body and HW of rats

At baseline, BW was not significantly different between the HT groups and control groups (P>0.05) (*Figure 2*). HT induced by PTU resulted in significant slowing of BW gain compared to rats in the control groups (P<0.05) (*Figures 2,3*). Interestingly, the HW of rats in HT groups was significantly lower than that in the control group (P<0.05) (*Figure 2*), but there was no significant difference in HWI between the two groups (P>0.05) (*Figure 2*).

Effects of PTU on SIRT1 of myocardium of rats

The SIRT1 content of the myocardium in the PTU groups was lower than that in the control groups (P<0.05) (*Figure 4*).

Correlation between relative SIRT1 content and thyroid hormone concentrations

The results of correlation analysis showed that the relative content of SIRT1 was correlated with thyroid hormones levels, particularly for TT$_3$ (P<0.05) (*Figure 5*).

Discussion

A previous study showed that HT had a promoting effect on SIRT2 (homologous protein of SIRT1) in nerve cells (20). However, the effect of HT on SIRT1 in the myocardium remained unclear. We evaluated the relationship between
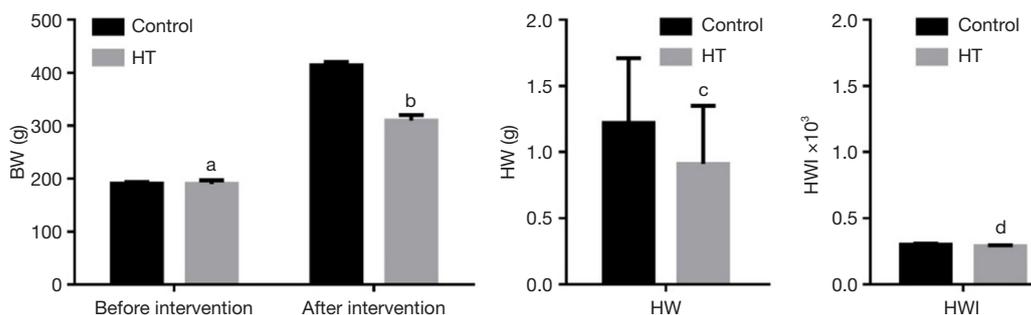
Figure 2 BW and HW in the different rat groups. a, P>0.05 *vs.* controls before intervention; b, P<0.05 *vs.* controls after intervention; c, P<0.05 *vs.* controls; d, P>0.05 *vs.* controls. HT, hypothyroidism; BW, body weight; HW, heart weight; HWI, heart weight index.
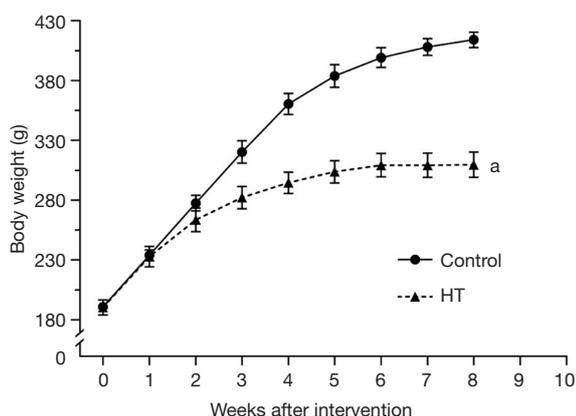
**Figure 3** The change of BW in different groups. a, P<0.05 *vs.* controls. HT, hypothyroidism.
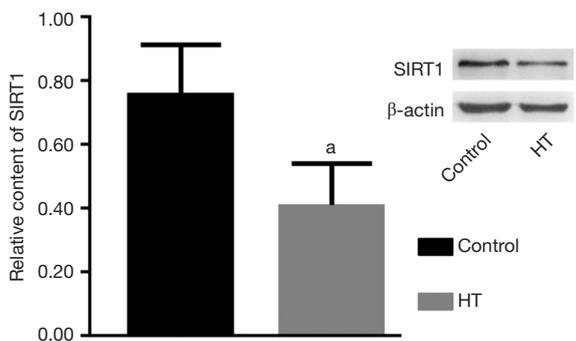
Figure 4 The relative protein content of SIRT1 in each group. a, P<0.05 *vs.* controls. HT, hypothyroidism; SIRT1, sirtuin1.

HT induced by PTU and SIRT1 content in rat hearts. The myocardial SIRT1 content of rats decreased significantly in HT. The rats showed slower weight gain and lower HW in HT. These results are consistent with those of previous studies (21,22).

Sirtuins are analogs of SIR2 and include seven subtypes (SIRT1–7) that exist as the oxidized form of nicotinamide adenine dinucleotide (NAD$^+$)-dependent protein deacetylase (1-3). A relationship between thyroid hormones and SIRT1 was observed in the present study. Previous studies have explored the relationship between thyroid hormones and SIRT1 in epidermal keratinocytes and hepatocytes, but not in the myocardium. A previous study showed that expression of SIRT1 in cultured human epidermal keratinocytes was increased after treatment with T_3 (23). Diiodothyronine (T_2) can rapidly increase the activity of SIRT1 in hepatocytes (24). Thyroid hormones increase the content of SIRT1, and SIRT1 may exert negative feedback regulation on thyroid hormones. In obese rats induced by diet, the suppression of hypothalamic SIRT1 significantly stimulates the hypothalamus-pituitary-thyroid axis and promotes the secretion of thyroid hormones (25). The mechanism of the decrease of SIRT1 after HT remains unclear. Additionally, how thyroid hormones increase SIRT1 content is unclear. SIRT1 has been shown to participate in myocardial protection. First, SIRT1 can increase the resistance to oxidative stress and ischemia/reperfusion injury via multiple pathways (4,26,27). Decreased SIRT1 in the heart may aggravate myocardial ischemia-reperfusion injury (28), cardiomyocyte apoptosis, and early-onset heart failure (29). Second, SIRT1 plays an important role in anti-atherosclerotic lesions (30) and reduces the oxidation of low-density lipoprotein (31). Third, SIRT1 can inhibit myocardial apoptosis by regulating the mitochondria in multiple manner (9,10,32-35). Therefore, SIRT1 is a myocardial protective factor, and its reduction adversely affects the myocardium. These studies demonstrated that HT plays an important role in heart failure, myocardial
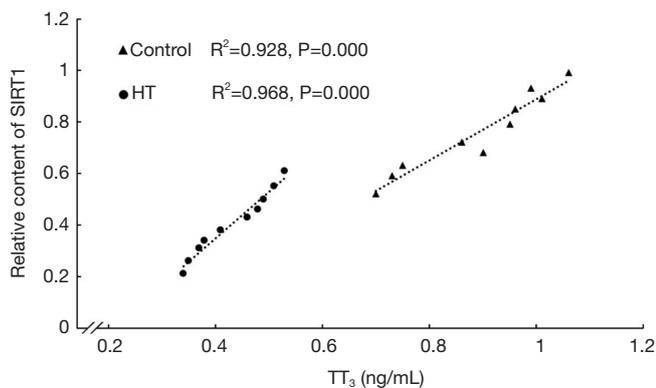
Figure 5 The correlation between SIRT1 relative content and thyroid hormones concentrations. HT, hypothyroidism; TT_3, total triiodothyronine; SIRT1, sirtuin1.

infarction, and other myocardial ischemia. The present study found that administration of thyroid hormone may improve myocardial ischemia and hypoxia as well as apoptosis by reducing HT and SIRT1. Our study also suggests that TH-induced atherosclerosis is associated with a reduction in SIRT1 by HT.

There were some limitations to our study. First, we did not observe myocardial tissue sections to determine whether there is a significant difference in morphology of myocardial cells between the two groups. Second, this study did not explore whether HT induced by PTU reduces the expression of SIRT1 at the transcriptional level. Finally, animal models were used, and we did not validate the results in cultured cells. Further studies are needed to examine the morphological changes in myocardial cells in the reduction of SIRT1 induced by HT and the effect of HT on the transcription of SIRT1. Whether increasing the content of SIRT1 or promoting the activity of SIRT1 in the myocardium can reduce myocardial damage caused by HT and whether this contributes to the protective effect of SIRT1 by increasing thyroid hormones requires further analysis. However, this study confirmed that PTU-induced HT leads to decreased levels of SIRT1 in cardiomyocytes, and this decrease was related to decreased TT_3. Based on our results, myocardium injury caused by HT may be associated with reduced SIRT1 in the heart.

Acknowledgments

Footnote

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