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The Possible Role of 3-Iodothyronamine in Browning of Inguinal White Adipose Tissue in Mice

Farelerde İnguinal Beyaz Yağ Dokusunun Kahverengileşmesinde 3-iyodotironaminin Olası Rolü

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Abstract

Purpose: In this study, we have investigated whether administration of a chronic low dose of 3-iodothyronamine (T1AM) could regulate the concentration of uncoupling protein 1 (UCP1) in mice inguinal white adipose tissue (IWAT).

Material and Method: Eighteen male mice were randomly divided into treatment (n=10) and control (n=8) groups. The experimental procedure was applied for seven days during which, the test group received T1AM dissolved in DMSO and saline, whereas the control group received only DMSO and normal saline. The inguinal white adipose tissue was analyzed for UCP1 and the serum was tested for non-esterified fatty acids concentration.

Results: There was a significant increase (P=0.01) in the UCP1 concentration (149±27.28) of test group when compared to the control (102±38.52). Interestingly the concentration levels of non-esterified fatty acids did not show any significant increase (P=0.14) between the two groups.

Discussion: 3-iodothyronamine increased the UCP1 concentration in IWAT in mice just like T_3 and it was also able to manipulate the white adipose tissue for the promotion of thermogenesis and weight loss. According to our findings, T1AM enhanced browning responses in white adipocytes just like T_3.

Keywords: 3-Iodothyronamine; inguinal white adipose tissue; plasticity; uncoupling protein 1

Özet

Amaç: Bu çalışmada kronik düşük doz 3-iyodotironamin (T1AM) verilmesinin fare inguinal beyaz yağ dokusunda (IWAT) uncoupling protein 1 (UCP1) düzeyini denetleme yetisini araştırdık.

Gereç ve Yöntem: On sekiz erkek fare rastgele şekilde tedavi (n=10) ve kontrol (n=8) gruplarına ayrılmıştır.Deneysel işlem yedi gün boyunca uygulanmıştır. Test grubu DMSO ve salin içinde eritilmiş T1AM alırken, kontrol grubuna sadece DMSO ve normal salin verilmiştir. İnguinal beyaz doku UCP1 açısından incelenmiş ve serumda esterifiye olmamış yağ asid konsantrasyonları ölçülmüştür.

Bulgular: Kontrol (102 ±38.52) ile karşılaştırıldığında test grubunda (149 ±27.28) anlamlı UCP1 konsantrasyon artışı (p=0.01) izlenmiştir. İlginç şekilde esterifiye olmamış yağ asid konsantrasyon düzeyleri her iki grup arasında anlamlı artış göstermemiştir (p=0.14).

Tartışma: T3'e benzer şekilde farelerde inguinal beyaz yağ dokusunda 3-iyodotironamin UCP1 konsantrasyonlarını arttırmıştır. Ayrıca, termogenez ve kilo kaybı için beyaz yağ dokusunu düzenleyebilmiştir. Bulgularımıza göre T1AM beyaz adipositlerde aynı T3 gibi kahverengileşme cevabını güçlendirmiştir.

Anahtar kelimeler: 3-iyodotironamin; inguinal beyaz yağ dokusu; plastisite; uncoupling protein

Introduction

Mitochondrial uncoupling protein 1 (UCP1), also called thermogenin, forms a channel in the inner mitochondrial membrane that allows protons to re-enter the mitochondrial matrix without passing through the ATP synthase complex. This permits continual oxidation of fuel (fatty acids in an adipocyte) without ATP synthesis, dissipating energy as heat and consuming dietary calories or stored fats. The aforementioned process is mediated in a specialized adipose tissue named brown adipose tissue (BAT) (the main

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site of adaptive thermogenesis), which is predominantly found in infants [1-3]. Recently BAT has become a research area attracting intense interest due to its presence in human adults as metabolically active tissue. Furthermore, the population of white adipocyte-like cells (brite/beige cells) can be transformed into brown adipocytes under certain stimuli like physiological (exposure to cold, development, gestation-lactation cycles), pharmacological (treatment with 3-adrenergic agonist, irisin, nitric oxide (NO), cannabinoid receptor 1 (CB_1) antagonists) and pathophysiological conditions [4-10]. TH signaling is required for normal BAT thermogenesis, the differentiation and development of BAT, and UCP1 expression. Additionally, there is evidence about TH's possible role in WAT browning [11-14]. Since 2004, when Scanlan and colleagues discovered 3-iodothyronamine (T1AM) as an endogenous metabolite and structural analog of thyroid hormone (TH), a new prospect opened up in thyroid metabolism research [15]. The subchronic T1AM treatment causes ketonuria and a significant loss of body fat indicating a shift in the metabolic pathways from carbohydrate to lipid oxidation. The treatment also triggers lipolytic pattern in rat adipose tissue and modulates mitochondrial FoF1-ATP synthase activity [16-20]. Taken together, these modulations in lipid metabolism provide a balance in energy homeostasis and thus have a great potential in therapeutic applications (especially for treating obesity). The present study was conducted to evaluate the role of T1AM in manipulating WAT brownification and the effect of its chronic low dosages on UCP1 concentration (BAT-specific biomarker) in IWAT of mice for the first time.

Materials and Methods

Animals

Eighteen male mice (28-30 g body weight) were selected for this study and divided into treatment (n=10) and control (n=8) groups. All animals were housed under constant temperature (20 °C) with a 12h/12h light/dark (L/D) cycle (lights on at 7:00 am). The animals were allowed to adapt to their environments for six days before the first experimental injection. Food and drinking water were given *ad libitum*. All experiments were performed in accordance with the guidelines for use of animals in research, approved by Veterinary School of Shiraz University, Iran. The body weights of the mice were determined on the first day of the experiment, the day of drug administration (before the drug was administered) and the eighth day (before their sacrifice).

Animal ethics

The experiment was performed with the approval of the state committee on animal ethics, Shiraz University, Shiraz, Iran (IACUC no: 4687/63). In addition, the recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the protection of animals used for experimental purposes, were considered.

Test procedure

The experimental procedure was applied for seven days and the first day of injection was considered as day 1. During the seven days, test group received T1AM dissolved in DMSO and saline while the control group received only DMSO and normal saline. T1AM (ethylamino-1, 1, 2, 2-d_4 hydrochloride) (Lot # STBD6692V) was purchased from IsoSciences (King of Prussia, USA). The animals in the test group were treated with intraperitoneal injections of 10 mg/Kg of T1AM (dissolved in 20% dimethyl sulfoxide (DMSO) and 80% physiological saline), once a day for seven days. The control mice were treated with T1AM-free intraperitoneal injections (80% normal saline plus 20% DMSO) under the same housing conditions for seven days. The mice were then sacrificed using ether and the inguinal adipose tissues (IWAT) were immediately removed as described earlier [21]. IWATs of all the mice were weighed and homogenized with the help of a homogenizer. The homogenates were centrifuged (20 min at 750 ×*g*) and the supernatants were removed for storage at -70 °C till further use.

Blood samples

Blood samples were collected on day 8 (24 h after the last injection) from the heart in sterile test tubes and allowed to clot for 30 min. The sera were separated by centrifugation at 750 *g* for 15 min and stored at -70 °C until assay.

Biochemical analysis

Uncoupling protein 1 (UCP1) was measured by double-antibody sandwich enzyme-linked immunosorbent assay using commercial mouse uncoupling protein (UCP1) ELISA kit (Shanghai Crystal Day Biotech Co., LTD). The serum non-esterified fatty acids (NEFA) were measured by kinetic, enzymatic and colorimetric methods using non-esterified fatty acids (NEFA) kit (Fortress Diagnostics Limited, Antrim, UK).

Statistical analysis

All data were statistically analyzed by SPSS/PC software (version 16). Independent-Samples t-test and the Mann-Whitney U test were used for the comparison of the mean values of UCP1 and NEFA concentrations, respectively. Two-way repeated measures ANOVA was applied for comparison of weight means before and after the experiment and statistical significance was defined as $P<0.05$.

Results

The results are presented as mean ±standard deviation (SD) in SI units. Table I represents the mean concentrations of UCP1 in inguinal white adipose tissue and NEFA in serum in control and test groups. There was significant a difference (P=0.01) in the UCP1 concentration between the control and test groups (102±38.52 versus 149±27.28) and no significant difference (P=0.14) in the concentration of NEFA between the control and test groups (2.57±0.68 versus 3.51±1.54).

Table II represents the mice weight (g), as mean ±SD, in control and test groups before (day 1) and after the procedure (day 8). There was no statistically significant interaction in the weight before (day 1) and after the procedure (day 8) in the control group and no significant interaction was observed in the weight before and after the procedure in the test group (P=0.08).

Table 1. Mean ±SD of UCP1 and NEFA concentrations in the control group mice that were treated with T1AM-free intraperitoneal injections (80% normal saline plus 20% DMSO) and test group that was administered T1AM (dissolved in 20% DMSO and 80% normal saline) by intraperitoneal injection of 10 mg/Kg once a day for seven days.

	Groups	
Biochemical Factors	**Control**	**est**
UCP1 (ng/mL) (p=0.01)	102±38.52	149±27.28
NEFA (mmol/L) (p=0.14)	2.57±0.68	3.51±1.54

Table 2. Mean ±SD of mice weight (g) in control and test groups before (day 1) and after the procedure (day 8) (p=0.08).

Groups	Weight Day 1	Weight Day 8
Control	30.43±2.44	30.57±2.14
Test	28.8±5.57	27.5±4.81

Discussion

In this study, T1AM was found to increase the UCP1 concentration in IWAT of mice. Under certain conditions adipose tissue can show plasticity, wherein one AT phenotype deposit is able to reversibly transform into the other with different morphological features [22]. Although all of the fat deposits have plasticity properties, there are heterogeneity and potential differences among them based on the anatomical sites and kind of species being discussed. For example, periovarian fat in rats, inguinal fat in mice, and numerous white fat pads in dogs display emergence of brown adipocytes among fat, which is considered and studied as typical white fat [7-9, 23]. The cellular mechanisms involved in this process are not clearly known yet. However, Moulin et al. showed that the expression of UCP1 (a biomarker of browning) is triggered by the overexpression of co-activator of peroxisome proliferators activated receptor (PPAR) and PGC-1 [24, 25].

It is well established that thyroid hormones (THs) regulate adipocyte plasticity, proliferation, and differentiation, because of which they can be used in obesity treatment to increase the metabolic rate and weight lose [14, 26-28). Their use, however, is limited because of their thyrotoxic effects on the heart, such as tachycardia and dysrhythmia. The identification of TH analogs that have fewer side effects in addition to lipolytic effect is therefore desirable. 3-Iodothyronamine (T1AM) is an endogenous biogenic amine, derived from TH. It has recently been shown to possess some metabolic effects similar to TH, which is considered as a novel chemical messenger. This makes T1AM a high potency metabolic hormone. The molecular targets of T1AM are currently unknown but Mariotti et al. [18] have shown a greater responsiveness of adipose tissue compared to the liver by the fifth day of T1AM administration (18). They also established that a high number of genes was altered in rat subcutaneous adipose tissue, especially the ones related to lipoprotein function and cholesterol homeostasis. It is determined that T1AM is able to alter the whole body and cellular metabolism. Moreover, the reports from some studies provide strong evidence about its mitochondrial effects, raising the question of whether or not T1AM could affect UCP1 and WAT browning as much as THs. Based on the fact that there exists no understanding on this subject, this study could improve our knowledge about the multilateral contribution of T1AM in adipose tissue metabolism. Therefore, we sought to measure UCP1 in IWAT of mice after administration of a chronic low dose of T1AM at 10 mg/kg/day over the course of seven days. As shown in Table I, the test group receiving 10 mg/kg/day T1AM had higher UCP1 concentration than the control group. UCP1 is a browning marker, which meant that T1AM was effective in inducing BAT property in typical WAT. Lee and colleagues demonstrated that T_3 induced UCP1 expression in human multipotent adipose-derived stem cells [14]. This takes place through thyroid receptor (TR) gene expression and regulates thermogenesis in BAT by TR isoform [26, 27]. It is well known that T1AM is not a ligand for TRs. Therefore, it can be stated that in our experiment the thyroid metabolite T1AM affects the browning just like thyroid hormones but via different receptor(s) and effector(s). It can be seen in Table I that although there was no significant difference in the NEFA concentration between the groups, T1AM of the test group was higher than the control. This observation is because of the lipolytic effect of T1AM which has been clearly proven by Haviland et al. [31]. In their study, the diet induced obese mice were injected a dose similar to that used in our study, which changed the metabolism into lipolysis and resulted in the increase of 3-hydroxybutyrate (lipid metabolite intermediate) and significant weight loss at ~8.3% body weight by day 9. This is in agreement with our result (Table II), where the test group mice lost weight (~1 g) while the control group exhibited a non-significant increase in weight. T1AM has been found to regulate the cAMP synthesis through an interaction with the trace amine-associated receptor subtype 1 (TAAR1) and it has also been reported as a more potent activator of TAAR1 receptor compared to other thyronamines [15]. Some earlier findings suggest that cAMP released by this receptor is dose dependent with T1AM [29]. Mitchel et al. [30] performed RT-PCR with rat TAAR1 specific primers and noticed that TAAR1 mRNA was present in various rat tissues including adipose tissue and that T1AM induced some of its lipolytic effects by activating this receptor [30]. This is further supported by Marrioti et al. [18], who used chronic T1AM treatment (the same dose as our study twice a day for five days) in a rat model and looked for gene expression changes [18]. They discovered elevated lipolysis and gene expression of beta-oxidation markers coupled with the decreased expression of adipogenesis markers in adipose tissue. Adipocytes lipolysis is also mediated by -adrenoceptors (-ARs) that activate adenylate cyclase leading to the phosphorylation of hormone-sensitive lipase and release of FFA as a consequence [32]. UCP1 is activated by FFA and thus its activity is directly coupled to lipolysis [33]. The similarities between T1AM and adrenergic system were not considered as a novel subject because in a previous experimental study it was demonstrated that increased glucose level by T1AM does not alter insulin response like adrenergic receptor stimulation in liver and pancreas [17]. These effects by T1AM were mediated by the TAAR1 receptor, which possessed remarkable homology with adrenergic receptors based on high amino acid similarity in the ligand-binding region [34]. Interestingly, a broad spectrum of ligands including trace

amines interacts with the adrenergic receptors and modulates different physiological functions [35], but neither T1AM nor 3-T1AM activates 2 adrenergic receptors [15]. Scanlan et al. [15] showed a rapid 10 °C fall in the body temperature of the mice following a single i.p. injection of T1AM, lasting up to 1 h after the injection until it peaked and returned back to normal level after 4-6 h. In a recent study by Fisher et al. [36], UCP1 gene was expressed after 72 h of cold exposure in IWAT in mice [36]. Although we did not measure the temperature of the mice, we assume that this hypothetical drop in temperature for seven days might help IWAT to produce UCP1 as adaptive thermogenesis.

Conclusion

Clearly, further work is required in this area to delineate the role of T1AM in WAT plasticity. Nevertheless, according to our findings, T1AM enhances the browning responses in white adipocytes just like T_3.

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Ethics

This study was accomplished under the approval of the State Committee on Animal Ethics, Shiraz University, Shiraz, Iran. The recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the standards in the protection of animals used for experimental purposes, were also followed.

Author Contributions

Concept: Nasrin Kazemipour, Saeed Nazifi, Dizayn: Neda Eskandarzade, Saeed Nazifi, Data Collection or Processing: Asma Jafarizade, Saeed Nazifi, Analysis or Interpretation: Neda Eskandarzade, Nasrin Kazemipour, Asma Jafarizade, Literature Search: Nasrin Kazemipour, Asma Jafarizade, Writing: Neda Eskandarzade, Saeed Nazifi

Conflict of Interest: The authors have no conflict of interest regarding this study.

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