Araştırma Makalesi   Research Article

Modulating Liver Cholesterol Metabolism by 3-Iodothyronamine

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Abstract

In this study, we attempt to find out whether chronic low dose 3-Iodothyronamine (an endogenous metabolite of thyroid hormone) administration could modulate liver de novo cholesterol synthesis, the same as thyroid hormones. Eighteen male mice were divided randomly into treatment (n=10) and control (n=8) groups. The experimental procedure was applied for 7 days during which test group received T1AM whereas the control group received dimethyl sulfoxide and normal saline. The liver was analyzed for HMG-CoA reductase concentration and hepatic lipase activity whiles cholesterol, LDL and HDL concentrations were measured in the blood serum. There was non-significant decrease in HMG-CoA reductase concentration (224±21.2 versus 187±32.5) in test group compared to control. Interestingly LDL and cholesterol concentrations exhibited significant decrease in test group versus the control. There was non-significant decrease in hepatic lipase activity (771±316 versus 645±317) in test group versus the control. It appears that T1AM reduced serum LDL and cholesterol just like T_3, in contrast, it decreased liver cholesterol biosynthesis contrary to THs.

Introduction

Inhibition of converting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-coA) to mevalonate (initiating step in cholesterol biosynthesis pathway) accomplished by HMG-coA reductase is the target of drug development for hypercholesterolemic patients. Statins are the best competitive inhibitors of this enzyme which is commonly used as a drug worldwide (Istvan and Deisenhofer, 2001). Although thyroid hormones stimulate this enzyme, hypothyroidism individuals are described as having hypercholesterolemia because of the regulatory role of THs on cholesterol profile through various ways (Bakker et al., 1998; Canaris et al., 2000; Muls et al., 1984; O'Brien et al., 1990). Besides T3, there are some peripheral thyroid hormones (THs) derivatives termed 'non-classical THs', which were previously described as inactive breakdown products. However, several reports recently postulated their genomic and non-genomic biological effects, which have been reviewed by Senese et al. (2014). For instance, 3-iodothyronamine (T1AM) is decarboxylated derivative of THs, a member of the new family of endogenous signaling molecules was discovered through the pioneering work of Scanlan et al.

(2004). The main receptors activated by T1AM are high affinity G protein-coupled receptors, including trace amine-associated receptor 1 (Scanlan et al., 2004; Zucchi et al., 2010) and possibly α2-adrenoceptors (Regard et al., 2007). Plasma membrane and vesicular biogenic amine transporters as well as different mitochondrial ligands are also considered as T1AM targets (Cumero et al., 2012; Snead et al., 2007; Venditti et al., 2011). However, it is well known that T1AM did not trigger THs receptors at all thus, administration of exogenous T1AM produced functional effects often opposite to those induced by T_3 (Liggett, 2004; Scanlan et al., 2004; Weatherman, 2007). Mariotti et al. (2014) confirmed gene expression patterns for the connections between T1AM and the genes that have a crucial role in cholesterol metabolism. We decided to evaluate the role of this THs structural analog (T1AM) in the modulation of cholesterol de novo synthesis and metabolism. 3-hydroxy-3-methylglutarylcoenzyme A (HMG-coA) reductase concentration was measured in hepatic tissue. Hepatic lipase activity was also assayed and LDL, cholesterol and HDL concentrations in the blood serum were measured.

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 12 h/12 h light/dark (L/D) schedule (lights on 7:00 am). Animals were allowed to adapt for 6 days before the first experimental injection. Food and drinking water were available *ad libitum.*

Animal ethics

This experiment 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.

Test procedure

The experimental procedure was applied for 7 days and the first day of injection was considered as day 1. During 7 days test group received T1AM whereas the control group received DMSO and normal saline. T1AM (ethylamino-1, 1, 2, 2-d4 hydrochloride) Lot # STBD6692V was purchased from IsoSciences (King of Prussia, USA). Animals in test group were treated with T1AM (dissolved in 20% dimethyl sulfoxide (DMSO) and 80% physiological saline) by intraperitoneal injection of 10 mg/Kg once a day for 7 days. Eight control mice were treated with T1AM-free intraperitoneal injections (80% normal saline plus 20% DMSO) under the same housing conditions. The mice were then sacrificed by ether and the liver was immediately removed followed by weighing and being homogenized by homogenizer. The homogenates were centrifuged (20 min at 750 g) and the supernatants were removed and stored at -70°C until use.

Blood samples

Blood samples were collected on day 8 (24 hours after the last injection) from heart in sterile test tubes and allowed to clot for 30 min. The sera were separated following centrifugation at 750 x g for 15 min and stored at -20°C until assay.

Biochemical analysis

HMG-CoA reductase was measured using double-antibody sandwich enzyme-linked immunosorbent technique using commercial mouse hydroxymethylglutaryl CoA reductase (HMG-CoA) ELISA kit (Shanghai Crystal Day Biotech Co., LTD). Hepatic lipase activity was measured using the enzymatic method worked by (Lott et al., 1986). The serum was analyzed for cholesterol using a modified Abell-Kendall/Levey-Brodie (A-K) method (Burtis and Ashwood, 1994).

Statistical analysis

All data were statistically analysed by SPSS/PC software (version 16). Mann–Whitney test was used for comparison of the HMG-CoA reductase and hepatic lipase means. Independent-Samples t-test was used for comparison of the cholesterol, LDL and HDL concentrations means between control and test groups. $P<0.05$ was considered as statistically significant.

Results

The results are shown as mean ± standard error (SE) in SI units. Figure 1 and 4 represent the mean concentrations of HMG-CoA reductase and hepatic lipase activity in control and test groups respectively. Figure 2, 3 and 5 show differences in LDL, cholesterol and HDL concentrations in control and test groups respectively.

Non-significant decrease was observed in the concentration of HMG-CoA reductase (224±21.2 versus 187±32.5), between control and test groups. There was significant difference in LDL concentration (0.76±0.11 versus 0.25±0.05), between control and test groups. There was significant difference in cholesterol concentration (2.64±0.1 versus 2.1±0.05), between control and test groups. There was non-significant decrease in hepatic lipase activity (771±316 versus 645±317), between control and test groups. No significant difference was observed in the concentration of HDL (1.8±0.11 versus 1.58±0.09), between control and test groups.

Discussion

In the present investigation we showed chronic low dose 3-iodothyronamine (T1AM) could decrease LDL probably through modulating de novo cholesterol synthesis. It is well established that hypothyroidism could not be separated from hypercholesterolemia, mainly due to disturbance of low density lipoprotein (LDL) clearance (Canaris et al., 2000; Muls et al., 1984; O'Brien et al., 1990). However, it is not surprising considering the regulatory effects of thyroid hormones on the LDL receptors and the activity of some key enzymes of lipoprotein metabolism. Promoter of the LDL receptor gene contains a thyroid hormone responsive element (TRE) that allows the triiodothyronine (T3) to upregulate the gene expression of the LDL receptor (Bakker et al., 1998).

Interestingly, the thyroid hormones increase the hepatic de novo cholesterol synthesis by inducing the HMG-CoA reductase that catalyzes conversion of HMG-CoA to mevalonate, the initiation reaction in the biosynthesis of cholesterol (Ness et al., 1973). Thyroid
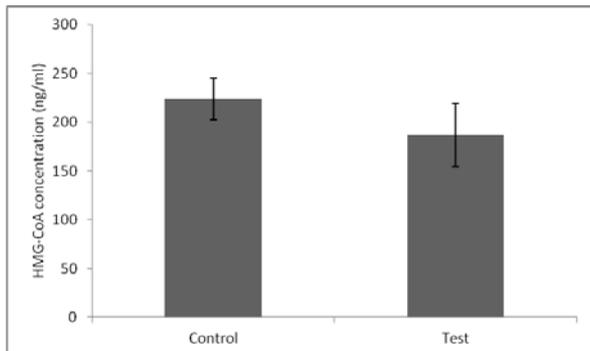
Figure 1. Hepatic HMG-CoA reductase concentration in control group mice which treated with T1AM-free intraperitoneal injections (80% normal saline plus 20% DMSO) and test group which administered T1AM (dissolved in 20% DMSO and 80% normal saline) by intraperitoneal injection of 10 mg/kg once a day for 7 days.
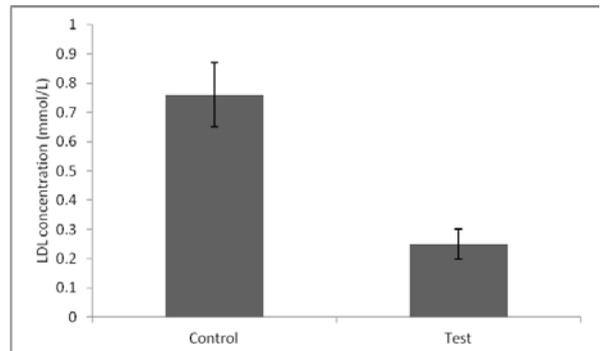

Figure 2. LDL concentration in control group mice which treated with T1AM-free intraperitoneal injections (80% normal saline plus 20% DMSO) and test group which administered T1AM (dissolved in 20% DMSO and 80% normal saline) by intraperitoneal injection of 10 mg/kg once a day for 7 days.
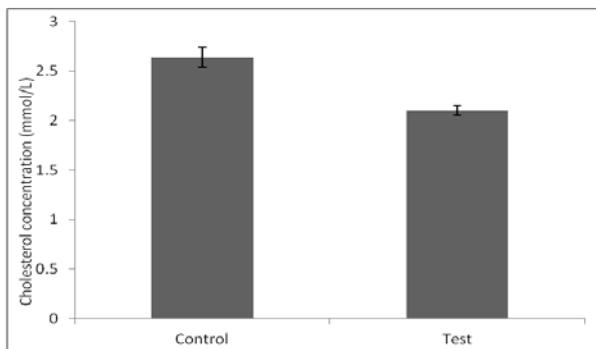

Figure 3. Cholesterol concentration in control group mice which treated with T1AM-free intraperitoneal injections (80% normal saline plus 20% DMSO) and test group which administered T1AM (dissolved in 20% DMSO and 80% normal saline) by intraperitoneal injection of 10 mg/Kg once a day for 7 days.

Figure 4. Hepatic lipase activity in control group mice which treated with T1AM-free intraperitoneal injections (80% normal saline plus 20% DMSO) and test group which administered T1AM (dissolved in 20% DMSO and 80% normal saline) by intraperitoneal injection of 10 mg/Kg once a day for 7 days.
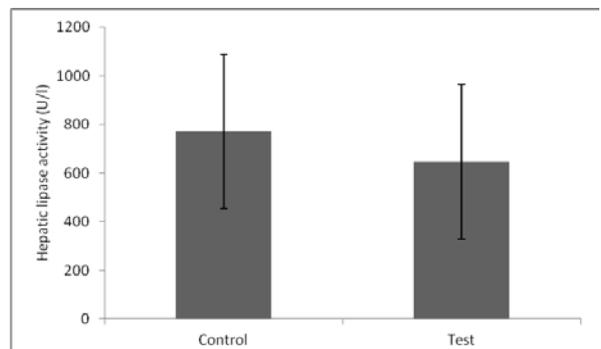
hormones also stimulate the cholesteryl ester transfer protein (CETP), a protein responsible for transporting cholesteryl esters from HDL_2 to the very low density lipoprotein (VLDL) and triglycerides (Lagrost, 1994). Up to year 2012 all of the literature reported decreased activity of LDL-receptor in hypothyroidism resulting in decrease of receptor-mediated catabolism of LDL being the only cause of the hypercholesterolemia (Angelin and Rudling, 2010; Liu and Brent, 2010). However, Goldberg et al. (2012) revealed novel complicated non-LDL receptor-mediated pathway of reducing circulating

cholesterol by thyroid hormones. They suggested that decreasing liver apoB production could be a new therapy for patients with genetic defects in the LDLR. Despite envisaging non-classical THs as negligible metabolites, recently, the bulk of articles showed many biological effects mediated by them. For example, Scanlan et al. (2004) documented multiple rapid-acting effects of 3-Iodothyronamine (T1AM) on carbohydrate and lipid metabolism without producing undesirable thyrotoxic effects on heart such as tachycardia and dysrhythmia for the first time. Additionally, T1AM
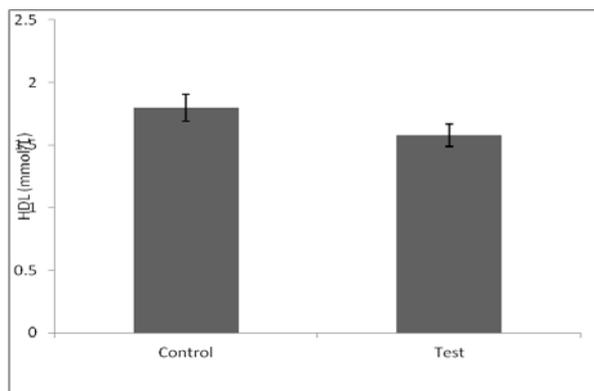
Figure 5. HDL concentration in control group mice which treated with T1AM-free intraperitoneal injections (80% normal saline plus 20% DMSO) and test group which administered T1AM (dissolved in 20% DMSO and 80% normal saline) by intraperitoneal injection of 10 mg/Kg once a day for 7 days.

appears to modulate gene expression, surprisingly some of them are not reproduced by T3. T1AM upregulate members of the PAR (Proline and Acidic amino acid Rich), a subfamily of transcription factors involved in the circadian transcription of genes encoding acyl-CoA thioesterases, leading to a cyclic release of fatty acids from thioesters. Produced fatty acids then act as ligands for PPARα (Peroxisome Proliferator-Activated Receptors α), and activate transcription of genes encoding proteins involved in the uptake and cholesterol metabolism (Gachon et al., 2011). Mariotti et al. (2014) highlighted the role of T1AM in expression of genes related to cholesterol metabolism and lipoproteins functions; however, there is a lack of research about the effect of T1AM on rate-limiting enzyme in cholesterol biosynthesis. So a decision was made to investigate this important subject in the hope that this study could improve our understanding about the similarities and differences in cholesterol synthesis alteration between thyroid hormones and T1AM. According to Figure 1, administration of exogenous T1AM produced non-significant reduction in HMG-coA reductase concentration in treatment group. Although it is not significant, it is lower in this group compared to control. This is not in agreement with the previous studies which demonstrated increasing effect of THs on HMG-coA reductase (Shin and Osborne, 2003). In Figure 2 and 3 it can be seen that the T1AM treatment group had markedly lower LDL and cholesterol concentrations compared to the other group. This is consistent with findings which revealed the gene like Ldlrap1was upregulated both in liver and adipose tissue by injection of T1AM (the same dose of as our study). Ldlrap gene

product is a linker protein for stabilizing LDLR and LDL association, thus helping the efficient endocytosis of the LDL-LDLR complex in hepatocytes (Mariotti et al., 2014; Michaely et al., 2004; Sirinian et al., 2005). It plays a major role in internalization of circulating LDLs (Low Density Lipoproteins) (Michaely et al., 2004). It seems that LDL's lowering effect of T1AM is similar to thyroid hormones. LDL lowering effect hypothesis of T1AM is proved by observing the upregulation of another gene in subcutaneous adipose tissue, Osbpl5 (oxysterol binding protein-like 5). This gene codifies a member of the oxysterol-binding protein (OSBP) family that controls oxysterol activity (Beh et al., 2001). Oxysterols, high oxygenated cholesterol derived products, are potent inhibitors of cholesterol biosynthesis (Gill et al., 2008). However, de novo synthesis of cholesterol is dominant in hepatic cells, as observed in early studies which reported that the rate of cholesterol synthesis in fat cells was only 4% of that of liver (Kovanen et al., 1975). Cholesterol synthesis is a very complex process as noted by Olivier and Krisans (2000) and perhaps T1AM reduced circulating cholesterol by affecting on both adipose tissue and liver through different mechanisms. Based on Figure 4, non-significant decrease in hepatic lipase activity was observed between control and test groups. Hepatic lipase, also called hepatic triglyceride lipase (HTGL), catalyzes the hydrolysis of fats. It is expressed in the liver and adrenal glands and is stimulated by thyroid hormones (Lithell et al., 1981). One of the principal functions of hepatic lipase is to convert intermediate-density lipoprotein (IDL) to low density lipoprotein (LDL) (Santamarina-Fojo et al., 1998). So it could be a marker for cardiovascular disease risk (Karackattu et al., 2006; Zambon et al., 2003). We observed difference between thyroid and thyroid metabolite in modulation of lipase activity. Perhaps the reduction in LDL concentration which has been observed in current study can be explained by reduction in lipase activity produced by T1AM, however, it was non-significant and must be verified in other studies which apply higher doses in a course longer than 7 days. This enzyme also hydrolyzes HDL_2 to HDL_3 (Kussi et al., 1980) and in the following study non-significant decrease was observed in the concentration of HDL (1.8 ± 0.11 versus 1.58 ± 0.09) between control and test groups (Figure 5). It seemed that lipase alteration in response to T1AM contradicts that of thyroid hormones.

Conclusion

Finally, it seems that cholesterol biosynthesis regulation of T1AM is complicated and additional investigations specifically targeted to elucidate the effect of chronic T1AM administration (applied more

than 7 days with higher doses) on this pathway in both liver and adipose tissues is necessary and must be taken into account.

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