Effect of Curcumin on the Thyroid Gland and the Plasticity of Inguinal Adipose Tissue in Experimental Model of Hypothyroidism in Rat: Histological Study

Thesis

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List of Contents

Title	Page No.

List of Abbreviations

ANP	Atrial natriuretic peptide
AT	Adipose tissue
BAT	Brown adipose tissue
BAX	BCL-2 associated X protein
BCL-2	B-cell lymphoma-2
BDMC	Bidemethoxycurcumin
BMI	Body mass index
BMPs	Bone morphogenic proteins
BNP	Brain natriuretic peptide
C/EBP α	CCAAT/enhancer binding protein alpha
CAMP	Cyclic adenine monophosphate
CD137	Cluster of differentiation 137
CD40	Cluster of differentiation 40
CIDEA	Cell death-inducing DFFA-like effector
DIT	Diiodotyrosine
DMC	Demethoxycurcumin
FDA	Food and Drug Administration
FGF21	Fibroblast growth factor 21
iBAT	Induced brown adipose tissue
IFN-γ	Interferon gamma
IL-1β	Interleukin1β
IL-6	Interleukin 6
MAPK	Mitogen activated protein kinase
MIT	Monoiodotyrosine
Myf5 +	Myogenic factor 5 positive
NE	Norepinephrine
NF-kB	Nuclear Factor Kappa B
PARP	Poly (ADP) ribose polymerase
PBS	Phosphate buffered saline
PGC- 1α	Peroxisome proliferator – activated receptor gamma co-activator alpha

List of Abbreviations

PKC ..... Protein kinase C
PPARγ ..... Peroxisome proliferator-activated receptor gamma
PRDM 16..... PR domain containing 16
PTU..... Propylthiouracil
rER..... Rough endoplasmic reticulum
SD ..... Standard deviation
sER ..... Smooth endoplasmic reticulum
ß-3AR ..... Beta3-adrenergic receptor
T3..... Triiodothyronine
T4..... Tetraiodothyronine
TGF-β1 ..... Transforming growth factor β1
TMEM26 ..... Transmembrane protein 26
TNF-α ..... Tumor necrosis factor alpha
TPO..... Thyroid peroxidase enzyme
TSH..... Thyroid stimulating hormone
UCP1 ..... Uncoupling protein 1
WAT ..... White adipose tissue

Abstract

Background and objectives: Obesity is closely related to hypothyroidism. Mild thyroid dysfunction is linked to significant changes in body weight. Curcumin is regarded as a preventive agent against obesity. This study aimed to explore the effect of curcumin on the structure of the thyroid gland and the inguinal adipose tissue in propylthiouracil (PTU) induced hypothyroid rat model.

Materials and methods: Thirty-six male Wistar rats (weighting 70-80 gm) were divided into three groups: ***Group I (Control, n=18):*** equally subdivided into: *Subgroup Ia (Euthyroid), Subgroup Ib (Euthyroid- corn oil):* received 1ml of corn oil and 3 sacrificed after 2 weeks and the other 3 after 6 weeks. *Subgroup Ic (Euthyroid- curcumin):* received 1ml of corn oil for 2 weeks followed by 1ml of curcumin dissolved in corn oil (100 mg/kg) for another 6 weeks. ***Group II (PTU- hypothyroid, n=12):*** equally subdivided into: *Subgroup IIa (PTU):* received daily 5 mg PTU in corn oil for 2 weeks and *Subgroup IIb (hypothyroid-Recovery):* received daily 5 mg PTU in corn oil for 2 weeks then left for recovery for 6 weeks. ***Group III (hypothyroid-curcumin):*** received daily 5 mg PTU in corn oil for 2 weeks followed by 1ml of curcumin for 6 weeks. Just before sacrifice, blood samples were collected from all rats to perform the thyroid function tests (free T3, free T4 and TSH). At sacrifice, the thyroid gland and the inguinal adipose tissue were dissected and processed for the different histological, ultrastructure, immune-histochemical and morphometric analyses.

Results: The follicles of the thyroid gland of *subgroup Ic* and *group III* revealed variable densities of colloid with peripheral scalloping. Depletion of the colloid with narrow or even obliterated follicular lumina and papillary projection were detected in *subgroup IIa*. Follicular lumina were partially filled with dense colloid in *subgroup IIb*. Areas of brown like adipocytes were detected among white adipocytes in *subgroups Ic, IIb and group III*. Corrugation of the cell membrane of the adipocytes was evident in *subgroup IIa*. Collagen fibers were scattered around the cell clusters in *subgroup Ic* and *group III* and appeared more dense in between the adipocytes of *subgroup IIb*. The findings were further confirmed by statistical analysis of the serological and morphometric results.

Conclusion: Curcumin administration either in euthyroid or hypothyroid rats resulted in the appearance of brown like adipocytes (beige cells) in the inguinal adipose tissue and a significant reduction of the mean weight of these rats. Moreover, curcumin exerted a stimulatory effect on the thyroid gland of both euthyroid and hypothyroid rats

Keywords: Curcumin; PTU; Thyroid gland; WAT; BAT; Beige cell

INTRODUCTION

Obesity and hypothyroidism are two common clinical conditions that have been linked together closely. Obesity is an accelerating worldwide health crisis associated with co-morbidities that include diabetes, hyperlipidemia, and hypertension. Moreover, clinical evidence suggested that mild thyroid dysfunction even in the form of subclinical hypothyroidism is linked to significant changes in body weight and represents a risk factor for overweight and obesity ***(Sanyal and Raychauduri, 2016).***

Although the relationship between obesity and hypothyroidism is well established, the primary cause is still controversial. As obesity was regarded as being secondary to thyroid dysfunction, recent views indicated that changes in thyroid‑stimulating hormone (TSH) could be secondary to obesity ***(Nannipieri et al., 2009).***

Propylthiouracil (PTU) is a thionamide anti-thyroid drug, which is the mainstay of pharmacologic treatment of Graves' disease. It exerts its anti-thyroid effects primarily by inhibiting thyroid hormone synthesis through interference with the organic binding of iodide into thyroglobulin, as well as through inhibiting the peripheral conversion of T4 to T3 ***(Emiliano et al., 2010).***

Several studies suggested that hypothyroidism induce oxidative stress in different tissues ***(Jena et al., 2012).*** On the other hand, thyroxine administration was found to increase the oxidative stress, therefore supplementation of exogenous antioxidant may be a preferable therapeutic agent against hypothyroid-induced oxidative stress ***(Mogulkoc et al., 2005).***

Curcumin is a naturally occurring curcuminoid of turmeric, which is a member of the ginger family (Zingiberaceae) and perhaps one of the most studied medicinal herbs ***(Shishodia et al., 2005).*** Curcumin has been shown to exhibit antioxidant, anti-inflammatory, anti-microbial, and anti-carcinogenic activities and has hypoglycemic and anti-rheumatic properties. Moreover, curcumin has been shown in various animal models and human studies to be extremely safe even at very high doses ***(Anand et al., 2008).***

Recently, curcumin was regarded to potentially prevent obesity through inhibition of adipogenesis and induction of adiponectin secretion ***(Wang et al., 2015).***

Some studies reported the effect of curcumin on the thyroid gland and the adipose tissues of the euthyroid rats ***(Papiez et al., 2008 and Wang et al., 2015).*** However, only few literatures are available about the curcumin influence on the structure of the thyroid gland and the adipose tissue in case of hypothyroidism.

Aim of the Work

This study was performed to detect the effect of curcumin on the structure of the thyroid gland and the inguinal adipose tissue of the hypothyroid rat.

THE ADIPOSE TISSUE

Types of adipose tissue

Adipose tissue is a major metabolic tissue that is classified into white adipose tissue (WAT) and brown adipose tissue (BAT), both are involved in energy balance but has different anatomical locations, functions, genetic expression and morphological structures ***(Park et al., 2014).***

White adipose tissue (WAT) is distributed throughout the body as visceral WAT around organs to provide protective padding and subcutaneous WAT under the skin to provide insulation from heat or cold. Brown adipose tissue is distributed around interscapular, axillary, paravertebral and perirenal sites and, it provides a key site of heat production (thermogenesis) ***(Park et al., 2014).***

White adipose tissue is normally formed of white adipocytes containing single, large lipid droplets that appear to comprise the majority of cell volume, while the cytoplasm and nucleus are found at the cell periphery giving signet ring appearance. Ultrastructural features of white adipocytes indicate that lipid droplets are not membrane bounded but they are formed of condensed layer of lipid reinforced by parallel vimentin filaments. In addition to the presence filamentous mitochondria, multiple sER, small Golgi apparatus, free ribosomes, few rER, microfilaments, and intermediate

filaments in their surrounding cytoplasm (fig. A) ***(Giordano et al., 2014 and Ross and Pawlina, 2015).***

On the other hand, BAT is formed of brown adipocytes containing multilocular multiple small lipid droplets with eccentric rounded nucleus. Ultrastructural features of brown adipocytes further include numerous large spherical mitochondria with numerous cristae, a small Golgi apparatus, few rER and sER (fig. A) ***(Ross and Pawlina, 2015).***

A new type of brown-like adipocyte is recently discovered that has distinct gene expression patterns differ from those of white or brown adipocytes. These new brown-like adipocytes are located within WAT, especially in inguinal WAT in animal models and are called beige cells. Under basal conditions these beige cells appear as white fat cells, but under different thermogenic stimuli they appear as brown-like fat cells with increased thermogenic genes ***(Reddy et al., 2014 and Cedikova et al., 2016).***

Moreover, there is a female-specific cell type, called pink adipocyte in mammary gland that arises during pregnancy and lactation periods and has a key role in the producion and secretion of milk. The name of "pink adipocytes" is given for these adipocytes as they are parenchymal cells capable of storing large amounts of lipids in female subcutaneous depot, in addition to macroscopic appearance of pregnant mammary gland, which appears pink in color (fig. A) ***(Giordano et al., 2014).***

White-to-pink trans-differentiation shaded light on breast cancer biology. It was suggested that the loss of expression of peroxisome proliferator-activated receptor gamma (PPARγ), a key factor for pink-to-white trans-differentiation by mammary secretory epithelial cells, created a pro-breast tumorigenic environment ***(Giordano et al., 2014).***

Adipose tissue in lactating mammary gland provides lipid and energy for milk production. It is the site for synthesis of different growth factors that modulates the response of the mammary gland function to steroid, proteins, and hormones ***(Ross and Pawlina, 2015).***

In this type of adipocytes, trans-differentiation of subcutaneous white adipocytes into milk producing alveolar epithelial cells occurs during gestation (adipo-epithelial trans-differentiation). This dynamic process is called alveologenesis. That involves mainly the parenchyma of mammary gland. After weaning, these pink adipocytes rapidly return back into normal adipocytes ***(Valencaka et al., 2017).***

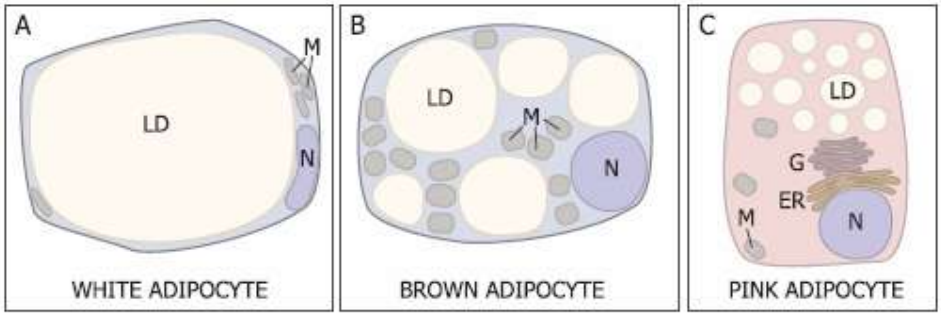

Fig. A: Schematic general cytology of the three cell types of the adipose tissue: white, brown and pink adipocytes. LD=Lipid droplet, M Mitochondrion, N Nucleus, G Golgi Apparatus, ER Endoplasmic reticulum ***(Valencaka et al., 2017).***

Development and differentiation of the adipose tissue

The timing of adipogenesis varies in different species. In humans, adipose tissue development (AT) is initiated during the second trimester while, in rodents it is intiated during the perinatal period. However, in both humans and rodents, subcutaneous AT is developed before visceral AT ***(Lee et al., 2014).***

During embryonic development in human, white adipocytes are derived from undifferentiated perivascular mesenchymal stem cells. Moreover, the regulation of adipocytes differentiation occurs through the expression of a transcription factor called PPARγ in complex with the retinoid X receptor, this complex promotes the maturation of early lipoblasts into mature adipocytes of WAT ***(Ross and Pawlina, 2015).***

Early lipoblasts look like fibroblasts with small lipid droplets and a thin external lamina. Mid-stage lipoblasts appear ovoid due to more lipid accumulation around the nucleus, and more apparent basal lamina. In the late stage of differentiation, small lipid droplets coalesce together to form a single large lipid droplet so, the cells increase in size and acquire more spherical shape. Also, together with white adipocytes, there are smaller population of cells called beige adipocytes which reside in WAT and have intermediate histological and metabolic features between white and brown adipocytes ***(Ross and Pawlina, 2015 and Mescher, 2018).***

On the other hand, ***Kajimura et al. (2015)*** stated that classical brown adipocytes of BAT depots are derived from a subpopulation of dermomyotomes. Myogenic progenitor cells synthesize several transcription factors that are considered as a " master switch " activating brown adipocyte differentiation and suppressing skeletal muscle development.

Myogenic progenitor cells will differentiate into early lipoblasts committed to brown adipocyte lineage development. Morphologically, Lipoblasts develop an external (basal) lamina and begin to accumulate numerous lipid droplets in their cytoplasm but the individual lipid droplets remain separate ***(Ross and Pawlina, 2015).***

During embryogenesis BAT develops before WAT, this is in accordance with the information about the principal role of BAT in non-shivering thermogenesis after birth, maintaining the body temperature of newborns ***(Ikeda et al., 2018).***

Functions of the adipose tissue

Adipose tissue was previously considered only as a site for the storage of triglycerides, cholesterol, and fat-soluble vitamins. Later on it is considered as a major endocrine and secretory organ, releasing in addition to fatty acids and other lipid moieties, a wide range of protein factors termed adipokines. The most biological relevant adipokines are leptin, adiponectin,