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Hepatic iodothyronine deiodinase type 1 activity is decreased in two ΔF508 cystic fibrosis mouse models

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Abstract

***Background*:** Abnormal thyroid status has been reported in cystic fibrosis (CF) patients, and this can possibly be correlated to neuromuscular symptoms. Iodothyronine deiodinase type 1 (D1) activity is an important determinant of thyroid status, and we chose to investigate D1 activity in CF liver. ***Methods*:** We have measured hepatic D1 activities in two ΔF508 CF mouse models. ***Results*:** Hepatic D1 activity was significantly reduced by 31% to 48% in homozygous ΔF508 mice compared with wild-type genotypes. ***Conclusions*:** A decreased hepatic D1 activity could be the biochemical basis of some of the abnormal thyroid parameters observed in cystic fibrosis patients.

Keywords: Cystic fibrosis; Liver; Iodothyronine deiodinase type 1; Thyroid hormone

1. Introduction

Progressive destruction of lung tissue due to recurrent endobronchial infections still is the main cause of morbidity and mortality in cystic fibrosis (CF) patients. In the past three decades, however, a number of studies have reported on an increased incidence of abnormal thyroid gland function and thyroid status in CF. Subclinical hypothyroidism, a condition characterized by elevated plasma levels of the pituitary thyrotropic factor thyroid stimulating hormone (TSH) with normal levels of circulating free thyroxine or 3,5,3′,5′-tetraiodothyronine (T4), has been reported to have a higher incidence in CF patients [1,2]. Other observations on thyroid parameters in cystic fibrosis vary although they all consistently indicate an altered thyroid status. Decreased serum T4 and 3,5,3′-triiodothyronine (T3) levels together with increased basal TSH levels have been reported [3], decreased serum T3 concentrations with normal T4 and TSH levels [4] and normal T4 but elevated T3 concentrations [5]. Normal T4, T3 and TSH serum levels, but increased levels of the biologically inactive metabolite reverse T3 or 3,3′,5′-triiodothyronine (rT3), have been measured in CF patients [6]; however, the latter observation is not corroborated by the decreased serum rT3 levels measured in CF patients with subclinical hypothyroidism [1].

The main endocrine secretion of the human thyroid gland is T4, generally believed to be a prohormone without or with only few biological actions. An important metabolic pathway of T4 is enzymatic deiodination by iodothyronine deiodinase activity, in which one or more iodine atoms are hydrolytically removed from the tyrosyl or inner ring (3-/5-position) or from the phenolic or outer ring (3′-/5′-position) of the iodothyronine molecule. The deiodinase family consists of three members, each of which are selenoenzymes with distinct substrate affinities and preferences for inner and/or outer ring deiodination [7,8]. Deiodinase type 1 (iodothyronine deiodinase type 1, D1), with the highest activities in human liver and kidneys, plays a central role in the monodeiodination of the outer ring of the T4 molecule, converting the prohormone to the bioactive

Abbreviations: D1, iodothyronine deiodinase type 1 (EC 3.8.1.4); PTU, 6-*n*-propyl-2-thiouracil; rT3, reverse T3 or 3,3′,5′-triiodothyronine; T3, 3,5,3′-triiodothyronine; T4, thyroxine or 3,5,3′,5′-tetraiodothyronine; TSH, thyroid stimulating hormone.

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thyroid hormone T3. D1 is therefore an important determinant of the systemic supply of T3 and thyroid status [9,10].

In general, through their effects on, e.g., sarcolemmal Na^+, K^+-ATPase and sarcoplasmic reticulum Ca^{2+}-ATPase activities, thyroid hormones are important regulators of skeletal muscle excitability and contractility [11,12]. It has been suggested that subclinical hypothyroidism is involved in neuromuscular symptoms such as muscle weakness and myalgias and increased lactate levels during exercise [13–17]. This correlates with the observed reduced oxidative work performance in forearm muscle of cystic fibrosis patients [18]. These neuromuscular symptoms could be secondary to an altered thyroid status. More importantly, perhaps, lung function and airway clearance in some cystic fibrosis patients could possibly be improved with therapeutic treatment aimed at restoring a normal thyroid status.

An altered hepatic D1 activity could explain some aspects, e.g. decreased serum T3 and increased rT3 levels, of the abnormal thyroid status in cystic fibrosis patients, as has also been suggested by others [2,3,6,19]. We therefore decided to investigate D1 activities in livers from two gene-targeted CF mouse models.

2. Material and methods

2.1. Mouse models

We have used two gene-targeted ΔF508 CF mouse models: the *cftr*^{tm1Eur} and the *cftr*^{tm2Cam} mouse, developed and described by van Doorninck et al. [20] and Colledge et al. [21], respectively. Both mouse models have the ΔF508 mutation inserted in the *cftr* gene. The *cftr*^{tm1Eur} mouse is constructed using a double homologous recombination procedure and contains no genomic alteration but the ΔF508 mutation. The *cftr*^{tm2Cam} mouse is developed using a targeting construct containing the 3-bp deletion between nucleotides 1522 and 1524, and a hypoxanthine phosphoribosyl transferase (HPRT) mini-gene selection cassette in intron 10 of the *CFTR* gene. Mice were bred at Erasmus University, Rotterdam, The Netherlands (*cftr*^{tm1Eur}) and in the University of Sheffield Field Laboratories, Sheffield, UK (*cftr*^{tm2Cam}), respectively, and had free access to standard laboratory animal feed and water. Animal breeding and use was approved by the local ethical review committees. Adult animals were used; homozygous ΔF508 mice were compared with wild-type littermates. Animals were killed by cervical dislocation, after which, livers were quickly excised, frozen in liquid nitrogen and then stored at −80 °C until further processing. Whole livers were homogenized in 10-ml ice-cold buffered solution (250 mM sucrose, 1 mM dithiothreitol, pH 8.0) using an Ultra-Turrax and a Potter–Elvehjem device. Homogenates were quickly frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.2. Materials

Reverse T3 (rT3) and 6-*n*-propyl-2-thiouracil (PTU) were purchased from Sigma (St. Louis, MO). Sephadex LH-20 was from Amersham Pharmacia Biotech (Uppsala, Sweden). Radioactively labeled [^{125}I]-rT3 (24.4 TBq/mmol) was from NEN Life Science Products (Boston, MA). Radiotracer was purified on a 10 wt.%/vol.% Sephadex LH-20 column shortly before use to remove free iodide as described earlier [22]. The purified hormone fraction collected in the 0.5 M NH_3/ethanol eluate was evaporated at 37 °C under a nitrogen atmosphere and then redissolved in the incubation medium. Protein was measured according to Lowry et al. [23] using BSA as a standard.

2.3. Iodothyronine deiodinase type 1 (D1)

Specific liver D1 (EC 3.8.1.4) activity was assayed in duplicate by incubating 10 μg liver homogenate protein for 15 min at 37 °C in 200 μl 100 mM phosphate buffer (pH 7.2), to which were added, 1 μM rT3, [^{125}I]-rT3, to a specific activity of 3 to $4{\cdot}10^{14}$ cpm/mol rT3, 2 mM EDTA and 10 mM dithiothreitol. The incubation was quenched by adding 100 μl 5 wt.%/vol.% ice-cold BSA. Quenched incubates were deproteinized with 500 μl 10 wt.%/vol.% ice-cold trichloroacetic acid followed by precipitation of denatured proteins at 1400 × *g* (15 min, 4 °C). To 0.5 ml of the supernatant thus obtained an equal volume of 0.1 M HCl was added, and liberated iodide was separated from the native iodothyronine with the use of Sephadex LH-20 column chromatography as described earlier [22], collecting $^{125}I^-$ in the first four 1-ml 0.1 M HCl eluates. Non-specific outer ring deiodination was determined in the presence of 100 μM PTU, a specific inhibitor of mammalian D1 activity. The specific D1 activity was thus defined as the PTU-sensitive outer ring deiodination of rT3 and was expressed as fmol rT3 deiodinated/min/mg protein. Our calculations included a correction factor of 2 to take into account the random labeling of the 3′- and 5′-positions of [^{125}I]-rT3.

2.4. Statistics

Data are presented as means ± S.D., with the number of different preparations in parentheses. Statistical significance was evaluated by Student's *t*-test or Welch's alternate *t*-test [24], where appropriate. Statistical significance was accepted at $P < 0.05$.

3. Results

No gross liver abnormalities in wild-type and homozygous mutant mice were observed. Indeed, most gene-targeted CF mice, *cftr* null mice, as well as ΔF508 homozygotes (including the mouse models we have used), have no liver and/or hepatobiliary pathologies reported [25]. The relative

Table 1
Specific hepatic D1 activities (in fmol/min/mg protein) in liver homogenates from gene-targeted CF mouse models

Mouse strain	Wild-type	Homozygote ΔF508/ΔF508	Δ (%)	P
cftr^{tm1Eur}	124 ± 24 (5)	86 ± 6 (5)	− 31	0.026
cftr^{tm2Cam}	23 ± 6 (4)	12 ± 4 (5)	− 48	0.013

protein content of the liver homogenates used in our assays, expressed as a percentage of the wet organ weight, were similar in both genotypes: 20 ± 5 (5) and 17 ± 3 (4) ($P = 0.33$) in *cftr*^{tm2Cam} wild-type and ΔF508 homozygote, and 20 ± 3 (5) and 22 ± 2 (5) ($P = 0.25$) in *cftr*^{tm1Eur} wild-type and ΔF508 homozygote, respectively. These values corroborate the absence of gross pathology, e.g., cirrhosis, or altered protein synthesis in CF mouse livers, and validate our calculations where enzyme activities were normalized for the protein content of the preparation.

Table 1 shows that the hepatic specific D1 activity in homozygote ΔF508 mice from both strains was reduced by 31% to 48% compared to the respective wild-type genotypes.

4. Discussion

Based on our observations, we suggest that a decreased hepatic D1 activity is the biochemical basis of some of the abnormal thyroid parameters in human cystic fibrosis patients.

The decreased activity of the selenoenzyme D1 could be secondary to intestinal malabsorption and low serum levels of the trace element selenium. Whole body analysis, however, only hinted at reduced selenium levels in a small group ($n = 4$) of animals from the *cftr*^{tm1UNC} knockout mouse strain [26]. Human data on serum selenium are even more equivocal: some studies report on decreased plasma selenium levels in cystic fibrosis patients [27–32], whereas others report serum selenium levels comparable to, or higher than, healthy subjects [28,32,33]. Shwachman clinical scores and whole blood selenium levels correlated negatively (i.e., patients with the highest clinical score possessed the lowest selenium concentration) in the study by Lloyd-Still and Ganther [32]. The reported differences in plasma selenium levels could well be related to differences in the nutritional status of individual CF patients.

Upon severe selenium deficiency, hepatic D1 activity in rat is decreased, although liver selenium levels and D1 activities do not tightly correlate [34]. In addition, in severely selenium deficient rats the drastically reduced hepatic D1 activities were not reflected in the moderate changes measured in thyroid hormone levels [35–37]. Plasma and liver selenium levels are poor predictors of circulating thyroid hormone concentrations.

We do not know of other reported data on direct measurements of D1 activity in CF. Still, the complex relationship between serum selenium levels in CF and selenoenzyme activity can be illustrated by the selenocysteine-containing enzyme, glutathione peroxidase (GSH-Px). In erythrocytes of 68 CF patients, the average GSH-Px activity was reduced by 20%, and this correlated positively with the reduced average plasma selenium level ($\Delta = -24\%$) [28]. Contrary, the average GSH-Px activity in a group of 20 CF patients was found to be in the normal range despite the low average whole blood selenium level which was drastically reduced by more than 40% [32]. Moreover, the GSH-Px activities measured individually correlated only weakly with the whole blood selenium levels in these patients. Castillo et al. [33] reported low serum selenium concentrations together with normal GSH-Px activities in a subset of CF patients with low serum tocopherol (vitamin E) levels. Normal selenium and GSH-Px parameters were established by these authors in patients with normal serum tocopherol levels. Concomitantly, reduced plasma selenium and tocopherol levels in CF were also measured by others [29,38]. This could indicate that the nutritional status is an important determinant in selenium availability and selenoenzyme, i.e., deiodinase, activity.

Initial results from this study were presented at the 27th Annual Meeting of the European Thyroid Association in Warsaw (2001).

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