Study of serum 3,5,3'-triiodothyronine sulfate concentration in patients with systemic non-thyroidal illness

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Sulfation is an important pathway of triiodothyronine (T3) metabolism. Increased serum T3 sulfate (T3S) values have been observed during fetal life and in pathological conditions such as hyperthyroidism and selenium deficiency. Similar variations have also been reported in a small number of patients with systemic non-thyroidal illness, but the underlying mechanisms have not been elucidated. In this study, serum T3S concentrations have been measured by a specific radioimmunoassay in 28 patients with end-stage neoplastic disease (ESND) and in 44 patients with chronic renal failure (CRF); 41 normal subjects served as controls. Both ESND and CRF patients had lower serum total T3 (TT3) and total T3 (rT3) than normal controls, while serum reverse T3 (rT3) was increased significantly in ESND (0.7 ± 0.5 nmol/l; p < 0.001 vs. controls) but not in CRF (0.3 ± 0.1 nmol/l). The TT3/rT3 ratio, an index of type I iodothyronine monodeiodinase (type I MD) activity, was reduced significantly in both groups of patients. Serum T3-binding globulin (TBG) was decreased in CRF but not in ESND patients. Serum T3S was significantly higher both in ESND (71 ± 32 pmol/l) and CRF (100 ± 24 pmol/l) than in controls (50 ± 16 pmol/l; p < 0.001). Serum T3S values showed a positive correlation with rT3 values and a negative correlation with both TT3 and FT3 values in ESND, but not in CRF. In the latter group a positive correlation was observed between T3S and TBG values. The T3S/FT3 ratio was higher both in CRF (18 ± 5) and in ESND (23 ± 18) as compared to controls (10 ± 4). Serum inorganic sulfate was increased and correlated positively with T3S values in CRF patients. In conclusion, the results of this study in a large series of patients confirm that patients with systemic non-thyroidal illness have increased serum T3S levels. The mechanisms responsible for these changes appear to be different in ESND and CRF patients. In ESND the increase in serum T3S levels is mainly related to reduced degradation of the hormone by type I MD, whereas in CRF it might be driven by the enhanced sulfate ion concentration, and could be partially dependent on the impaired renal excretion of T3S. Because T3S can be reconverted to T3, it is possible that increased T3S concentrations contribute to maintenance of the euthyroid state in systemic non-thyroidal disease.

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Sulfation of the phenolic hydroxy of T3 plays an important role in hormone metabolism. It is estimated that 17–20% of T3 is metabolized via this pathway in normal adults (1, 2). Sulfation increases the polarity of T3, favoring its excretion in urine and bile. Furthermore, deiodination of T3 by type I iodothyronine monodeiodinase (type I MD) is greatly facilitated by sulfocojugation of the hormone (3). Triiodothyronine sulfate (T3S) does not bind to T3 receptors in nuclei, and per se has no biological activity (2). However, studies in the rat have shown that T3S can be reconverted to T3 by the intestinal flora or by sulfatas located in several tissues, including liver, kidney and brain (4, 5). These pathways explain the thyromimetic effect of T3S when injected in rats (6).

Triiodothyronine sulfate is detectable in the circulation of normal adults and experimental animals. Its serum concentration is increased during fetal life and in several pathological conditions, including selenium deficiency and hyperthyroidism (1, 7, 8). Administration of drugs that inhibit type I MD (e.g., sodium ipodate and propylthiouarcil) also increases serum T3S levels (1, 9). Preliminary observations in a limited number of unselected patients with systemic non-thyroidal illness suggested that serum T3S might be elevated under these conditions (1).

The aim of the present study was to evaluate serum T3S levels in a large group of patients with different types of systemic non-thyroidal illness, and in particular in patients with end-stage neoplastic disease (ESND) or chronic renal failure (CRF). This was done in order to
gather insight into mechanisms that lead to serum T₃S changes in these patients.

Materials and methods

Patients and controls

Two groups of patients with non-thyroidal illness were included in the study: 28 ESND patients (age range 57–76 years), and 44 CRF patients undergoing regular hemodialysis (age range 33–76 years).

All ESND patients had a low T₃ syndrome, characterized by serum total T₃ levels lower than 1.5 nmol/l. Neoplastic diseases in this group including carcinomas of the hypopharynx, tonsil, larynx, lung and pancreas, lymphomas and melanoma. Only ESND patients with normal kidney function were included in this study.

In CRF patients, hemodiafiltration with a bicarbonate bath (30–35 mEq/l) and lactate solution substitution fluid was performed, with a blood flow of 300–400 ml/min. a dialysate flow of 700 ml/min and an ultrafiltration rate of 70 ± 20 ml/min. Polysulphone. PAN. PMMA and cellulose acetate hollow-fiber devices with a surface area between 1.3 and 1.8 m² were used. Those CRF patients with coexistent diabetes mellitus, respiratory failure, congestive heart disease, liver cirrhosis and neoplastic diseases were excluded.

None of the patients were taking drugs known to influence thyroid status during the 3 weeks preceding the time of blood sampling.

Forty-one normal subjects (age range 24–50 years) were used as controls.

Laboratory methods and hormonal assays

Serum sulfate was determined with a colorimetric assay as described previously (10). The normal value in our laboratory, tested in 50 healthy volunteers, is 0.3 ± 0.2 mmol/l (mean ± SD). Serum T₃S levels were measured by RIA as described previously (1) using a highly specific rabbit anti-T₃S antibody, with intra- and interassay coefficients of variation of 7.8% and 11%, respectively. The relative reactivity of various iodothyronines and sodium sulfate with the anti-T₃S antibody was ≤0.001%.

Serum TSH was measured by an ultrasensitive time-resolved immunofluorimetric assay (AutoDELFIA hTSH Ultra kit, Wallac Sollentuna, Sweden). Serum total T₃ (TT₃) and T₄ (TT₄) were measured using indirect methods (Method Spac Byk-Sangtec Diagnostic, Dietzenbach, Germany). Reverse T₃ (rT₃) and thyroxine-binding globulin (TBG) values were measured by specific RIAs (BIDATA reverse T₃ kit, BIDATA Spa, Guidonia Montecelio, Italy; TBG RIA, Biocode sa, Sclessin, Belgium). The FT₃ concentration was determined by a competitive RIA technique (AMERLEX-MAB® FT₃ kit, Johnson & Johnson Clinical Diagnostics Ltd, Milan, Italy).

Normal values in our laboratory are as follows: TSH, 0.4–3.7 mU/l; TT₃, 54–154 nmol/l; TT₄, 1.5–3.2 nmol/l; rT₃, 0.14–0.54 nmol/l; TBG, 8.6–30.5 mg/l; FT₃, 3.8–8.4 pmol/l.

Statistical analysis

Hormonal values were expressed as means ± SD, unless specified otherwise. Results were analyzed by Student’s t-test, ANOVA or Mann–Whitney U test, as appropriate, and by linear regression.

Results

Table 1 summarizes the hormonal and biochemical data of patients enrolled in the study. Mean serum T₃S concentrations were increased significantly both in ESND (71 ± 32 pmol/l, range 33–150) and in CRF (100 ± 24 pmol/l, range 56–175) patients with respect to controls (50 ± 16 pmol/l, range 13–79). Eight out of 28 patients in the ESND group and 37/44 in the CRF group had serum T₃S values higher than the upper limit in the controls. In the ESND group, the mean serum TT₄ concentration was slightly lower and the mean serum TT₃ concentration was markedly lower than in normal controls. In the CRF group, both mean TT₄ and TT₃ concentrations were markedly reduced, and to a similar extent, as compared to controls. The mean serum rT₃ value was increased significantly in ESND patients but not in CRF patients with respect to the controls. The mean FT₃ value was reduced significantly in ESND patients as compared to controls. The TT₃/rT₃ ratio, an index of type I MD activity, was reduced significantly both in ESND and in CRF patients with respect to normal controls. On the other hand, the T₃S/FT₃ ratio was increased significantly in both groups of patients. Median TSH serum values did not differ in the three study groups. A decrease in mean serum TBG concentration was observed in CRF patients but not in ESND patients as compared to normal controls.

Table 1. Hormonal and biochemical data of end-stage neoplastic disease (ESND) and chronic renal failure (CRF) patients as compared to normal controls.

<table>
<thead>
<tr>
<th></th>
<th>ESND (N = 28)</th>
<th>CRF (N = 44)</th>
<th>Normal (N = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₃S (pmol/l)</td>
<td>71.0 ± 32.4***</td>
<td>100.3 ± 24.3***</td>
<td>50 ± 16</td>
</tr>
<tr>
<td>rT₃ (nmol/l)</td>
<td>0.7 ± 0.5***</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>TT₃ (nmol/l)</td>
<td>1.0 ± 0.33***</td>
<td>1.3 ± 0.30***</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>TT₄ (nmol/l)</td>
<td>104 ± 29*</td>
<td>79 ± 26***</td>
<td>119 ± 24</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>0.7* (0.1–5.0)</td>
<td>1.1 (0.1–4.8)</td>
<td>1.0 (0.4–2.3)</td>
</tr>
<tr>
<td>TBG (mg/l)</td>
<td>18.5 ± 4.0</td>
<td>15.7 ± 4.7</td>
<td>18.4 ± 5.3</td>
</tr>
<tr>
<td>FT₃ (pmol/l)</td>
<td>3.8 ± 1.1*</td>
<td>5.6 ± 1.6</td>
<td>5.7 ± 1.1</td>
</tr>
<tr>
<td>T₃S/FT₃</td>
<td>23 ± 18**</td>
<td>18 ± 5***</td>
<td>10 ± 4</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SD (range), unless specified otherwise. Student’s t-test for unpaired variates (vs normal): ***p < 0.001 and *p < 0.05.

Median.
In ESND patients, T\(_3\)S values showed a highly significant direct correlation with rT\(_3\) levels (r = 0.8, p < 0.001), while an inverse correlation with both TT\(_3\) levels (r = 0.6, p < 0.005) (Fig. 1) and FT\(_3\) levels (r = 0.5, p < 0.01) was observed. At variance, no correlation could be found between T\(_3\)S values and rT\(_3\), TT\(_3\) or FT\(_3\) values in CRF patients (Fig. 2).

Serum T\(_3\)S and TGB concentrations were correlated directly in the CRF group (r = 0.4, p < 0.005) but not in the ESND group (Fig. 3). With regard to this correlation, it is worth noting that the intercept value on the y-axis was much lower in CRF than in ESND patients, owing to the reduced serum TGB values in some CRF patients.

The mean concentration of inorganic sulfate in sera of CRF patients was 10.9 ± 3.3 (± s.e.) mmol/L, while in normal subjects it was 0.3 ± 0.2 mmol/L. Serum T\(_3\)S values were correlated positively with inorganic sulfate values (p < 0.02) in CRF patients.

Discussion

Sulfate conjugates of thyroid hormones have been identified in serum and body fluids of experimental animals for decades (see Ref. 11 for a review). Recently, the development of specific and sensitive radioimmunoassays for sulfated iodothyronines provided new insight into the sulfation pathway of thyroid hormones (12-14). Serum levels of sulfated iodothyronines are relatively low in normal adults but increase under various physiological and pathological conditions (1, 7, 8, 12-14). This increase is usually associated with a reduction in the activity of type I MD, because sulfated iodothyronines are much better substrates for type I MD than the parental hormone (15). However, type I MD activity is not the only determinant of sulfated iodothyronine concentration in serum. For example, low serum T\(_3\)S levels may be found in spite of a reduced activity of type I MD in female rats, in which undetectable serum levels of T\(_3\)S may result from a reduced activity of T\(_3\) sulfotransferase in the liver (16). On the other hand, hyperthyroid patients have high serum levels of T\(_3\)S, in spite of an increased activity of type I MD (1). This could be explained by an increased availability of T\(_3\) for the sulfotransferase reaction (2). Therefore, serum concentrations of T\(_3\)S are regulated by changes in the activity of different enzymes and by the availability of hormone precursor.

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**Fig. 1.** Serum triiodothyronine sulfate (T\(_3\)S), reverse T\(_3\) (rT\(_3\)) and total T\(_3\) (TT\(_3\)) values in end-stage neoplastic disease (ESND) patients. A significant positive correlation was found between T\(_3\)S and rT\(_3\) concentrations (upper panel, p < 0.001), whereas an inverse correlation was found between T\(_3\)S and TT\(_3\) concentrations (lower panel, p < 0.005).

**Fig. 2.** Serum triiodothyronine sulfate (T\(_3\)S), reverse T\(_3\) (rT\(_3\)) and total T\(_3\) (TT\(_3\)) values in chronic renal failure (CRF) patients. No correlation was found between T\(_3\)S and rT\(_3\) (upper panel) or TT\(_3\) (lower panel) concentrations.
Increased serum T₃S concentration has been reported in some patients with systemic non-thyroidal illness, but the mechanisms responsible for this increase have not been investigated (1). In these patients, significant alterations in the production, distribution and metabolism of thyroid hormones are observed, in the absence of underlying disorders of the hypothalamus, pituitary or thyroid gland (17). The most common feature is represented by a reduced serum T₁ concentration (low T₁ syndrome) associated with increased rT₁ values. A decreased deiodination of T₄ to T₃ and of rT₁ to 3,3'-T₂ by type I MD, due to factors that act directly at the enzyme level or inhibit the cellular transport of iodothyronines and their subsequent deiodination, is involved in these changes (17).

In the present study changes in serum T₃S levels were investigated in two types of severe non-thyroidal illness (ESND and CRF). The ESND patients had a typical low T₁ syndrome. In this group, the increased serum T₃S levels were correlated directly with rT₃ levels and correlated inversely with FT₁ and TT₃ concentrations. This suggests that in ESND patients the increase in serum T₃S is mainly due to a reduction of type I MD activity, which explains the simultaneous occurrence of high T₃S and rT₁ levels (reduced degradation) and low T₁ values (reduced generation). Despite low serum TT₁ and FT₁ levels, T₃S concentrations were high, indicating that in ESND patients the reduced availability of precursor T₁ did not hamper the increase in serum T₃S levels. Because no relationship was observed between serum T₃S and TBG levels, it would appear that a change in the transport protein is not a major determinant of serum T₃S levels in ESND patients.

In agreement with most reports in the literature (18–25), CRF patients had markedly decreased TT₁ and TT₃ values, modestly reduced TBG and normal rT₁ concentrations. In uremic patients, these abnormalities are due to the accumulation of toxic constituents and/or to chronic malnutrition, which alter the hypothalamus–pituitary–thyroid axis at various levels (26). A decreased TSH secretion with a loss of the nocturnal TSH surge has also been described (27). In our uremic patients the TT₁/rT₁ ratio, taken as an index of type I MD activity, was reduced with respect to normal controls but not to such an extent as in ESND patients. Despite a lesser reduction of the TT₁/rT₁ ratio, in CRF patients the serum T₃S levels were even higher than in ESND patients. In keeping with this finding, no direct or inverse correlation was found between serum T₃S levels and rT₁, TT₁ or FT₁ concentrations. These data suggest that mechanisms other than the reduced type I monodeiodination contribute importantly to the increase in serum T₃S levels observed in CRF patients. In this regard, several possible explanations can be considered. The accumulation of T₃S in serum from CRF patients might in part result from impaired renal excretion of T₃S. Only a minor fraction of T₁ can be detected as T₃S in the urine of adults and experimental animals (28, 29). However, the amount of T₃S in the glomerular filtrate could be greater than that found in the urine, due to re-absorption and subsequent deiodination of T₃S at the tubular level, in a manner similar to that proposed for other iodothyronines (30). Indeed, kidney tissue exhibits high levels of type I MD activity, and the type I MD mRNA has been localized to the kidney proximal tubule (31). Therefore, the glomerular filtration rate could be more important for the clearance of T₁ than would be estimated by measuring the T₃S concentration in urine. The increase of T₃S might also be driven by the elevated serum concentration of inorganic sulphate, which in uremic patients results from a decreased renal excretion of this ion (32). The finding that serum inorganic sulfate levels were correlated with those of T₃S is in keeping with this hypothesis but is not a direct proof because the same mechanism (e.g. reduced urinary clearance) may be responsible for the increase of both substances. Finally, an increased activity of T₁-sulphotransferase might produce the elevation of T₃S in chronic renal failure. Because little is known about the regulation of this enzymatic activity in adults, this hypothesis remains a
matter for future studies. Triiodothyronine sulfate binds to TBG (1), and T₃S levels were correlated directly with those of TBG in CRF patients. Because serum TBG levels were reduced in some CRF patients, this change in transport protein tended to contrast the increase in serum T₃S.

In conclusion, the results of the present study in a large series of patients confirm and expand our previous report (1), showing that patients with systemic non-thyroidal illness have increased serum T₃S concentrations. Additionally, they provide insight into the pathogenic mechanisms of these changes, indicating that in ESND patients the increase in serum T₃S values is principally related to its decreased degradation by type I MD, whereas in CRF additional factors may be important, including an impaired renal excretion of T₃S and an increased serum sulfate concentration.

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