# Differentiated thyroid carcinoma : diagnostic and therapeutic studies
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# Chapter 5

## Lithium as Adjuvant to Radioiodine Therapy in Differentiated Thyroid Carcinoma, Clinical and In Vitro Studies

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# Abstract

*Objective:* Lithium has been reported to increase radioiodide (RaI) dose in benign thyroid disease and differentiated thyroid carcinoma (DTC). It is not known if lithium influences the *outcome* of RaI therapy in DTC. We therefore studied the clinical effects of RaI without and with lithiumcarbonate in patients with proven metastatic DTC. In addition, controversy exists on the mechanism by which lithium increases RaI dose in DTC. We performed an in vitro study specifically aimed at lithium effects on the sodium iodide symporter (NIS).

*Design: Clinical study:* 12 patients were selected with metastases of DTC who had received previous RaI therapy without lithium (control) that had not influenced tumor progression, despite RaI accumulation in metastases. The patients received 1200 mg lithiumcarbonate/day followed by 6000 MBq RaI. Outcome parameters were RaI uptake, serum thyroglobulin (Tg) levels and radiological dimensions of metastases as compared between RaI with lithium and control. *In vitro study:* Iodide uptake was studied in the benign rat thyroid cell line FRTL-5, in the polarized non-thyroid MDCK cell-line, stably transfected with hNIS to study lithium effects on NIS in a non-thyroid background and the human follicular thyroid carcinoma cell line FTC133-hNIS to study lithium effects in a background of DTC. Lithiumchloride was added in concentrations up to 2 mM for 0-48 hours. Both steady state iodide uptake (30 min) and initial rate (2 min) were studied using a specific activity of 100 mCi/mmol I, the latter experiment to determine lithium effects on substrate dependency. Iodide efflux studies were performed as well.

*Results:* Despite an increased uptake of RaI in 7 patients, no beneficial effect of RaI with lithium was observed on the clinical course as assessed by serum Tg measurements and radiographically.

In the in vitro studies, no effects of lithiumchloride on iodide uptake or efflux were observed.

*Conclusions:* We conclude that the addition of lithium to RaI did not have beneficial effects on the clinical course in 12 patients with metastatic DTC. No beneficial effects of lithium on iodide uptake were observed in vitro. Therefore, the clinical value of lithium in DTC remains subject of debate.

## Introduction

Although the role of RaI therapy in recurrent or metastatic thyroid cancer is beyond dispute, the remission rate in metastases treated with I-131 is limited [1-3]. Therefore, strategies to enhance the tumor dose of RaI are worthwhile.

Lithium salts have been introduced decades ago for the treatment of psychiatric disorders [4]. Lithium salts have been associated with an increased trapping of iodide by the thyroid gland [5,6]. This property of lithium led to the assumption that lithium may enhance the dose of RaI in benign and malignant thyroid disorders. Indeed, increased RaI retention by lithium has been confirmed in Graves hyperthyroidism leading to a higher therapeutic efficacy [7-11], although this could not be confirmed in other studies [12,13]. In addition, lithium has been reported to increase tumor dosages of RaI in DTC [5,14-17]. These studies vary in the time course of lithium application: some studies initiated lithium administration 2 days prior to RaI therapy [12,17] whereas others started lithium only at the instant of RaI therapy [5,10,14].

Despite the observation of increased RaI uptake in DTC, no studies have been published to our knowledge in which the effects of the addition of lithium to RaI on the *clinical course* of patients were investigated. We therefore studied the clinical effects of RaI without and with lithiumcarbonate in 12 patients with proven metastatic DTC.

The mechanism of the enhanced RaI trapping by lithium salts in DTC is presently unclear. Thyroid carcinomas that accumulate iodide have in common with benign thyroid diseases that they express the sodium iodide symporter (NIS) that is responsible for iodide uptake [18], whereas thyroid cancer differs from normal thyroid in numerous other aspects, including the loss of follicular architecture and the loss of expression of many proteins involved in thyroid hormone synthesis [19]. Therefore, the most obvious explanation for lithium effects on iodide trapping in benign and malignant thyroid disease would be to enhance NIS function. In the literature however, variable effects of lithium salts on iodide uptake in vitro or in animal studies are reported. Some studies found that lithium salts *inhibit* the uptake of iodide, iodotyrosin coupling and the release of thyroid hormone [20-23]. Other studies found unaltered uptake [8,9,24,25] or increased iodide uptake [26].

As most of these studies were performed before the cloning of NIS, we wanted to study the effects of lithium salts on NIS function in the background of normal thyroid physiology, in a non-thyroid background and in the background of thyroid carcinoma. As this objective cannot be addressed easily in patients, we performed in vitro studies, studying lithium uptake in thyroid and non-thyroid cell lines with endogenous NIS expression or stably transfected with hNIS.

## Patients, Materials and Methods

### Clinical study

After the publication of the study of Koong et al [17], it was decided to apply the treatment schedule of this study in patients with metastases of DTC that had been scheduled for RaI therapy and who had had an unfavorable response to prior RaI therapy despite the fact that their metastatic lesions accumulated RaI as revealed by whole body scintigraphy (WBS), 7 days after radioiodide therapy. This first RaI therapy served as a control. Patients who were selected had to have undergone total thyroidectomy and RaI ablative therapy. The presence of metastases of thyroid carcinoma was established by measurable serum Tg levels and the presence of metastatic sites at post-therapeutic whole body scintigraphy, X-ray, CT or MRI after prior radioactive iodine therapy.

The objectives of this study were to investigate if addition of lithium to RaI has beneficial effects on radioiodine uptake and the clinical course of the patients. Outcome measures were the uptake of RaI on post-therapy WBS, progression of serum Tg levels after RaI therapy and the change in dimensions of the metastatic sites at X-ray, CT or MRI.

Twelve patients were included in the protocol (2 males, 10 females). Their clinical characteristics are presented in Table 1. The mean age at diagnosis of thyroid carcinoma was 59 years. Most patients had papillary thyroid carcinoma. In 10 of the patients, metastases were already present at the time of diagnosis of thyroid carcinoma, most of them pulmonary. Before the RaI therapy combined with lithiumcarbonate and the control RaI therapy were performed, all patients had received extensive therapies; RaI therapy had been administered in a mean cumulative dosage of 28 GBq (Table 1). Six of the 12 patients had received additional non-RaI therapies during the course of their disease. However, none of these therapies had been applied within a 1-year period prior or after the historical control RaI therapy or the RaI therapy combined with lithiumcarbonate.

### *Protocol*

Four weeks before RaI therapies, patients were routinely switched from T4 to $T_3$ therapy. $T_3$ was discontinued two weeks before RaI therapy. A low iodide diet was started 1 week prior to the RaI therapy. RaI was administered orally as an activity of 6000 MBq $Na^{131}I$. Seven days after RaI administration, whole body scintigraphy was performed. During the second RaI therapy, lithiumcarbonate (Litarex, Dumex, Baarn, The Netherlands) was prescribed according to the schedule of Koong [17]. Lithiumcarbonate 564 mg was given twice (bid) with a 12 h interval. Plasma lithium levels were measured by atomic absorption spectrometry. The dose was adjusted if

necessary to achieve a lithium concentration of 0.6-1.2 mmol/L. Lithium was continued during 7 days after the RaI administration. To investigate the effects of lithiumcarbonate on RaI uptake, ideally a randomized crossover design with a washout period should be performed. The crossover design would be necessary to account for the continuing rise in serum TSH levels during the period of $T_4$ withdrawal. This would have implicated a prolonged period of $T_4$ withdrawal and consequently high TSH levels. This was considered not ethical because the RaI therapy was scheduled anyway and a prolonged period of increased TSH levels could theoretically have unfavorable effects on tumor progression [27-29].

**Table 1.** Characteristics of 12 patients with metastases of differentiated thyroid arcinoma

| | |
|---|---|
| Patients (n) | 12 |
| Females / Males (n) | 10/2 |
| Age at diagnosis (y) | 59 ± 11 |
| Tumor Histology (n) | |
| *Papillary* | 7 |
| Follicular variant | 2 |
| *Follicular* | 3 |
| Stage at Diagnosis (n) | |
| *T 1–3 and M-0* | 2 |
| *T-4 and M-0* | 0 |
| *M-1* | 10 |
| Metastases at Diagnosis | |
| *Lungs* | 8 |
| *Bone* | 5 |
| *Soft-tissues* | 2 |
| Cumulative Activity I-131 (GBq) | 28.1 ± 11.1 |
| Metastases at Therapy | |
| *Lungs* | 10 |
| *Bone* | 7 |
| *Soft-tissues* | 2 |
| Additional Therapies | |
| *Surgery* | 2 |
| *Embolization* | 4 |
| *External irradiation* | 4 |

Na$^{131}$I whole-body scintigraphy was performed 7 days after the oral administration of 6000 MBq of $^{131}$I (Mallinckrodt BV, Petten, The Netherlands). The run speed of the dual-head gamma camera (Toshiba GCA 7200, equipped with a high-energy collimator) was 15 cm per minute (matrix size 256×256). WBS was followed by anterior and posterior planar images of the head and neck and chest region (matrix size 256×256, preset time 10 min). Quantitative assessment of I-131 uptake was performed by calculating uptake in 2 regions of interest by 2 observers who were unaware of treatment modality. These regions were carefully chosen in such a way that they had not been subjected to other treatment modalities. Two regions were chosen to assess whether a potential effect of lithium was uniform or not. Quantitative uptake on WBS performed after lithium was compared with control in corresponding regions of interest, and expressed as 'increased', 'stable', 'decreased' or 'mixed'. 'Mixed' was used when the result of lithium in the 2 regions of interest differed.

Thyroglobulin increments are expressed as the differences in the natural logarithms (Ln) of the Tg values during suppressive $T_4$ therapy observed at the end of the observation period after RaI therapy and the last Tg value during $T_4$ before RaI, divided by the duration of the observation period; in formula: Delta LnTg:

$(LnTg_{end}-LnTg_{start})$/months.

Radiological measures were scored semi-quantitatively as: 'stable', 'progression', 'regression' or 'cure'. ×256, preset time 10 min).

*Laboratory measurements*

Serum TSH was determined with on a Modular Analytics E-170 system (Roche Diagnostic Systems, Basle, Switzerland), intra-assay variability: 0.88-10.66%, inter-assay variability: 0.91-12.05%). Serum Tg was measured. Serum Tg was determined with IRMA (Tg kit, Brahms, Berlin Germany) on a Wallac gammacounter (Wallac, Turku, Finland), intra-assay variability: 0.14-13.9%, inter-assay variability: 12.3-17.4 %). Serum Tg antibodies were determined with IRMA (Sorin Biomedica, Amsterdam, The Netherlands) on a Wallac gammacounter (Wallac, Turku, Finland) intra-assay variability: 3.6-4.1%, inter-assay variability: 11.6%).

## In vitro studies

*Cell lines and culturing conditions*

Three cell-lines were studied: The rat thyroid FRTL-5 cell-line derived from the ATCC (ATCC, Manassas, New York) expresses endogenously NIS which is subjected to TSH regulation [30]. FRTL-5 were grown in Ham's F-12 media (Life Technologies, Inc.) supplemented with 5% calf serum, 1 mM non-essential amino acids (Life Technologies, Inc.), 10 mM glutamine, 100 units/ml penicillin, 100 μg/ml

streptomycin, and a six-hormone mixture (6H) containing insulin (1.3 µM), hydro-cortisone (1 µM), transferrin (60 pM), L-glycyl-histidyl-lysine (2.5 µM), somatostatin (6.1 nM), and TSH (1 mU/ml) as reported previously [31].

Recent studies suggest striking similarities between polarized protein sorting in thyrocytes and MDCK epithelial cells. We have therefore used MDCK clones stably transfected with hNIS [32](donated by N. Carrasco, Albert Einstein College of Medicine, New York) to study direct effects of lithium on NIS in a non-thyroid background.

To study if lithium influences NIS function in the background of a thyroid carcinoma, the follicular thyroid carcinoma cell line FTC133 was used. FTC133 (kindly donated by Dr. Goretzki and Dr. Simon, University of Düsseldorf, Germany) was derived from a 42-year-old male with metastatic follicular thyroid carcinoma [27]. We have stably transfected this cell line with hNIS [33,34]. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) and modified HAM-F12 medium 1:1 supplemented with 10% fetal bovine serum, penicillin/streptomycin and geneticin to maintain an advantageous environment for transfected cells, in a humidified incubator at 37°C and 5% $CO_2$. Lithiumchloride was added to the culturing fluids in various concentrations and time schedules as indicated.

*In vitro iodide uptake*

For uptake experiments, cells were grown in 12-well plates. LiCl was added in concentrations ranging from 0 to 2 mM either 48 hours prior to the uptake studies or at the moment of uptake studies (acute). Culturing media were carefully checked for pH after addition of Lithium. Prior to the uptake studies, the cells were washed 3 times in Hanks Balanced Salt Solution (HBSS), buffered with 10 mM Hepes (pH 7.5) Thereafter, HBSS containing 20 µM Na$^{125}$I with a specific activity of 100 mCi/mmol was added to the cells. Cells were incubated at 37 °C in a humidified atmosphere.

Three types of uptake studies were performed: steady state, initial rate and efflux studies.

In all experiments, reactions were terminated by aspirating the radioactive mixture and washing three times with the ice cold HBSS. Accumulated $^{125}$I was determined by permeabilizing the cells with 500 ul ethanol for 20 min at –20 $^0$C and quantitating the released radioisotope in a γ counter. The DNA content of each well was subsequently determined after trichloroacetic acid precipitation, by the diphenylamine method [18]. Based on the specific activity of the substrates, the efficiency of the γ-counter, and the DNA content of each well, iodide uptake was expressed as picomoles of substrate transported per microgram of DNA or as percentage of control conditions.

For steady state experiments, the radioactive cells were incubated for 30 minutes with the radioactive solutions.

In the initial rate experiments, the effect of substrate concentration on uptake was determined by incubating washed cells for 2 min in medium containing 9 concentrations of iodide, ranging between 0.625 and 160 μmol/L. Uptake reactions were then terminated and substrate uptake was quantitated as indicated above.

Iodide efflux was studied in a subsequent experiment; after addition of HBSS with 20 μM $Na^{125}I$ with a specific activity of 100 mCi/mmol during 30 min in the presence or absence of LiCl in concentrations from 50-2000 umol/L, the radioactive supernatant was removed and HBSS with or without lithium was added to the cells for 5-min intervals up to 30 min after removal of the radioactive supernatant. Radioactivity was counted in all fluids. The sum of all radioactivity counts in all washing fluids was considered the accumulated radioactivity at the beginning of the efflux.

All experiments were performed in hexaplicate.

*Immunofluorescence*

FRTL-5 cells in the presence of TSH were seeded onto poly-(lysine)-coated coverslips. Cells were cultured with or without LiCl 2 mM for 48 hours. Cells were washed 3× with PBS/CM, fixed with 2% paraformaldehyde in PBS for 20 min at RT, and rinsed with PBS/CM. Cells were permeabilized with 0.1% Triton in PBS/CM plus 0.2% BSA (PBS/CM/TB) for 10 min at RT. Cells were quenched with 50 mM $NH_4Cl$ in PBS/CM for 10 min at RT and rinsed with PBS/CM/TB. Cells were incubated with 8 nM anti-rat NIS antibodies [35](donated by N. Carrasco), washed, and incubated with 1:700 dilution of fluorescein-labeled goat anti-rabbit antibodies (Vector Laboratories). After washing, cells on the coverslips were mounted onto microscope slides using an antifade kit from Molecular Probes. Coverslips were sealed with quick-dry nail polish and allowed to dry in the dark for 2 h at RT and stored at 4 °C. NIS immunofluorescence was analyzed with a Bio-Rad Radiance 2000 Laser Scanning Confocal MRC 600, equipped with a Nikon Eclipse epifluorescent microscope.

*Statistical analyses*

Continuous data are expressed as mean ± SD when distributed normally, as tested by the Kolmogorov-Smirnov test, otherwise as medians and ranges. Continuous data between groups were compared with a paired Students T-test or the Wilcoxon test, in case data were not distributed normally. Proportional data were compared with the Chi-square test. In the iodide efflux studies, half-life of accumulated radioactivity was calculated by linear regression analysis of the radioactivity-time curve. A p value of $< 0.05$ was considered significant.

## Results

Clinical study

The control RaI therapy and the RaI therapy with lithiumcarbonate were not different with regard to serum TSH and Tg levels during $T_4$ withdrawal. (Table 2). No adverse events were observed during lithiumcarbonate administration. All 12 patients had lithium levels > 0.6 mmol/L. In two patients, the lithiumcarbonate dose had to be increased to 564 mg three times per day (tid) to achieve these concentrations. The post-therapeutic increments in Tg levels are given in Table 2. Uptake of RaI was increased after addition of lithium in 7 patients. Two patients had a mixed pattern, some lesion showing increased uptake, other stable or decreased uptake. Median increments in the natural logarithm of Tg levels did not differ significantly between the first RaI therapy (0.08 vs. 0.11, p=0.228). The number of subjects with positive or negative Tg increments after RaI therapy did not differ either between RaI therapies without and with lithiumcarbonate. The same pattern was observed for the radiological evaluation of metastatic sites: the number of subjects with stabile, progressive or regressive metastatic sites was not different after the historical control RaI therapy as compared with RaI combined with lithium. Therefore, we were unable to document a benefit of the administration of lithium in these patients.

**Table 2.** Effects of RaI therapy with 6000 MBq I-131 on clinical course in patients with progressive differentiated thyroid carcinoma without (control) or with addition of lithiumcarbonate (lithium)

|  | Control | Lithium | p-value [1] |
|---|---|---|---|
| Serum TSH at therapy (mU L$^{-1}$) | 96 ± 56 | 99 ± 87 | 0.831 [&] |
| Serum thyroglobulin Levels (μg L$^{-1}$) |  |  |  |
| *During withdrawal* | 2320 (104-277960) | 3518 (668-1310000) | 0.328 [&] |
| *Increment Post-Therapy* |  |  |  |
| (Delta LnTg(Ln μg L$^{-1}$ month$^{-1}$)) | 0.08 (-0.08-0.26) | 0.11 (-0.19-1.04) | 0.228 [&] |
| Delta Ln Tg positive/negative (n) | 9 / 3 | 10 / 2 | 0.615 [*] |
| Whole body scintigraphy |  |  |  |
| *Iodide uptake in ROI vs. Lithium vs. Control* |  |  |  |
| *Increased (n patients)* |  | 5 |  |
| *Stable 1* |  | 1 |  |
| *Decreased* |  | 3 |  |
| *Mixed [2]* |  | 2 |  |
| Radiological evaluation (X-ray, CT, MRI) |  |  |  |
| *Regression (n patients)* | 3 | 2 | 0.091 [*] |
| *Stabile* | 5 | 1 |  |
| *Progression* | 4 | 9 |  |

[1]: lithium vs. control; [2]: 2 regions of interest with different effects of lithium; [*]: Chi-square test; [&]: Wilcoxon –test

In vitro study

*Steady state Iodide uptake*

We studied the 30 min accumulation of iodide in FRTL5, MDCK-hNIS and FTC133 hNIS after acute or 48 hours incubation with LiCl in concentrations of 50, 100, 500, 1000 and 2000 umol/L. We did not observe any effect of either acute or 48 hour addition of lithiumchloride in any concentration on steady state iodide uptake in the 3 cell lines.

The results for 500, 1000 and 2000 umol/L are shown for FRTL5, MDCK-hNIS and FTC133 hNIS  (Figure 1a).
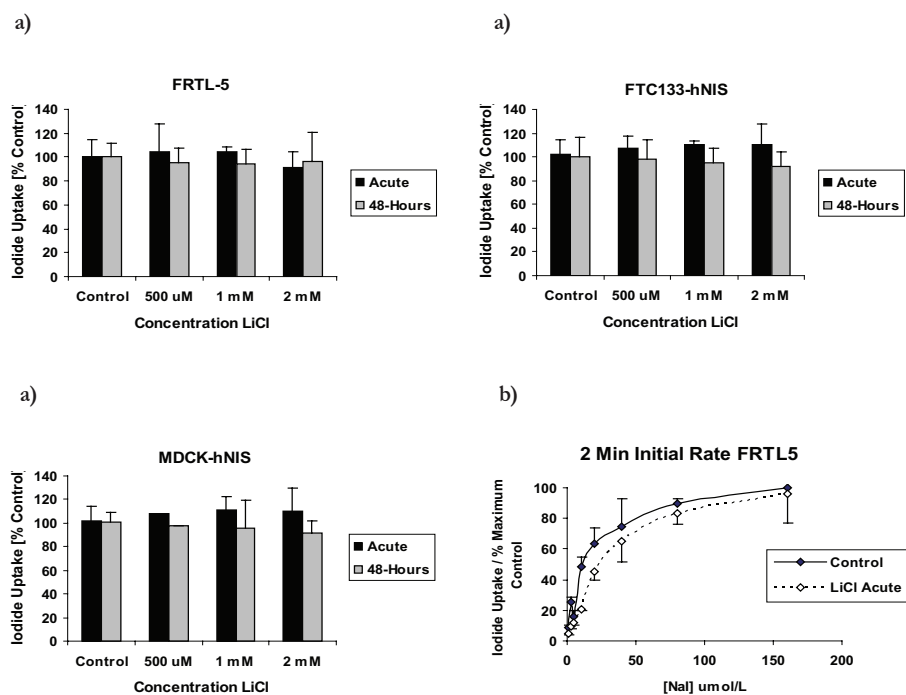
a)

a)



b)



**Figure 1. a.** Acute and 48 hours effects of  500, 1000 and 2000 uM LiCl on iodide uptake in 3 cell-lines: FRTL-5, MDCK-hNIS and FTC133-hNIS. Incubation media consisted of HBSS with $20\,\mu M$ $Na^{125}I$ with a specific activity of $100\,mCi/mmol$. Experiments were terminated after 30 minutes. Iodide uptake was expressed as percentage of control uptake. Mean values for iodide uptake without lithium were: FRTL-5: $12.3\pm6.6$ pmol/ug DNA, for MDCK-hNIS: $49.5\pm8.3$ pmol/ug DNA and FTC133-hNIS: $23.4\pm0.5$ pmol/ ug DNA. **b.** Two minutes iodide uptake by FRTL-5. Incubation media contained $Na^{125}I$ in concentrations from 0.625 uM to 160 uM, all with a specific activity of $100\,mCi/mmol$. All uptake values were expressed as a percentage of the maximum uptake in FRTL5 without lithium.

*Initial rate iodide uptake*

We studied the 2 minutes iodide uptake and the effects of substrate concentration in FRTL-5, MDCK-hNIS and FTC133 hNIS after acute or 48 hours incubation with LiCl in concentrations of 50, 100, 500, 1000 and 2000 umol/L. We did not observe any effect of either acute or 48 hour addition of lithium salts in any concentration on initial rate uptake of the 3 cell lines. The results for acute addition of 2 mM LiCl are given in Figure 1b.
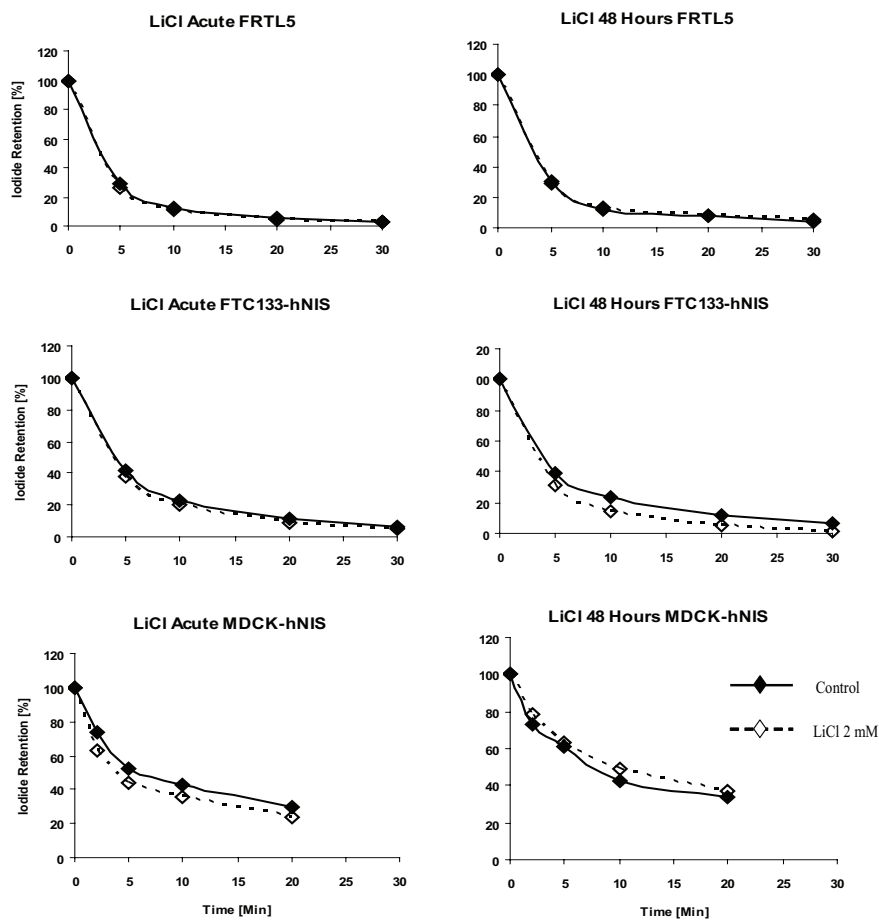


**Figure 2.** Influence of LiCl 2 mM, added during 48 hours or during the efflux experiment (acute) on iodide efflux in FRTL-5, MDCK-hNIS or FTC133-hNIS. Iodide efflux was studied after addition of HBSS with 20 μM Na$^{125}$I with a specific activity of 100 mCi/mmol during 30 min in the presence or absence of LiCl. Thereafter, the radioactive supernatant was removed and HBSS with or without lithium was added to the cells for 5-min intervals up to 30min after removal of the radioactive supernatant.

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*Iodide efflux*

To study whether the absence of an effect of lithium salts on iodide uptake may be theoretically explained by an effect of similar magnitude on iodide efflux, we studied iodide efflux or retention in FRTL5, MDCK-hNIS and FTC133 hNIS after acute or 48 hours incubation with LiCl in concentrations of 50, 100, 500, 1000 and 2000 umol/L. We did not observe any effect of either acute or 48 hour addition of lithium salts in any concentration on steady state iodide uptake in the 3 cell lines.

The results for acute and 48 hours addition of 2 mM LiCl for FRTL5, MDCK-hNIS and FTC133-hNIS are shown for FRTL5 (Figure 2).

*NIS immunofluorescence*

No effects of the addition of 2 mM LiCl for 48 hours on NIS staining were observed (Figure 3).
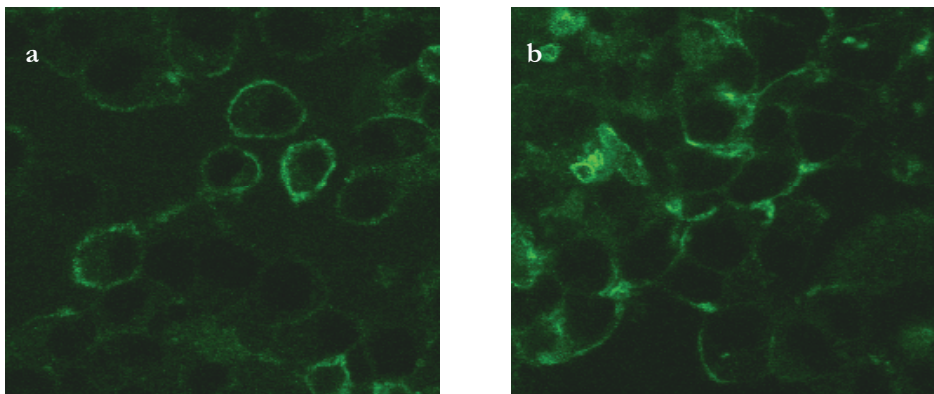


**Figure 3.** NIS immunostaining of FRTL-5 cells, cultured during 48 hours without (a) or with (b) LiCl 2 mM. No effect on immunofluorescence was seen. Magnification 60 x.

**Discussion**

We performed the present study to investigate whether the addition of lithium to RaI in patients with metastasized DTC has beneficial effects on the clinical course of the disease. In addition, we studied whether the reported beneficial effects of lithium on RaI uptake in patients with benign or malignant thyroid diseases may be explained by a direct effect of lithium on NIS or alternatively that the effects must be attributed to other mechanisms as suggested in a number of studies [20,21,23]. In the clinical study, we did not find any evidence for a positive effect of lithiumcarbonate

on the clinical course of DTC. Several explanations for the lack of success can be hypothesized. First, the category of patients could have been different from the study of Koong et al [36]. However, in both studies patients with iodide accumulating metastases were included. In both studies, papillary carcinomas were predominant and most patients had pulmonary metastases. Second, in our study, the clinical course was compared after two *high dose* RaI therapies with a longer interval than in the study of Koong et al. It can be hypothesized that the longer time interval may have given rise to changes in biological tumor characteristics or alternatively, that the historical control RaI therapy may have selected radioresistant tumor cells [37]. However, in all patients, RaI accumulating lesions were present after the second RaI therapy as well. In addition, although Tg levels were progressive in most patients, their long-term increment rates were not altered substantially after the first RaI therapy, so it is unlikely that this explanation is true. Third, it could be hypothesized that the response of thyroid carcinoma cells to lithium combined with high activities of RaI is different from lithium combined with tracer doses: with high doses of RaI, thyroid cancer cell necrosis could lead to a faster release of radioactivity from the cells [14]: however, if this were true in our patient group, this would have lead to a favorable response on RaI. A fourth explanation could be that even if lithium had led to higher iodide retention in our patients, no additional therapeutic effect of RaI therapy was achieved. Efficacy of RaI therapy is the result of tumor dose on the one hand and radiosensitivity on the other hand. If lithium had resulted in an increased tumor dose, this could still have been insufficient to establish growth arrest of thyroid carcinoma.

In addition, some patients underwent alternative treatments like embolization or external irradiation that might confound the potential effect of lithium. However, we carefully chose indicator metastases that were not subjected to these alternative therapies. In the experimental studies we investigated whether the beneficial effects of lithium salts on RaI uptake in patients with benign or malignant thyroid diseases may be attributed to a direct effect on NIS or that the effects must be attributed to other mechanisms as suggested in a number of studies [20,21,23]. This hypothesis was based on the fact that benign thyroid disease and iodide accumulating thyroid carcinoma have in common the expression of NIS, whereas virtually every other aspect of thyroid hormone physiology is different. In addition, we studied the effects of addition of lithium to RaI therapy on the clinical course in 12 patients with metastatic DTC. In the in vitro experiments, we included 3 cell-lines: FRTL5, in which NIS expression is subjected to normal regulation. MDCK-hNIS is a polarized cell-line in which trafficking of thyroid proteins resembles that in normal thyroid cells [32] but where lithium effects on NIS can be studied in the absence of normal thyroid regulation. FTC133-hNIS is a follicular thyroid carcinoma cell line, stably transfected with hNIS [34], in which effects of lithium salts on NIS in a background of thyroid carcinoma can be studied.

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In our experiments, no effects of lithiumchloride were found on iodide uptake, neither when added acutely, nor when added 48 hours before the uptake experiments. Uptake was studied both in steady state and initial rate experiments. To exclude the theoretical possibility that lithium salts may affect uptake and efflux to the same magnitude, efflux studies were performed as well, again with no effect of lithiumchloride. These results are the first reported on in vitro effects of lithium salts on NIS function in a benign or malignant thyroid background and in a non-thyroid background.

Although of course we have not studied all steps of iodide physiology, we believe that an explanation via NIS is highly unlikely and thereby confirm earlier studies in which no effect of lithium salts on iodide uptake were found [8,9,20,22-25]. Haberkorn et al. [38] did not find an effect of lithium salts on iodide trapping in NIS transfected thyroid carcinoma in an animal study. In another study in NIS transfected colon carcinoma cells, even an inhibiting effect of lithium was found [39]. It is suggested that for the enhancement of iodide trapping by lithium intact organification is necessary which then is inhibited by lithium [38]. This may explain the absence of lithium effects in thyroid- or non-thyroid tumors with a short half-life and absence of organification.

In conclusion, our data indicate that if a beneficial effect of lithium in thyroid carcinoma would be present, it would not be by enhancing NIS activity. The clinical data presented in this study raise doubt if there is a beneficial effect at all in enhancing the effects of RaI treatment in thyroid carcinoma. Therefore, the clinical value of lithium in DTC remains a subject of debate.

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