IN VITRO NUCLEAR MEDICINE

Disparate Effects of Heparin on Free Thyroxine as Measured by Two Radioimmunoassays: Concise Communication

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Free thyroxine (FT_4) has been measured in two groups of patients, before and after heparin, using equilibrium dialysis (ED) and two radioimmunoassays, RIA-I and RIA-II. In Group A, nine patients were tested before and after 12–24 hr of intravenous heparin. FT_4 (ED) rose from 1.9 ± 0.5 to 2.8 ± 3.0 ng/dl, and RIA-I from 1.37 ± 0.37 to 1.89 ± 1.21 ng/dl, whereas RIA-II results fell from 0.97 ± 0.38 to 0.66 ± 0.32 ng/dl. In Group B, ten patients were tested before and after 15 min of intravenous heparin. FT_4 (ED) rose from 1.7 ± 0.7 to 3.2 ± 1.6 ng/dl ($P < 0.02$), and RIA-I rose from 1.3 ± 0.46 to 2.02 ± 1.01 ng/dl ($P < 0.05$), whereas RIA-II results fell from 1.07 ± 0.38 to 0.63 ± 0.31 ng/dl ($P < 0.02$). The correlation coefficient between FT_4 (ED) and RIA-I in 38 paired results was 0.96, but there was no correlation between FT_4 (ED) and RIA-II results.

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Previous investigators have demonstrated that heparin causes a rise in free thyroxine (FT_4) as measured by equilibrium dialysis (ED) (*1–5*). With the introduction of at least four commercial radioimmunoassays (RIA) for FT_4, FT_4 measurements have become accepted as one of the best routine thyroid function tests. Investigations in our laboratory and others (*6–8*) have indicated that FT_4 levels determined by RIA may be of particular value in patients hospitalized for various severe nonthyroidal illnesses in whom conventional thyroid function tests tend to be abnormal. However, very little information is presently available on possible effects of various drugs on FT_4 levels as measured by these new methods. We undertook a study to evaluate the effect of heparin on FT_4 as measured by two RIA kits.

PATIENTS STUDIED AND METHODS

Two groups of patients were studied.

Group A. Nine patients (5 male and 4 female), age range 29–71 yr (mean 48), with a diagnosis of pulmonary embolus or deep venous thrombosis, received an intravenous bolus of 5,000 units heparin followed by 1,000 units/hr. Blood samples were obtained before heparin was started and after 12–24 hr of intravenous heparin.

Group B. Ten patients (7 male and 3 female), age range 51–79 yr (mean 61), were scheduled for cardiac catheterization. These patients received heparin in a dose of 45 units/kg intravenously to prevent clotting problems during the procedure. Blood was obtained before, and 15 min after, heparin was administered, but before contrast material was injected.

All patients gave written informed consent to be included in the study, which was approved by the Stanford University Medical School Human Subjects Committee. The following tests were performed on all pre- and post-heparin samples: FT_4 was measured by equilibrium dialysis (*9*) and by two RIA kits: RIA-I, GammaCoat T_4 (I-125) Total and/or Free T_4 RIA kit (*10*),* and RIA-II, Amerlex Free T_4 RIA kit.† RIA-I uses a sequential assay, with tubes coated with anti-T_4 antibody. In the first brief incubation, FT_4 in serum or standard binds to the antibody-coated tubes. In the second step, after aspiration of the rest of the serum, the antibody-binding sites remaining free are quantitated by incubation with

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TABLE 1.

Group	Number		Free T_4 (ED) Normal range (1.0–3.1 ng/dl) Mean ± s.d.	Free T_4 (RIA-I) Normal range (0.8–2.3 ng/dl) Mean ± s.d.	Free T_4 (RIA-II) Normal range (0.68–1.8 ng/dl) Mean ± s.d.	Total T_4 Normal range (5–11 μg/dl) Mean ± s.d.	TBG binding capacity Normal range (15–25 μg/dl) Mean ± s.d.	Total T_3 Normal range (70–200 ng/dl) Mean ± s.d.
A	9	Pre-	1.72 ± 0.5	1.37 ± 0.37	0.97 ± 0.38	6.4 ± 2.9	15.4 ± 3.8	86 ± 48
		Post-	2.8 ± 3.0	1.89 ± 1.21	0.66 ± 0.32	5.8 ± 0.7	14.9 ± 4.7	85 ± 14
B	10	Pre-	1.72 ± 0.7	1.30 ± 0.46	1.07 ± 0.38	5.8 ± 2.2	17.5 ± 3.0	84 ± 30
		Post-	3.2 ± 1.6*	2.02 ± 1.01†	0.63 ± 0.31*	5.7 ± 0.4	16.9 ± 3.9	99 ± 21

* $p < 0.02$.
† $p < 0.05$.

T_4 (I-125). RIA-II represents a competitive-binding RIA, in which serum or standard is simultaneously incubated with an anti-T_4 antibody, immobilized on polymer particles, and a T_4 (I-125) analog that is said not to interact with serum proteins.

Total T_4, T_3, and TSH were measured by double-antibody RIAs like those described in the literature (*6,12,13*). T_4 binding capacity was determined by Spinsep-TBG.‡ Pre- and postheparin values were compared by Student's t-test. The statistical significance of the results was the same whether or not the two patients with the most dramatic changes in FT_4 levels were included. Correlation coefficients for FT_4 (equilibrium dialysis) versus FT_4 (RIA-I) or FT_4 (RIA-II) were calculated by least-squares regression analysis.

There was sufficient blood in the samples from patients (who had rises in FT_4) to study the effect of adding heparin in vitro to their preheparin samples. Up to 12 μU/ml—an order of magnitude greater than that used clinically—was added.

RESULTS

Table 1 shows comparison of FT_4, as measured by ED and the two different RIAs, along with the total T_4, T_3, and the T_4 binding capacities for all pre- and postheparin specimens. Mean FT_4 values obtained by ED or RIA-I in Group A appeared to rise after heparin, but the differences were not statistically significant (Fig. 1). In Group B, postheparin FT_4 values were significantly higher: mean FT_4 by ED rose from 1.72 ± 0.7 to 3.2 ± 1.6 ng/dl (mean ± s.d., $P < 0.02$) and by RIA-I from 1.3 ± 0.46 to 2.02 ± 1.0 ng/dl (mean ± s.d., $p < 0.05$) (Fig. 2).

In contrast, postheparin FT_4 concentrations as measured by RIA-II were generally lower than the corresponding preheparin values, with ten of 19 patients having subnormal FT_4 values after heparin. The most disparate results were found in the two patients who had the largest increase in FT_4 by ED (2.25 to 7.9 ng/dl or 2.37 to 9.02 ng/dl) or by RIA-I (2.1 to 6.1 ng/dl or 2.1 to 5.0 ng/dl), but FT_4 by RIA-II declined (1.7 to 0.65 ng/dl or 0.7 to 0.2 ng/dl). In addition, one euthyroid patient with subnormal T_4-binding proteins but a normal baseline FT_4 by ED and RIA-I had a subnormal baseline FT_4 by RIA-II. One patient was found to be hypothyroid (TSH 60 μU/ml) with low FT_4 levels by all three methods.

There was a very good correlation between FT_4 levels by ED and RIA-I. The correlation coefficients were $r = 0.75$ for preheparin values, 0.98 for postheparin values, and 0.96 ($p < 0.001$) for all 38 matched studies (pre- and postheparin). Regression analysis for ED versus RIA-II gave $r = 0.58$ for preheparin values, but there was no correlation with the postheparin FT_4 values.

Total T_4, T_3, the T_4 binding capacity, and TSH re-

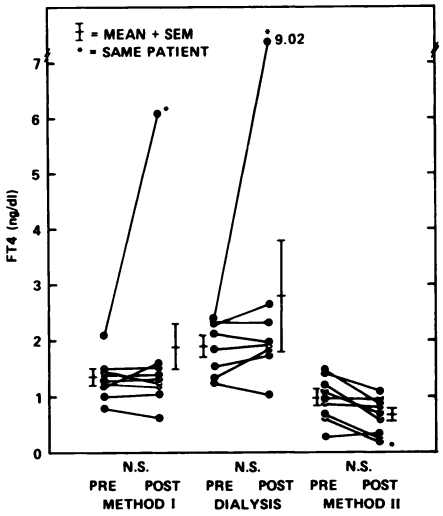

FIG. 1. Individual FT_4 measurements before and 24 hr after heparin in nine patients measured by RIA-I, equilibrium dialysis, and RIA-II.

mained essentially unchanged; 18 of the 19 patients had TSH values of <2 μU/ml.

There was no change in FT_4 by either radioimmunoassay after addition of heparin in vitro.

DISCUSSION

When FT_4 was measured by ED, we observed a significant rise in FT_4 15 min after a bolus of heparin (Group B), and an increase in FT_4 concentrations in two of nine patients on long-term heparin anticoagulation therapy (Group A). Compared with earlier investigations measuring postheparin FT_4 levels in patients with renal failure or deep venous thrombosis (*1*,*2*,*5*), our observations show a similar trend, although quantitatively the changes in FT_4 concentrations are less pronounced. While two patients, one in each group, had dramatic rises in FT_4, the postheparin FT_4 values of only three out of 19 patients were clearly in the hyperthyroid range.

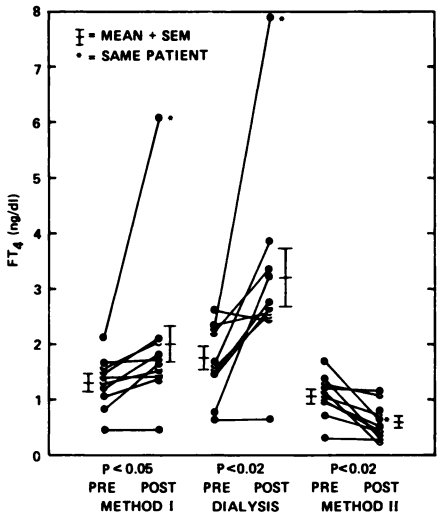

FIG. 2. Individual FT_4 measurements before and 15 min after heparin in ten patients measured by RIA-I, equilibrium dialysis, and RIA-II.

As illustrated by Figs. 1 and 2 and an overall correlation coefficient of r = 0.96, pre- and postheparin values obtained by RIA-I* in all cases paralleled very closely those determined by ED, with a rise or no change of FT_4 after heparin.

However, RIA-II produced discordant results: FT_4 fell after heparin administration in 16 of 19 patients. Thus, using two recently developed RIAs for FT_4, both being designed to measure the same free hormone in the serum, we have found diametrically opposite results. Since ED is generally regarded as the reference procedure for quantitation of FT_4 by which other methods are judged, it appears that in this particular situation only the results obtained by RIA-I reflect accurately the actual FT_4 levels, whereas the postheparin FT_4 values produced by RIA-II (Amersham) are too low and misleading. Other limitations of RIA-II have been observed in euthyroid but severely ill patients with abnormally binding protein concentrations, or in euthyroid patients with inherited T_4 excess and abnormal binding to albumin (*14*).

The clinical implications of the elevated FT_4 levels after heparin, as measured by ED or RIA-I, remain unresolved, since there is generally no evidence of transient hyperthyroidism. None of our patients appeared hyperthyroid. In spite of this, Hershman et al. (*2*) showed a reciprocal fall in TSH along with the rise in FT_4, suggesting biochemical hyperthyroidism. Thomson et al. (*3*) were unable to confirm this finding, and our data do not allow comment because of suppressed TSH values (<2 μU) in 18 of the 19 patients before and after heparin. Further evidence that the rise in FT_4 is relevant has been a blunted TSH response to intravenous TRH. Gelfand et al. (*5*) found this in five patients on heparin, compared with the results in the same patients tested before heparin was given. It is also possible that the reduced response to TRH in heparinized patients was because the first dose of TRH caused a slight rise in thyroid hormones and subsequent pituitary suppression, as has been described previously (*15*). Thomson et al. (*4*) showed a smaller response to intravenous TRH in patients treated with heparin for 24 hr compared with those tested after 7 days of heparin or 7 days of warfarin but no heparin. The effect of ill health *per se* causing a blunting of the TSH response cannot be excluded in that study. Addition of heparin in vitro in clinically relevant amounts did not affect the results of FT_4 measurement by either RIA method, and this has been found by equilibrium dialysis in the past (*2*).

The objective of our study was to determine whether FT_4 results, when these were measured by the newer, widely used, RIAs, are altered by heparin to such an

extent as to compromise the diagnostic accuracy of FT_4 measurements for assessing thyroid status. The conclusion is that if thyroid function is to be evaluated in patients who require heparin, the test should be done before heparin is started. If the blood is drawn on a euthyroid heparinized patient, that patient may be misclassified as hyperthyroid, or a hypothyroid as normal, if RIA-I is used, and a normal patient would be misdiagnosed as hypothyroid if RIA-II is used. It might be argued that since T_4, FT_4 index, and T_3 are not altered by heparin, these may be more appropriate tests, but it is widely recognized that each of these tests may be subnormal in sick patients (*7,8,16,17*) and, therefore, less satisfactory than FT_4 measurements.

FOOTNOTES

* Clinical Assays, Cambridge, MA.
† Amersham, Arlington Heights, IL.
‡ Nuclear Diagnostics, Inc., Troy, MI.

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