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RADIOIODINATION AND QUALITY CONTROL OF HUMAN THYROTROPIN FOR RADIOIMMUNOASSAY

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This work reports the radioiodination of human thyrotropin (hTSH) in our laboratory (IPEN) and evaluates its quality in comparison with a commercial product. The radioiodination yield obtained in 20 experiments ranged from 18.5 to 56.3%, while the purification recovery ranged from 75.5 to 124.0% and the specific activity ranged from 1.01 to 3.10 MBq μg^{-1}. The values for the distribution coefficient revealed in the purification of radioiodinated hTSH ranged from 0.232 to 0.371. When tested concomitantly in the same radioimmunoassay system, the IPEN and the commercial tracer presented parallel standard curves. A highly significant correlation ion was observed between the quality control samples estimated through both curves ($p < 0.001$). These results confirm the quality of the hTSH radioiodinated at IPEN and suggest the acquirement of self-sufficiency in this "in vitro" nuclear technology.

INTRODUCTION

The RIA laboratory of the Instituto de Pesquisas Energéticas e Nucleares (IPEN) was the pioneer in Brazil in the development of RIA of circulating hormones for

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clinical and experimental applications, preparing their own tracers from imported biological reagents[1].

Since then that laboratory has prepared and assessed the quality of ^{125}I-labeled hormones for RIA use[2-7] even purifying some hypophyseal hormones for radioiodination[8,9].

The aim of this work is to report the preparation and the quality evaluation of the hTSH radioiodinated at that laboratory. This study forms part of a project developed at IPEN to prepare radioiodinated hormones with confirmed quality in order to replace imported products.

EXPERIMENTAL

The hTSH (5 µg in 10 µl of 0.05M sodium phosphate buffer, pH 7.4), kindly provided by the National Institute of Diabetes and Digestive and Kidney Diseases - NIDDKD - (Baltimore, USA), and supplied by Radioassay Systems Laboratories - RSL - (Carson, USA), were labeled with Na^{125}I (approximately 29.6 MBq) from (New England Nuclear - Dupont, USA; and Amersham, UK). A modification of the conventional method of Hunter and Greenwood[10] was used, employing 10 µl of Chloramine T (5 mg ml^{-1}) and 20 µl of sodium metabisulfite (10 mg ml^{-1}).

Purification was achieved by passing the iodination mixture through a 2 cm x 45 cm column, packed with Sephadex G-100, obtained from Pharmacia (Uppsala, Sweden), equilibrated and eluted with 0.05M phosphate, pH 7.4, with 0.1% bovine serum albumin (BSA) at 4 ^{o}C. Fractions of 2 ml were collected with a flow rate of 12 ml h^{-1}, counted for radioactivity and further evaluated for binding to excess of the specific antibody, also supplied by the NIDDKD (anti-hTSH-3).

Separation of antibody-bound from free tracer was performed by precipitation with polyethylene glycol - PEG 6000 (500 µl, 25% wt./vol.), purchased from Atlas (São Paulo, Brazil). The fractions related to the ^{125}I-hTSH, identified by its distribution coefficient (Kd)[11] in the molecular sieve chromatogram, were pooled and divided in aliquots of 0.5 ml. They were stored at -20 ^{o}C to be used as tracer within 90 d.

The specific activity (SA) of the ^{125}I-hTSH was estimated by column recovery[12], considering the actual amount of Na^{125}I used, which was determined from the efficiency of the γ-counters (Nuclear Chicago Automatic Gamma Counting System, 200 samples and ANSR of Abbott Laboratories, 240 samples, USA). For the former counter the efficiency was estimated by the coincidence method[13] that divides the counts into two groups. One group, called the "singles" (Ns), results from measurement of a single γ-ray (35.5 keV). The other group, called the "coincidence" (Nc), results from the simultaneous measurement of the 27.5 X-ray and 35.5 γ-ray = 63 keV. For the second counter the value specified in its operator's manual (65%) was considered. The yield of iodination was calculated from the integrated area of the ^{125}I-hTSH peak revealed in the molecular sieve chromatogram.

Some of these ^{125}I-hTSH preparations were tested together with commercial tracers in the same RIA system. We employed the second antibody RIA kit also purchased from RSL. These tests were performed at the Central de Radioimunoensaio de São Paulo (CRIESP), a national reference laboratory of radioimmunoassay analysis.

The following parameters of the standard curves constructed with IPEN and those tracers from the RSL kit were analyzed: non-specific binding (NSB), zero binding

(B_0), effective 50% dose (ED_{50}) and limit of precision (corresponding to 80% and 20% doses). Besides, the internal quality control (QC) samples with low, medium and high hTSH contents were also estimated in these assays. The linear regression between the concentrations measured with IPEN and RSL tracers, as well as the respective correlation coefficient were determined.

RESULTS

Table 1 shows the results of 20 radioiodinations and the respective purifications performed in our laboratory with the hTSH provided by NIDDKD or by RSL. The labeling yields ranged from 18.5 to 56.3% and the recovery of the purifications ranged from 75.5 to 124.0%. The SA of the tracers was very variable, changing according to the quantity of $Na^{125}I$ used. the Kd value of the tracers ranged from 0.232 to 0.371%.

Figure 1 shows the elution pattern of one occasional purification of the radioiodinated NIDDKD-hTSH. Fractions which presented higher binding to the specific antibody by PEG precipitation (corresponding to the hatched area) were pooled and stored at -20 ^{o}C until use as the tracer in the RIAs. Figure 2 shows the same pattern obtained in the purification of the radioiodinated hTSH from RSL.

In Figure 3 the RIA standard curve constructed with a tracer prepared at IPEN with the NIDDKD-hTSH is compared with the commercial tracer from the RSL RIA Kit. It can be observed that the two curves are practically superimposable, presenting very close values. Similarly, very close results were obtained when the unlabeled hTSH purchased from RSL was radioiodinated in our labo-

TABLE 1

Results of the NIDDKD and RSL hTSH labeled and purified at IPEN laboratory. Tracers 1 to 13 were prepared from the NIDDKD-hTSH-I-6 and those 14 to 18 with the NIADDKD-hTSH-I-7

Tracer No.	hTSH	$Na^{125}I$ used activity, MBq	Recovery, %	Yield, %	SA, Bq μg^{-1}	K_d
1	NIDDKD	24.75	115.6	38.8	3.10	0.343
2	NIDDKD	19.54	112.4	45.5	1.84	0.365
3	NIDDKD	20.35	112.7	56.3	2.31	0.244
4	NIDDKD	32.41	91.5	20.1	1.35	0.259
5	NIDDKD	30.52	104.7	26.1	1.75	0.277
6	NIDDKD	18.87	75.5	26.7	1.06	0.250
7	NIDDKD	27.90	79.2	33.3	1.99	0.265
8	NIDDKD	34.15	99.6	18.6	1.34	0.271
9	NIDDKD	34.60	99.0	33.0	2.28	0.364
10	NIDDKD	27.19	124.0	40.2	2.28	0.371
11	NIDDKD	28.71	114.6	18.5	1.08	0.297
12	NIDDKD	37.92	99.3	37.6	2.84	0.297
13	NIDDKD	26.49	96.0	21.1	1.12	0.327
14	NIDDKD	19.54	93.2	25.8	1.01	0.291
15	NIDDKD	30.78	98.7	31.2	1.92	0.296
16	NIDDKD	25.46	112.9	28.1	1.43	0.316
17	NIDDKD	25.75	119.4	52.1	2.68	0.296
18	NIDDKD	33.34	105.9	22.9	1.53	0.328
19	RSL	28.53	89.7	41.5	2.37	0.232
20	RSL	22.79	91.0	58.2	2.76	0.241

ratory and submitted to the same comparative evaluation with the tracer of the RSL hTSH RIA Kit (Fig. 4).

The results of the comparative study of the hTSH RIAs performed with both tracers prepared at IPEN and supplied by RSL are shown in Table 2. Very close values were found

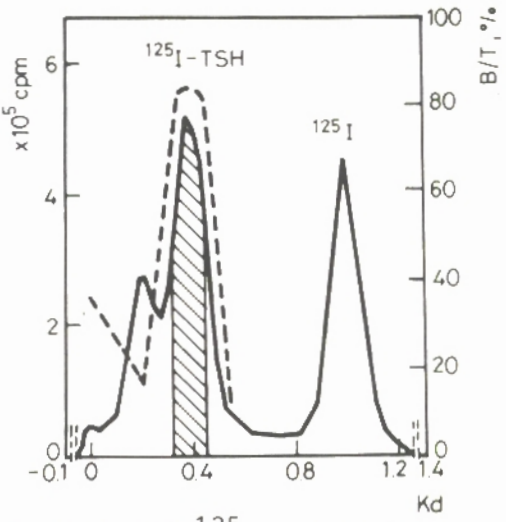

Fig. 1. Purification of ^{125}I-NIDDKD-hTSH prepared at IPEN by molecular sieve chromatography on Sephadex G-100. Each fraction was counted for radioactivity (solid line) and assessed for binding with excess of antibody (dotted line)

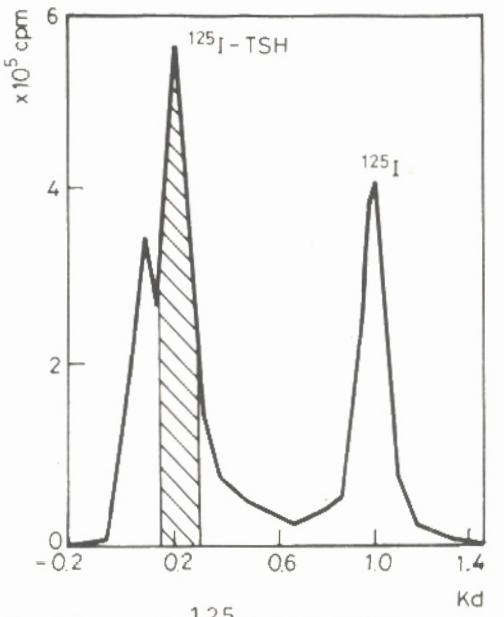

Fig. 2. Purification of ^{125}I-RSL-hTSH prepared at IPEN by molecular sieve chromatography on Sephadex G-100

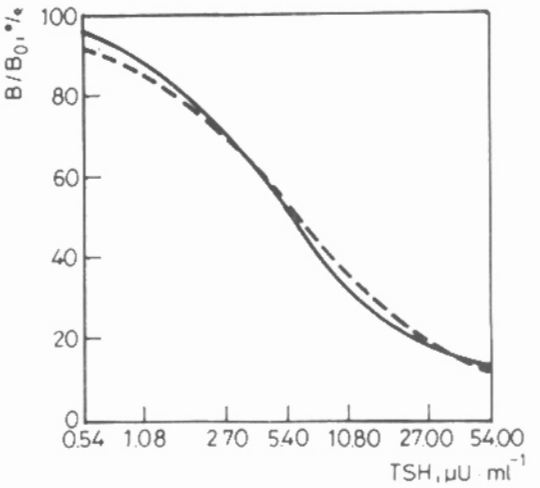

Fig. 3. Comparison between RIA standard curves made up with the tracer prepared at IPEN with the NIDDKD-hTSH (dotted line) and that from the RSL hTSH RIA Kit (solid line)

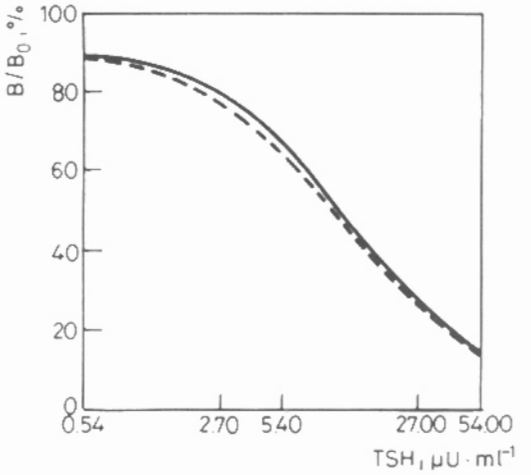

Fig. 4. Comparison between RIA standard curves made up *with the tracer prepared at IPEN with the RSL-*hTSH (dotted line) and that one from the RSL hTSH RIA Kit (solid line)

TABLE 2

Results of the hTSH RIA performed concomitantly with the RSL hTSH RIA Kit employing the tracers from the self-kit (above) or prepared at IPEN (below). The seven first tracers of the table prepared at IPEN employed hTSH from NIDDKD, while the last one employed hTSH supplied by RSL

Tracer No.	NSB, %	B_0, %	ED_{50} $\mu U\ ml^{-1}$	80-20% dose $\mu U\ ml^{-1}$	QC low 1.3-3.4 $\mu U\ ml^{-1}$	QC medium 6.9-12.7 $\mu U\ ml^{-1}$	QC high 22.0-48.0 $\mu U\ ml^{-1}$
1	2.0	38.0	7.1	2.4-26.5	1.3	11.1	37.9
	3.0	28.0	6.2	1.9-21.0	1.5	9.1	25.9
2	2.0	38.0	7.0	2.3-19.0	1.1	12.9	38.5
	2.0	45.0	6.5	2.0-19.0	1.2	9.9	38.2
3	2.0	35.0	8.4	2.1-35.0	0.9	8.7	38.5
	3.0	21.0	8.4	2.8-40.0	0.9	8.5	37.8
4	4.0	25.0	6.6	2.0-27.0	1.6	12.9	40.1
	4.0	27.0	7.6	1.9-35.0	0.6	8.6	42.0
5	2.2	27.0	5.0	1.9-20.0	1.4	6.6	28.7
	3.2	30.0	5.0	1.4-17.0	1.1	6.3	26.5
6	2.7	29.0	5.8	1.8-25.0	1.7	8.8	31.1
	3.2	28.0	6.4	1.5-28.0	1.0	6.9	30.2
7	1.2	37.0	9.2	1.9-42.0	1.7	10.1	45.0
	3.8	36.0	5.8	1.4-19.0	2.4	9.6	54.0
8	3.9	28.0	11.0	2.5-37.0	1.7	11.1	42.8
	2.0	35.0	9.4	2.0-37.0	1.5	14.2	40.4

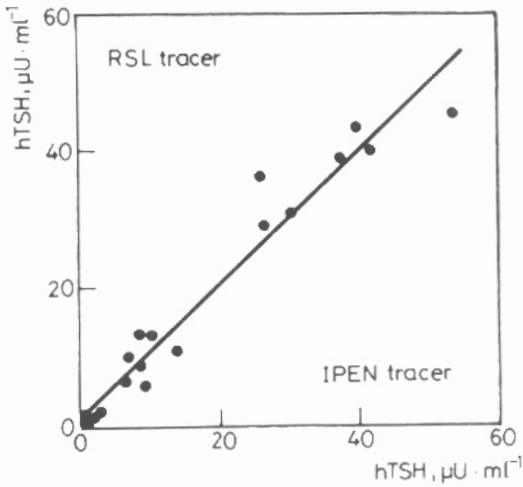

Fig. 5. Relation between the hTSH concentration of the QC samples measured in the RIAs performed with the tracers prepared at IPEN and those from the RSL hTSH RIA Kit

for the parameters of the standard curves and for the hTSH concentration determined in the internal QC samples.

Figure 5 illustrates the positive and significant correlation between the hTSH concentration of the QC samples estimated by the RSL hTSH RIA Kit employing the tracers from its kits and the one prepared at IPEN ($r = 0.9778$, $p < 0.001$), expressed by the equation: RSL = 0.9592 IPEN + 1.3884.

DISCUSSION

Although other methods have been used to radioiodinate hTSH[14], the classical method of chloramine T has been employed[15-17] and it is the technique recommended by the NIDDKD[18].

The ^{125}I-hTSH prepared at IPEN from the NIDDKD-hTSH was perfectly comparable with the commercial product from RSL, when used in the performance of the RIA. Besides, the same results were obtained when unlabeled hTSH from RSL was labeled in our laboratory.

This agreement reveals a comparable quality of our tracers with that of the commercial product. It also indicates that the radioiodination conditions employed in our laboratory for preparing the ^{125}I-hTSH can be considered satisfactory.

In spite of the newer and more sensitive techniques for hTSH measurement, such as immunoradiometric assays[19,20] and fluorescent immunoassays[21,22], the RIA is still used in clinical and research laboratories. Its use was reported in 1991 in a proficiency testing program in endocrinology held in New York State (USA)[23]. In the developing countries it has been expected to have a long life span[24] mainly for the diagnosis of thyroid disorders, which do not require a very sensitive hTSH determination.

Being a well established method, hTSH RIA has also been used for testing other "in-house" assay reagents as the second antibody[25] and magnetisable solid phase antibodies[26]. Besides, it has been employed for monitoring the purification of the hypophyseal hormone in our laboratory and for evaluating the tracer prepared with this hTSH[9].

Therefore, the quality of hTSH radioiodinated at our laboratory was confirmed, suggesting the acquirement of self-sufficiency in this "in vitro" nuclear technology.

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REFERENCES

1. O.Z. Higa, I.T.T. Souza, B.L. Wajchenberg, H. Pinto, R.R. Pieroni, Radioimmunoassay and Related Procedures in Clinical Medicine and Research, IAEA, Vienna, 1974, p. 291 (IAEA-SM-177/84).

2. V.C. Borghi, B.L. Wajchenberg, Horm. Metab. Res., 14 (1982) 384.

3. P. Bartolini, M.T.C.P. Ribela, I. Schwarz, A.J.G. Santos, V.C. Borghi, WORLD Federation of Nuclear Medicine and Bilogy: Proceedings of the IV. World Congress of the WORLD Federation of Nuclear Medicine and Biology, Buenos Aires, 02-07 November, 1986, p. 133.

4. M.T.C.P. Ribela, E.A. Araújo, E.K. Gimbo, I. Schwarz, L. Morganti, V.C. Borghi, P. Bartolini, Arq. Biol. Tecnol., 30 (1987) 36.

5. M.G.P. Ginabreda, V.C. Borhi, A. Betarello, Arq. Biol. Tecnol., 32 (1989) 226.

6. V.C. Borghi, M. Nascimento, L.A.C. De Marco, B.L. Wajchenberg, Horm. Metab. Res., 22 (1990) 256.

7. V.C. Borghi, M. Nascimento, B.L. Wajchenberg, Developments in Radioimmunoassay and Related Procedures: Perspectives in Developing Countries, IAEA, Vienna, 1992, p. 369.

8. E.K. Gimbo, M.T.C.P. Ribela, V.C. Borghi, I. Schwarz, L.E.M. Dias, E.A. Araújo, P. Bartolini, Publicação Instituto de Pesquisas Energéticas e Nucleares, 181 (1988).

9. V.C. Borghi, L.H. Lin, Publicação Academia de Ciências do Estado de São Paulo, 60 (1988) 69.

10. W.M. Hunter, F.C. Greenwood, Nature, 194 (1962) 495.

11. R.E. Martenson, J. Biol. Chem., 253 (1978) 8887.

12. P. Engeblienne, G.J. Slegers, Immunol. Methods, 56 (1983) 135.

13. D.L. Horrocks, Clin. Chem., 21 (1975) 370.

14. M.C. Donnell, J.W. Peterson, Clin. Chem., 25 (1979) 1065.

15. J.H. Romaldini, H.F. Rodrigues, L.M. Tanaka, V.L. Teixeira, L.C.F. Reis, Arq. Bras, Endocrinol. Metabol., 24 (1980) 93.

16. J.J. Hagan, J.I. Tu, D.R.K. Murty, E.L. Nickoloff, Clin. Chem., 29 (1983) 1870.

17. M.T.C.P. Ribela, P. Bartolini, Anal. Biochem., 174 (1988) 693.

18. Pituitary Hormones and Antisera Center, Human Thyroid Stimulating Hormone Radioimmunoassay, Maryland, (Technical Report 209).

19. G. Caldwell, S.M. Gow, V.M. Sweeting, H.A. Kellett, G.J. Beckett, J. Seth, A.D. Toft, Lancet, 1 (1985) 1117.

20. D.S. Ross, Ann. Intern. Med., 104 (1986) 718.

21. H.L. Kaihola, K. Irjala, J. Viari, V. Nanto, Clin. Chem., 31 (1985) 1706.

22. E. Reichstein, R.C. Morton, E. Diamandis, Clin. Biochem., 22 (1989) 23.

23. R. Rej, P. Drake, Scand. J. Clin. Lab. Invest., 51 (suppl. 205) (1991) 47.

24. R.D. Piyasena, P.L. Airey, R.D. Ganatra, M. Nofal, IAEA Bulletin, 1 (1989) 5.

25. S.R. Silva, V.C. Borghi, B.L. Wajchenberg, Resumenes del IX. Congresso Argentino de Biologia y Medicina Nuclear, Buenos Aires - Argentina, 1991, p. 14.

26. R. Edwards, H.J. Hope, P. Suprarop, Developments in Radioimmunoassay and Related Procedures: Perspectives in Developing Countries, IAEA, Vienna, 1992, p. 177.