COMPARISON OF THREE METHODS USED FOR MEASUREMENT OF RADIOIODINE FIXATION IN THYROID GLAND OF MICE

by

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The aim of this work is to compare the results of estimation of radioiodine uptake using three methods in a study on mice, and to test reliability of the radioiodine uptake estimation by gamma camera. The study is conducted on 21 white, Swiss-type mice of both sex at age of 10 weeks, weighing between 25 g and 34 g. The mice were injected intraperitoneally with 0.37 0.03 MBq of radioiodine ^{131}I. After 72 hours the mice were anesthesized, and radioactivity of thyroid region was measured by gamma camera (the 1st method, in situ). After the measurement, the animals were sacrificed, their thyroid glands were carefully excised together with adjacent trachea and placed at the bottom of a test tube. The radioactivity of the excised tissue was then measured by both gamma camera (the 2nd method) and gamma counter (the 3rd method). This method is treated as a standard and the most accurate. In the study we used Siemens e_cam gamma camera and Wallac Wizard 1470 Automatic Gamma counter. The radioiodine fixation determined using those three methods was 25.25 7.32%, 26.08 8.55% and 25.74 7.18%, without statisticaly significant difference s between methods ($p > 0.05$). The high correlation between the three methods of measuring radioiodine fixation in thyroid gland was observed: (1) the correlation coefficient between the fixation rate obtained by gamma camera *in situ* and the fixation rate obtained by measuring the radioactivity of extirpated thyroids by gamma camera was 0.869 ($p < 0.01$); (2) the correlation coefficient between fixation rate obtained by gamma camera *in situ* and the fixation rate obtained by measuring radioactivity of extirpated thyroids by gamma counter was 0.890 ($p < 0.01$); (3) the correlation coefficient between fixation rate obtained by measuring radioactivity of extirpated thyroids by gamma camera and the fixation rate obtained by measuring radioactivity of extirpated thyroids by gamma counter was 0.835 ($p < 0.01$).

Key words: thyroid gland, radioiodine uptake test, gamma camera

INTRODUCTION

One of the oldest diagnostic tests in nuclear medicine, the radioiodine uptake test (RAIU) was initially performed by J.G. Hamilton in 1938. This test gives information about intensity and velocity of iodine metabolism in thyroid gland, *i. e.*, about the functional status of the gland. Nowadays, the test is mostly used for making or confirming diagnosis of subacute thyroiditis, as well as for determination of therapeutic dose of radioactive iodine for benign thyroid diseases. Besides, the test is sometimes employed after thyroidectomy in patients with differentiated thyroid cancers, in order to estimate the amount of residual thyroid tissue and calculate optimal ablative dose of radioiodine ^{131}I [1].

Some of (the) newer studies used the radioiodine uptake test for determining borderline values of thyroid gland normal function in certain geographical regions [2], as well as for the quantification of impact made by table salt iodination on incidence of thyroid diseases [3].

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The main point of the Radioiodine Uptake Test is calculating oral dose fraction of radioiodine ^{131}I which is fixed for thyroid tissue after certain time. Typically, the measurement is scheduled 3 and 24 hours after the oral intake of radioiodine, in order to determine two physiological parameters: the uptake of iodine in thyroid gland (based on measured radioactivity after 3 hours) and the retention of radioiodine in thyroid tissue, *i. e*., its metabolic activity (based on measured radioactivity after 24 hours). [4]

A human thyroid gland with normal function binds on average from 8% to 20% of radioiodine ^{131}I in the first 3 hours, and from 20% to 45% in the first 24 hours [5]. The rest of radioiodine is excreted mostly in urine. Both urinary excretion and thyroid uptake of radioiodine are significantly influenced by diuretics, [6, 7] and the direction of this influence is species specific [8].

The thyroid gland binds up to 790 mGy per MBq of the administered ^{131}I radioactivity during the test, depending on the fixation rate in the thyroid. The other organs absorb much lower dose (*e. g*. up to 0.049 mGy/MBq for adrenals [9]). Although the radioiodine ^{131}I has unfavorable physical characteristics, its high tissue selectivity considerably adds to the safety of this diagnostic procedure.

The radioiodine ^{123}I has more favorable physical characteristics than radioiodine ^{131}I, and the radiation risk is lower when it is used for the fixation test instead of the radioiodine ^{131}I. However, the half-life of the radioiodine ^{123}I is relatively short (about 13 hours), and its availability is limited (it is produced by cyclotrone), making the radioiodine ^{131}I still the most widely used isotope.

There were also some attempts to evaluate the thyroid function by rapid technetium test (^{99m}Tc-pertechnetate) with low radiation risk, instead of using the radioiodine ^{131}I fixation, but short half-life of ^{99m}Tc-pertechnetate (6 hours) and its capacity to evaluate only function of NaI symporter (*i. e*. to measure only iodine intake and not metabolic activity of thyreocytes), precluded its wide-spread use in diagnostics [10].

Although the radioiodine fixation test in clinical practice is used less frequently than various imaging methods, it is still a valuable diagnostic tool. Apart from being used with specially designed systems, the radioodine fixation test could be conducted with gamma scintilation camera, a device which is available in every department for nuclear medicine. However, the reliability studies of the radioiodine fixation test conducted with gamma camera are very rare. The aim of our study was to test the reliability of measurements of radioiodine fixation in thyroid gland made by gamma camera in vivo, through their comparison with the results of radioiodine fixation measurements in extirpated thyroid glands by gamma camera or gamma counter.

MATERIALS AND METHODS

Experimental animals

The study was conducted on 21 white, Swiss-type mice of both sex at age of 10 weeks, weighing between 25 g and 34 g, which were bought from animal farm of Military-Medical Academy, Belgrade. The animals were kept in separate cages, exposed to 12-hours light and dark cycles, at temperature 21 20 °C, with free access to water and food *ad libitum*. The experiment was conducted in compliance with the directive EU(86/609/EEC) [11] and with permission of local Ethics Committee from Medical Faculty, University of Kragujevac, Serbia.

Radioiodine application and radioactivity measurements

The mice were injected intraperitoneally with 0.37 0.03 MBq of radioiodine ^{131}I (the injection volume was 0.5 mL). The applied dose of radioactivity was calculated as a difference between radioactivity of the solution in a syringe before (full syringe) and after (empty syringe) the injection. After the administration of ^{131}I, the mice were transferred to the same cages.

All measurements were performed with gamma camera SIEMENS e.cam Dual Haed (Siemens, 1997. USA) with high-energy colimator, at 5 cm distance and for 15 minutes, using ^{131}I photopeak of 364 keV, with window 20% and matrix 256 × 256 pixels. The measurements were further processed by gamma counter Wallac Wizard 1470 Automatic (PerkinElmer Life Sciences, Wallac Oy, 2005, Finland), using the same ^{131}I photopeak of 364 keV, with duration of one minute. All measurements were corrected for background radioactivity.

The relative efficacy of measurement of gamma counter is about 50% for ^{131}I gamma quants energy of 364 keV. According to the product declaration, the gamma camera equiped with high-energy colimators used in the study (Siemens e.cam Dual Head) has overall sensitivity of 3.65 cpm/kBq* for ^{131}I.

After 72 hours the mice were anesthesized by thiopenton sodium, and we conducted *in vivo* measurement of thyroid radioactivity by gamma camera. After that, mice were sacrified and their thyroid glands were carefully extirpated together with the adjacent trachea (fig. 1), and placed at the bottom of a test tube. The radioactivity of the excised tissue was then measured by gamma camera, under the same conditions as previously (*in vitro* measurement on gamma camera).

After the measurements by gamma camera were completed, the recordings were processed by a special software. The regions of interest (ROI) in quadrate

*cpm means counts per minute

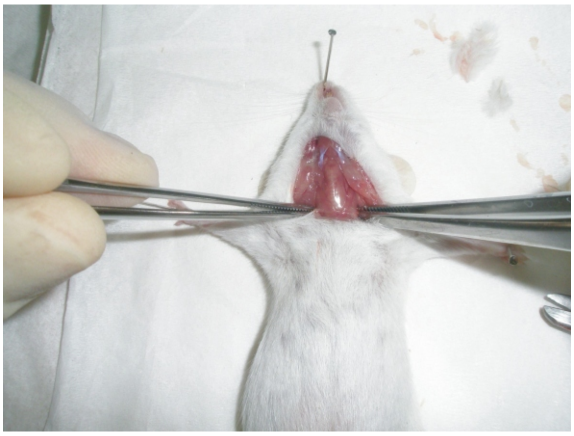

Figure 1. Mouse thyroid gland in situ immediately before the extirpation

shapes, 50 × 50 pixels large, were delineated around the contours of "full syringe", "empty syringe", mouse thyroids in situ and extirpated thyroids in test tubes, as well as around the background picture (fig. 2). The background radioactivity was then subtracted from the radioactivity of the "full syringe", "empty syringe", mouse thyroids in situ and extirpated thyroids in the test tubes.

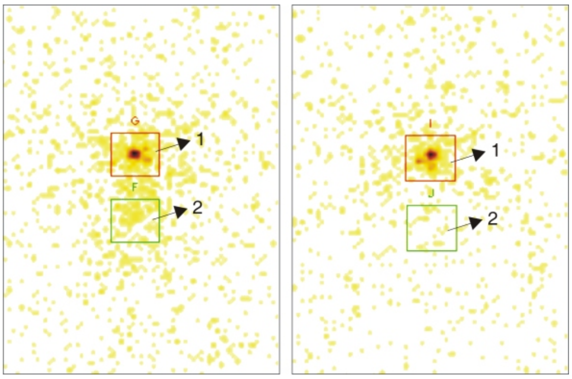

Figure 2. The ROI around the mouse thyroid (1) and the ROI for background (2). The left panel shows thyroid in situ, and the right panel shows extirpated thyroid

The radioactivity of the test tubes was then measured in gamma counter Wallac Wizard 1470 Automatic during 1 minute (*in vitro* measurement in gamma counter), under the same conditions as "full syringe" and "empty syringe" were measured previously. The volumes of the extirpated tissues were about 0.5 mL. Those volumes were similar to the volumes of the "full syringe", which provided for the same efficacy of detection by gamma camera as well as gamma counter.

Based on these results, the radioiodine fixation for each mouse was calculated in the following way: after correction of background radioactivity, the number of impulses counted for "empty syringe" was subtracted from the number of impulses counted for "full syringe". This is amount of administered radioactivity. Besides, the correction for radioactive decay of ^{131}I was made, since 72 hours elapsed from administration of radioactivity to the measurement. Equation (1) shows those calculations

$$f \quad 1.3\frac{A_t}{A_0}100\,[\%] \qquad (1)$$

where A_t is the radioactivity of thyroid after the time $t = 72$ hours, A_0 – the administered radioactivity in the time t_0, and 1.3 – the correction factor for radioactive decay of ^{131}I after 72 hours

The statistical calculations were made by SPSS software, version 18. The data were primarily described by central tendency measure and by measure of variability (mean and standard deviation). The significance of differences between three methods used for estimation of radioiodine fixation was tested by Student's *t*-test. The strength of correlation between the results of measurements by the three methods was tested by Pearson's correlation coefficient. The results were considered statistically significant if probability of null hypothesis was less than 0.05.

RESULTS

The results of all measurements and calculations are shown in tabs. 1 and 2. Table 1 contains results obtained by gamma camera both *in vivo* and *in vitro*. Table 2 contains values of measurements obtained by gamma counter *in vitro*. Figure 3 shows radioiodine fixation values obtained by all three methods of measurement.

The calculated radioiodine fixation values, *i. e.* percentages of binding of ^{131}I for thyroid tisue obtained by the three types of measurements are shown in the last columns of tabs. 1 and 2.

The radioiodine fixation in mouse thyroid measured by gamma camera *in vivo*, from the thyroid

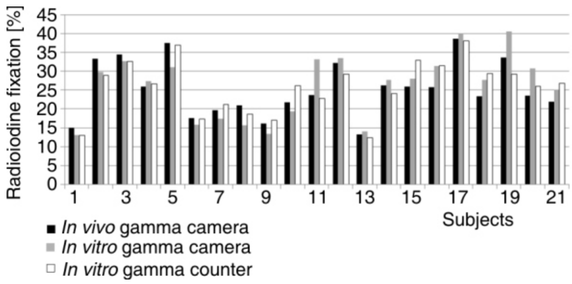

Figure 3. The radioiodine fixation in mouse thyroid measured by three methods: (1) by gamma camera *in vivo*, from the thyroid gland in situ; (2) by gamma camera *in vitro*, from the extirpated thyroid gland; and (3) by gamma counter *in vitro*, from the extirpated thyroid gland

Table 1. The results of *in vivo* and *in vitro* measurements of radioactivity by the gamma camera

Subject No.	Administered radioactivity [counts]	Thyroid radioactivity *in vivo* [counts]	Thyroid radioactivity *in vitro* [counts]	Radioiodine fixation estimated from *in vivo* measurement [%]	Radioiodine fixation estimated from *in vitro* measurement [%]
1	18390	2750	2400	14.95	13.05
2	18140	6040	5410	33.30	29.82
3	19310	6640	6310	34.39	32.68
4	18220	4730	5000	25.96	27.44
5	20590	7700	6400	37.40	31.08
6	18760	3310	2970	17.64	15.83
7	20230	3980	3520	19.67	17.40
8	20550	4310	3230	20.97	15.72
9	18900	3050	2530	16.14	13.39
10	20300	4410	3920	21.72	19.31
11	18930	4480	6280	23.67	33.17
12	18780	6040	6280	32.16	33.44
13	15260	2010	2150	13.17	14.09
14	18120	4750	5020	26.21	27.70
15	22250	5780	6220	25.98	27.96
16	18050	4660	5670	25.82	31.41
17	21590	8350	8640	38.68	40.20
18	23250	5420	6450	23.31	27.74
19	16400	5520	6650	33.66	40.55
20	19170	4520	5900	23.58	30.78
21	18360	4020	4600	21.90	25.05
Mean SD				25.25 7.32	26.08 8.55

Table 2. The results of *in vitro* measurements of radioactivity by the gamma counter

Subject No.	Administered radioactivity [counts]	Thyroid radioactivity *in vitro* [counts]	Radioiodine fixation estimated from *in vitro* measurement [%]
1	2253525	292302	12.97
2	2478326	717787	28.96
3	2367138	770092	32.53
4	2225324	593059	26.65
5	2289267	843492	36.85
6	2229377	386331	17.33
7	2303506	486230	21.11
8	2240810	515550	18.54
9	2173727	370144	17.03
10	2331414	609011	26.12
11	2206749	503232	22.80
12	2355927	688607	29.23
13	1912427	235493	12.31
14	2341824	565624	24.15
15	2056977	677449	32.93
16	1849613	582827	31.51
17	2564505	975551	38.04
18	2423172	711772	29.37
19	2324964	678541	29.19
20	2375559	618991	26.06
21	2134338	570792	26.74
Mean SD			25.74 7.18

gland *in situ* was 25.25% ± 7.32%. The radioiodine fixation in mouse thyroid measured by gamma camera *in vitro*, from the extirpated thyroid gland, was 26.08% 8.55%. The radioiodine fixation in mouse thyroid measured by gamma counter *in vitro*, from the extirpated thyroid gland, was 25.74% 7.18%. There are not statisticaly significant differences between those three methods ($p > 0.05$).

The results were also analysed by the Pearson's correlation test (tab. 3). We have found high correlation

Table 3. The results of determined Pearson's correlation coefficient

Correlations				
		Radioiodine fixation obtained by gamma camera *in vivo*	Radioiodine fixation obtained by gamma camera *in vitro*	Radioiodine fixation obtained by gamma counter *in vitro*
Radioiodine fixation obtained by gamma camera *in vivo*	Pearson correlation	1	0.869*	0.890*
	Sig. (1-tailed)		0.000	0.000
	N	21	21	21
Radioiodine fixation obtained by gamma camera *in vitro*	Pearson correlation	0.869*	1	0.835*
	Sig. (1-tailed)	0.000		0.000
	N	21	21	21
Radioiodine fixation obtained by gamma counter *in vitro*	Pearson correlation	0.890*	0.835*	1
	Sig. (1-tailed)	0.000	0.000	
	N	21	21	21

* Correlation is significant at the 0.01 level (1-tailed)

between these three methods of measuring radioiodine fixation in thyroid gland: (1) the correlation coefficient between the fixation rate obtained by gamma camera *in vivo* and the fixation rate obtained by measuring extirpated thyroids by gamma camera was 0.869, (2) the correlation coefficient between the fixation rate obtained by gamma camera *in vivo* and the fixation rate obtained by measuring extirpated thyroids by gamma counter was 0.890, and (3) the correlation coefficient between the fixation rate obtained by measuring extirpated thyroids by gamma camera and the fixation rate obtained by measuring extirpated thyroids by gamma counter was 0.835. All abovementioned values of correlation coefficient were statistically significant ($p < 0.01$).

DISCUSSION

Measuring fixation of radioiodine in thyroid gland by gamma camera is easy and comfortable for both patients and physicians, thanks to the useful and simple software tools implemented in computer systems which accompany the camera. However, the obtained values of radioiodine fixation depend on many objective factors, like methodology of measurement, performance of the gamma camera detector, software precision when delineating the ROI, *etc*. On the other hand, there are also subjective factors influencing measurement results, like knowledge and skills of a health worker conducting the test, right positioning of a patient, choosing appropriate ROI, *etc*. This was the reason why we decided to test reliability of radioiodine fixation measurement by gamma camera in comparison with measurement by gamma counter, which is more accurate method.

In comparison with the measurements by gamma camera, the measurements by gamma counter are more exact, since they are conducted using near 4π geometry, with much thicker cristal, and almost in direct contact. Knowing that error of measurement depends on absolute count of impulses, measurement by gamma counter is much more precise, because number of impulses was more than thousand times higher from that obtained by gamma camera. The gamma camera measures only impulses from one plane, while gamma counter captures almost all photons irradiated from the tissue. [12]

The thickness of the cristal in gamma camera is 3/8 inches, while the thickness of the cristal in gamma counter is 2 inches, making efficacy of the measurement better. Besides, the gamma camera is collimated in order to obtain a better resolution of the picture, and many photons are lost in the collimator's septa; such loss is not hapening with the gamma counter [8]. In other words, gamma counter is much more efficacious device for detection of gamma quants with energy of 364 keV. It captures much more quants from the same sample than if the measurement was made by gamma camera.

In our study, the measurements by gamma camera were made from the distance of only 5 cm, in order to achieve a higher efficacy of the detection, and taking into account a small volume of mice thyroid gland. The distance was very small in comparison with measurements in humans, but it was proportional to the size of the gland. Increase of the distance to 30 cm would decrease the number of captured impulses. The optimal distance of gamma camera from the neck depends on the volume of the thyroid gland (the target volume), as shown by other authors [13].

Besides, since the geometry of measurements was the same in all measurements conducted in this study, the chosen distance of 5 cm could not affect differences between the measurement results [14].

The measuring procedures on gamma camera and gamma counter make compromise between chosen amount of ^{131}I radioactivity which is administered (it directly affects the count rate) and duration of measurement on one side, and possible adverse effects of radioactivity on patients and health workers on the other side. The aim is to minimise uncertainty of measurements, and maximise safety of the procedure for both health workers and environment in general [15].

The radioactivity that we applied to mice was 0.37 MBq   0.03 MBq, which provided sufficient count rate even with short measurement period, without significant risk for investigators. The measurements by gamma camera lasted for 15 minutes, and from one "full syringe" more than 20000 impulses were collected. With such high number of impulses, the uncertainty of measurement is below 3%, with the reliability level of more than 99.7%. Measuring of "empty syringe", thyroid glands in situ and extirpated thyroids by gamma camera collected for 15 minutes more than 2000 impulses, giving uncertainty of measurement below 5%, and reliability level of more than 95% [16]. On the other hand, such small amount of radioactivity bears no risk of adverse effects or contamination of environment.

CONCLUSIONS

Our study showed that differences in fixation rate obtained by gamma camera *in vivo* and *in vitro* and gamma counter *in vitro* were not statistically significant.

In spite of higher number of captured impulses by gamma counter than by gamma camera, the measured radioiodine fixation rates by gamma camera from the thyroid gland *in situ*, by gamma camera from the extirpated thyroid gland, and by gamma counter from the extirpated thyroid gland correlate well. Our results confirm high reliability of measuring radioiodine fixation in thyroid gland by gamma camera.

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AUTHOR CONTRIBUTIONS

Theoretical analysis was carried out by M. D. Matović and M. Ž. Jeremić. Experiments were carried out by M. Ž. Jeremić, S. M. Janković, M. Z. Milošev, M. L. Novaković, V. D. Spasojević-Tišma, and V. D. Urošević. All authors analyzed and discussed the results. The manuscript was written by M. Ž. Jeremić and M. D. Matović. Figures and tables were prepared by M. Ž. Jeremić and V. D. Urošević.

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КОМПАРАЦИЈА ТРИ МЕТОДЕ КОРИШЋЕНЕ ЗА МЕРЕЊЕ РАДИОЈОДНЕ ФИКСАЦИЈЕ У ТИРЕОИДНОЈ ЖЛЕЗДИ МИШЕВА

Циљ овог рада је да се упореде вредности тестова фиксација добијених прорачуном на основу три метода мерења у студији на мишевима. Истраживање је спроведено на 21 белом швајцарском мишу оба пола, у узрасту од 10 недеља, тежине између 25 g и 34 *g*. Мишевима су интраперитонеалним убризгавањем апликоване приближно исте дозе ^{131}I, просечне активности 0.37 MBq 0.03 MBq. Након 72 сата мишеви су анестезирани и мерена им је радиоактивност тироидног региона на гама камери (први метод, *in situ*). Након тога су жртвовани и пажљиво им је одстрањена тироидна жлезда заједно са суседном трахејом и смештена на дно серумске епрувете. Радиоактивност тако одстрањеног ткива је мерена и на гама камери (други метод) и у гама бројачу (трећи метод). Овај метод је узет за стандард као најтачнији. У студији су коришћене гама камере Siemens e.cam Dual Head (Siemens, 1997, USA) и гама бројач Wallac Wizard 1470 Automatic (Wallac Oy, 2005. Finland).

Радиојодна фиксација одређена коришћењем ове три методе је била 25.25% 7.32%, 26.08% 8.55% и 25.74% 7.18%, без статистички сигнификантне разлике између метода ($p > 0.05$). Добијен је висок коефицијент корелације између три метода мерења теста радиојодне фиксације: (1) коефицијент корелације између вредности фиксација добијених мерењима на гама камери *in situ* и екстирпираних тироидеја на гама камери је био 0.869 ($p < 0.01$); (2) коефицијент корелације између вредности фиксација добијених мерењима на гама камери *in situ* и екстирпираних тироидеја у гама бројачу је био 0.890 ($p < 0.01$); (3) коефицијент корелације између вредности фиксација добијених мерењима екстирпираних тироидеја на гама камери и екстирпираних тироидеја у гама бројачу био је 0.835 ($p < 0.01$).

Кључне речи: штитаста жлезда, тест радиојодне фиксације, гама камера