^{111}In OR ^{125}I LABELED EPIDERMAL GROWTH FACTOR FOR THE *IN VIVO* LOCALIZATION OF EGF RECEPTORS: *IN VIVO* STABILITY

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SUMMARY: Radio iodinated epidermal growth factor (^{125}I-EGF) used for the detection EGF receptors in implanted tumors in mice showed rapid in vivo de-iodination. To increase its stability EGF was labeled with ^{111}In and its biodistribution in normal NMRI mice was determined and compared to ^{125}I-EGF. The highest accumulation was observed in the thyroid (max at 6 h: 1045.2 ± 376.4 % /g) with ^{125}I-EGF. ^{111}In-EGF demonstrated prolonged blood clearance and whole body retention compared to ^{125}I-EGF. High renal activity (23.79 ± 3.41% /g) indicated excretion via kidneys. ^{111}In labeling increased the stability of the radiotracer. Further studies in tumor implanted nude mice are warranted to demonstrate the biological efficacy of ^{111}In labeling.

Key Words: ^{111}In-EGF, ^{125}I-EGF, EGF receptors, ITLC.

INTRODUCTION

Although ^{131}I dose not have the necessary ideal imaging characteristics it is the most often used radioisotope compared to ^{123}I, ^{99m}Tc and ^{111}In in labeling monoclonal antibodies for *in vivo* studies (7). Its long half-life of 8 days though detrimental from the standpoint of radiation dosimetry is advantageous when the tumoral uptake is prolonged and a follow-up of one week is necessary (2, 7). For *in vitro* and animal studies ^{125}I ($T_{1/2}$= 60 days) is preferred with the understanding that it will be replaced by ^{131}I in human applications. The *in vivo* stability of the label may present a serious problem. Several methods have been proposed to stabilize the label against *in vivo* cleavage by enzymes (3, 8, 10, 13).

Epidermal growth factor (EGF) receptors have been demonstrated on cultured breast carcinoma cell lines (9) and biopsy samples from human breast cancers (6, 11). We have been using ^{131}I labeled human EGF for the localization of EGF receptors in nude mice implanted with human breast carcinoma xenografts (12). In recent experiments we have observed rapid *in vivo* de-iodination resulting in high blood background, high thyroid uptake and increased urinary excretion with no significant accumulation in tumoral tissues. In a previous communication by our group ^{125}I-EGF was coupled to mouse albumin by using the cyanuric chloride method of Sinn *et al.* (13) in order to stabilize it against *in vivo* de-iodination (4). In the present investigation EGF was labeled with ^{111}In ($T_{1/2}$ = 2.8 days) and its biodistribution and whole-body retention in normal mice was compared to ^{125}I-EGF.

MATERIALS AND METHODS

Epidermal growth factor was bought from Bissendorf, F.R.G. ^{125}I-EGF and ^{111}In as $^{111}InCl_3$ were purchased from Amersham, England. Mouse albumin was purchased from Sigma Chem Co., USA.

^{125}I-EGF was diluted with saline to give 740 kBg/0.2 ml for animal experiment. To stabilize ^{125}I label mouse albumin was dissolved in this solution to a concentration of 2%.

Labeling EGF with ^{111}In

130 μg EGF stock containing 100 μm EGF was dissolved in 50 μl distilled water. 100 μl $^{111}InCl_3$ (15 MBq) was added and mixed well. The mixture was incubated at R. T. for 30 min. It was diluted to 4 ml to give a concentration of 740 kBq/0.2 ml for the animal studies.

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Chromatographic quality control

Impregnated-Thin- Layer Chromatography (ITLC) was utilized with ITCL-SG mini-strips (Gelman Instrument, USA) and saline as a solvent to determine the amount of free ^{125}I in ^{125}I-EGF (15). Free iodide migrated with the solvent front (R_f = 1.0) while radio iodinated compound remained at the origin (R_f = 0.0). The stability of the label was checked up to 8 days after calibration date.

Animal studies

25 NMRI mice were each I.V. injected with 0.2 ml (740 kBq) ^{125}I-EGF solution through the tail vein. They were sacrificed in groups of 5 at 1,3,6,12 and 24 h post-injection. Some blood, thyroid, lungs, liver, kidneys, spleen, femur, muscle and stomach were removed. So of the blood was centrifuged to separate the serum. They were all weighed and counted against a standard prepared from 1/100 dilution of the injected solution in a gamma counter (Model: BF 5300, Berthold, Germany). Percent uptake by each organ and per gram tissue and the means with standard deviations of 5 animals were all calculated. The same procedure was followed with ^{111}In-EGF except that the times of sacrifice were 1, 3, 6, 24 and 48 h post-injection.

The radio activities of the whole animals were determined at the same intervals up to the time of sacrifice by the use of a whole body counter against a standard having 100% of the injected solution. % retention of radioactivity was calculated for each animal and the mean values were plotted as a function of time.

RESULTS

ITLC analysis of ^{125}I-EGF indicated a high labeling yield (>99%) at the time of arrival. Upon storage at 4°C the amount of free ^{125}I-incrased up to 4.1±2.4% at 8 days which was within acceptable limits. The animal experiments were performed the first two days when the amount of ^{125}I was <1%. The results of biodistribution studies are summarized in Tables 1 and 2 for ^{125}I- and ^{111}In-EGF, respectively. With ^{125}I-EGF radioactivity levels in all the organs decreased as time progressed except for thyroid activity which reached a maximum at 6 h with 1045.2±376.4%/g, indicating the presence of free ^{125}I-*in vivo*. The only organ that concentrated large amounts of radioactivity was thyroid. ^{111}In-EGF demonstrated different biodistribution than ^{125}I-EGF. Blood

Table 1: Biodistribution of ^{125}-EGF in normal mice (%/g tissue).

	Time (h)				
Organ	1	3	6	12	24
Blood	5.53±1.63	2.87±1.42	0.984±0.411	0.0811±0.0123	0.061±0.0099
Serum	6.16±1.54	3.11±1.46	1.09±0.50	0.0650±0.0176	0.0565±0.0176
Thyroid	140.8±59.0	206.7±19.2	1045.2±376.4	320.9±276.1	125.9±93.1
Lungs	3.41±1.01	1.80±0.73	0.666±0.291	0.0799±0.0637	0.0439±0.0094
Liver	4.46±2.13	1.02±0.45	0.396±0.119	0.0558±0.0171	0.0327±0.086
Kidneys	7.05±1.56	2.49±0.90	0.983±0.235	0.185±0.031	0.0855±0.0500
Spleen	2.29±0.65	0.867±0.740	0.394±0.115	0.0578±0.0152	0.0488±0.0050
Femur	1.76±0.56	0.943±0.425	0.364±0.099	0.0566±0.0032	0.0499±0.0155
Muscle	0.86±0.31	0.586±0.291	0.196±0.034	0.0282±0.0082	0.0359±0.0068
Stomach	3.28±0.91	2.13±1.11	0.718±0.227	0.0716±0.0406	0.0460±0.0048

Table 2: Biodistribution of ^{111}-EGF in normal mice (%/g tissue).

	Time (h)				
Organ	1	3	6	24	48
Blood	11.95±1.76	5.59±0.73	4.35±0.20	1.37±0.31	0.486±0.006
Serum	21.01±3.52	10.49±1.17	9.11±0.89	2.54±0.77	0.621±0.177
Thyroid	2.98±0.55	3.30±0.84	3.22±0.48	2.70±0.42	3.40±0.90
Lungs	4.98±0.17	3.33±0.12	3.20±0.09	2.20±0.36	2.42±0.45
Liver	3.69±0.49	3.20±0.29	4.31±0.07	4.52±0.63	5.80±0.86
Kidneys	20.60±7.07	21.82±5.26	23.79±3.41	21.71±2.03	18.40±1.27
Spleen	2.43±0.27	2.35±0.61	2.74±0.61	3.72±1.11	3.35±0.71
Femur	3.12±0.13	3.30±0.30	3.88±0.53	3.75±0.27	4.38±0.53
Muscle	1.19±0.18	1.20±0.09	1.15±0.20	0.916±0.217	0.910±0.045
Stomach	1.58±0.10	1.58±0.08	1.57±0.19	1.53±0.14	1.51±0.18

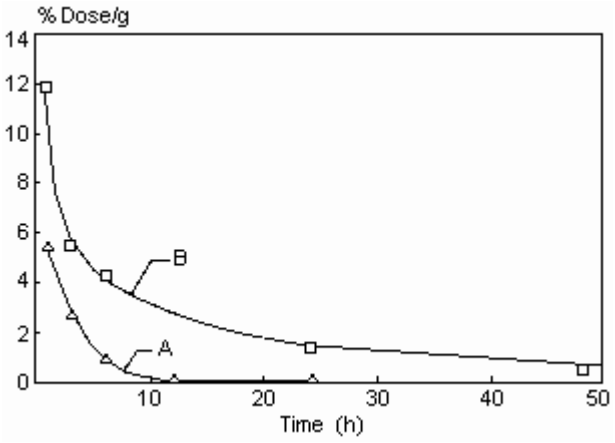

Figure 1: Blood clearance of ^{125}I-EGF (A) and ^{111}In-EGF (B) in normal mice.

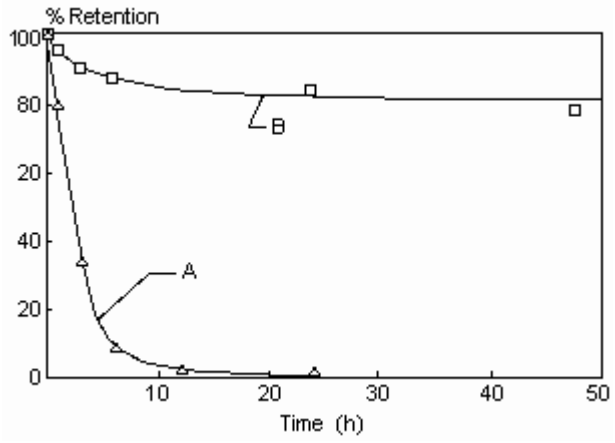

Figure 2: Whole-body retention of ^{125}I-EGF (A) and ^{111}In-EGF (B) in normal mice.

radioactivity levels were higher. The high uptake by the kidneys (23.79±3.41%/g at 6 h) might indicate urinary excretion of free $^{111}In^{+3}$ ion. The uptake by other organs remained at almost the same level over the observed time range. Figure 1 displays the time activity curve of blood disappearance for both ^{125}I-EGF and ^{111}In-EGF. The blood clearance of ^{125}I-EGF ($T_{1/2}$=2.2 h) was faster compared to ^{111}In-EGF ($T_{1/2}$=3 h). The whole-body retention of both radio-tracers are presented in Figure 2. ^{111}In-EGF had a prolonged retention (too long for $T_{1/2}$ calculation) compared to ^{125}I-EGF ($T_{1/2}$=2 h).

DISCUSSION

Although ^{125}I-EGF is stable *in vitro*, the radio-iodine bond dissociates *in vivo* forming free ^{125}I-iodide ion which accumulates in the thyroid as demonstrated by the biodistribution studies in normal mice. Because of this no single organ apart from thyroid concentrated it to appreciable quantities. The absence of tumoral uptake reported earlier (4,12) can be attributed to enzymatic cleavage of radio-iodine bond *in vivo*. The rapid clearance of radioactivity and high thyroidal uptake was also reported for radiodinated somatostatin analogue used in the detection of somatostatin receptors in tumor bearing rats (1). Coupling EGF to serum albumin is one way of stabilizing this bond. Labeling EGF with another radioisotope such as ^{111}In might serve the same purpose. As demonstrated in the present study the blood clearance and the whole-body retention are both prolonged with ^{111}In-EGF compared to ^{125}I-EGF. Indium ion does not accumulate in the thyroid like iodide ion. When administered as $^{111}InCl_3$ it is bound to transferrin in the plasma. It accumulates in the liver and bone marrow (5,14). Our animal studies show that there is no preferential uptake in the liver or femur. However, trans-chelation is also possible in the plasma. Electrophoretic analyses of plasma samples obtained from mice administered with ^{111}In labeled antimelanoma monoclonal antibodies indicated that ^{111}In was transferred from Mab to plasma proteins (5). Biodistribution studies in tumor implanted animals will give definite evidence against trans-chelation.

Although ^{111}In has a shorter physical half-life than ^{131}I, it might be sufficient for the visualization of implanted tumors. The prolongation of blood clearance and whole-body retention might facilitate its tumoral uptake. Further studies are warranted in tumor implanted nude mice to show the biological efficacy of ^{111}In-EGF.

ACKNOWLEDGEMENT

The first author is indepted to Alexander von humboldt Stiftung for a research fellowship. The technical assistance of Mrs. Sigrun Möllenstadt is gratefully acknowledge

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