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ORIGINAL ARTICLE

V. K. Khetarpal · L. S. Storbeck

Biological disposition of intravenously administered ^{131}I-labeled anti-EGF-receptor antibody (RG 83852) in the rat

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Abstract RG 83852 is a murine monoclonal antibody that preferentially inhibits the high-affinity binding of epidermal growth factor (EGF) to its receptor. Since overexpression of EGF receptor has been implicated in some human malignancies, the antibody is under investigation as a potential anticancer agent. The present work characterized the tissue distribution and elimination of ^{131}I-labeled antibody in rats following i.v. administration. ^{131}I-RG 83852 was given in a 2.22 mg/kg dose to rats, and 4, 24, 48, and 72 h afterwards ^{131}I activity excreted in the urine and feces and that present in various tissues was determined. The plasma contained the highest concentration of radioactivity at all times. At 4 h the plasma contained about 12% of the injected dose (ID)/ml, and radioactivity in this compartment accounted for almost 70% ID. The plasma elimination of ^{131}I-derived activity occurred linearily at a rate of about 0.48% ID/h. Except in the thyroid, the concentration of ^{131}I activity in all tissues was much lower than in the plasma (tissue-to-plasma ratio $\leq$0.1). In the thyroid, accumulation of radioactivity (4% ID at 24 h) was presumably due to trapping of ^{131}I released from the antibody as a result of biodegradation. The urinary excretion occurred at a rate of about 0.5% ID/h; the fecal excretion was minimal. The biodistribution results are consistent with the protein structure of the antibody. Based on the available disposition data, it is proposed that elimination of the antibody involves degradation, a process that follows zero-order kinetics, followed by excretion of the labeled product(s) in the urine.

Key words EGF-receptor antibody · Antibody elimination Tissue distribution

V. K. Khetarpal (✉) · L. S. Storbeck
Drug Disposition, Rhône-Poulenc Rorer, 500 Arcola Road, Collegeville, PA 19426, USA
Fax (+1) 215-454-5800

Introduction

The overexpression of epidermal growth factor receptor (EGFR) has been documented in several types of malignancies, mainly of epithelial origin, and it has been proposed that overexpression of EGFR plays a role in tumorigenesis [7, 10, 13, 17, 18, 21]. RG 83852 is a murine monoclonal antibody that preferentially inhibits the high-affinity binding of epidermal growth factor (EGF) to its receptor [1]. The monoclonal antibody is isotype IgG_{2a}, having a molecular weight of approximately 160,000 daltons, as estimated by TSK sizing HPLC [1]. Originally developed as a tool for studying the binding site and biochemistry of the EGFR [11, 17], the anti-EGFR antibody was found to have inhibitory effects on the growth of certain human tumor cells [1, 9, 14, 15]. Additionally, the in vivo anti-tumor activity of the antibody was demonstrated in nude mice to which KB tumor cells were inoculated s.c. [1]. In this model, the antibody, given either i.v. or i.p., resulted in significant retardation of tumor growth. Also, combined treatment with the antibody and Adriamycin or cisplatin resulted in tumor reduction that was greater than that accounted for by the additive effect [1]. By using ^{125}I-labeled antibody, it was shown that 7–8% of the i.v.- or i.p.-administered antibody accumulated in the tumor [1]. These data prompted the development of RG 83852 as a therapeutic agent against human tumors of epidermal cell origin, particularly in conjunction with other chemotherapeutic agents. In the initial clinical trials planned, ^{131}I-labeled RG 83852 was used to perform gamma-scintigraphy in tumors for drug localization. The purpose of the present investigation was to characterize the biodistribution and excretion of the antibody in the rat using ^{131}I-RG 83852. In addition to the delineation of elimination kinetics, these data were considered critical for estimating radiation dosimetry to support the clinical efficacy studies.

Materials and methods

Test material

RG 83852 was prepared by the Biotechnology Division of Rhône-Poulenc Rorer (King of Prussia, Pa.). It was purified from murine ascites that were produced by inoculating mice with a cloned hybridoma cell that secretes the antibody. The antibody was labeled with ^{131}I by the chloramine T method [8] at the Sloan-Kettering Institute, New York, N.Y. The product appeared as a single peak on an HPLC molecular sizing column with retention time characteristic of immunoglobulin. The labeled antibody was contained in buffered aqueous solution (1.59 mg/ml) and, on the day of receipt, had a specific activity of 1.333 mCi/mg protein. On the day of dosing, the antibody was diluted with sterile saline to give a final concentration of 0.389 mg/ml.

Determination of radioactivity

^{131}I activity in the dosing solution and in various biological samples was measured by counting gamma radiation using a Cobra (Model 5005) Gamma Counter (Packard Instrument Co., Downers Grove, Ill.). The counter was equipped with a program for automatic correction due to radioactive decay of ^{131}I (half-life = 8.04 days). All measurements were obtained as counts per minute (cpm). The counting efficiency of the gamma counter was approximately 10%.

Animals and treatment

Male Sprague-Dawley rats each weighing about 175 g were obtained from Taconic Farms (New York) and maintained on standard food (Rat Purina Chow) and water, both given ad libitum. After 7 days of acclimation, rats were fasted overnight and then 16 (176–183 g) were each given a single i.v. treatment with about 2.25 mg ^{131}I-RG 83852/kg. The projected dose range for clinical efficacy trials in humans was 0.1 mg/kg to 7.0 mg/kg. The antibody dose contained in approximately 1 ml was injected slowly (average injection time, 1 min) in the tail vein of each rat. The exact weight of the dosing solution administered was calculated from the weight of the syringe before and after dosing. Subsequent to dosing, rats were housed individually in metabolism cages (Nalgene, Rochester, N.Y.) designed for the separate collection of urine and feces.

Sample collection

Four rats were used for each of the following sampling times: 4, 24, 48, and 72 h after dosing. Both urine and the fecal samples were collected up to the time of sacrifice and then pooled and analyzed as single samples for each rat. At the time of sacrifice, the rat was first anesthetized with ether and about 6 ml of blood was withdrawn from the inferior vena cava. The animals were then killed by overexposure to ether. The following tissues were excised in toto: liver, gastrointestinal tract, kidneys, lungs, heart, spleen, testes, seminal vesicles, prostate glands, urinary bladder, pancreas, thymus, thyroid, adrenal glands, and brain. Samples of bone marrow (from femur), fat (retroperitoneal), skeletal muscle (thigh), and skin (dorsal, including hair) were also removed. The gastrointestinal tract, along with its contents, was divided into stomach, small intestine, cecum, and large intestine.

Sample analysis

Urine, plasma, and cage washes were diluted with water, and ^{131}I activity was measured in replicate 100-µl aliquots. The feces was mixed with about 5 vol. of water and homogenized using a polytron (Brinkmann Instruments, Westbury, N.Y.). Three aliquots of about 1 ml were weighed and the radioactivity was measured in each sample. To measure ^{131}I activity in blood, duplicate 0.5-ml aliquots were mixed with 9.5 ml of 1 *N* potassium hydroxide (KOH). The mixture was allowed to stand at room temperature for about 72 h to digest cells and the radioactivity was measured in duplicate aliquots of 50 µl. The ^{131}I activity in bone marrow, seminal vesicles, prostate glands, and urinary bladder was determined directly by placing the tissue in a polypropylene tube, weighing it, and then counting radioactivity in the gamma counter. All other tissues were weighed, mixed with 4 or 10 vol. of 1 *N* KOH, and weighed again. After standing in KOH solution for 3 or 4 days the tissues were homogenized using a polytron. The ^{131}I activity was measured in accurately weighed triplicate aliquots of the homogenate. The radioactivity present in each tissue was calculated based on the total amount of homogenate and the average radioactivity measured in triplicate aliquots.

Table 1 Concentration of ^{131}I-derived activity in the blood and plasma compartments of rats following i.v. administration of ^{131}I-RG 83852 (mean ± SD; n = 4/sampling time)

Time post-dose (h)	Antibody concentration (% ID/ml)		Blood/plasma concentration ratio
	Blood	Plasma	
4	6.89±0.43	11.89±1.11	0.58±0.03
24	6.37±0.38	10.31±0.78	0.62±0.05
48	4.69±0.26	8.27±0.47	0.57±0.01
72	3.70±0.03	6.42±0.08	0.58±0.01

Data analysis

The total activity (counts/minute) calculated from the weight of dosing solution injected into the animal was considered to be the injected dose (ID). The cumulative excretion of ^{131}I activity in the urine and feces was expressed as a percentage of the injected dose (% ID). In the plasma and blood, the percentage of radioactivity remaining at each time point was based on the determination of radioactivity (% ID/ml) and the volume of each compartment in the Sprague-Dawley rats. The values used for the blood and plasma volumes were 6.08 ml/100 g [3] and 3.26 ml/100 g [20], respectively. For organs removed in toto, calculation of the percentage of dose associated with the tissue was based on actual weight of the tissue removed. For skin, skeletal muscle, and fat, total amount of the tissue was based on reported values for the proportion of the body weight constituted by these tissues. The values used were: skin 16%, fat 7%, and skeletal muscle 50% [16]. For bone marrow, the percentage of dose associated with the tissue was not calculated.

Results

The amount of radioactivity administered to each rat averaged about 96.9×10^6 cpm, and the mean (±SD) antibody dose was calculated to be 2.22 (±0.08) mg/kg.

The concentrations of ^{131}I activity determined in plasma and blood are given in Table 1 and the estimated total antibody remaining in these compartments at various times are shown in Fig. 1. Also shown in Table 1 are the blood-to-plasma ratios. The plasma contained a high concentration of ^{131}I activity at 4 h (11.89% ID/ml), which declined linearily at an average rate of 0.081% ID/h per ml. This elimination rate corresponded to the total loss of 0.48% ID/h from the plasma. Most of the radioactivity in blood could be accounted for by the plasma fraction.

The concentration of ^{131}I activity vs time curves obtained for various tissues are shown in Fig. 2. The total

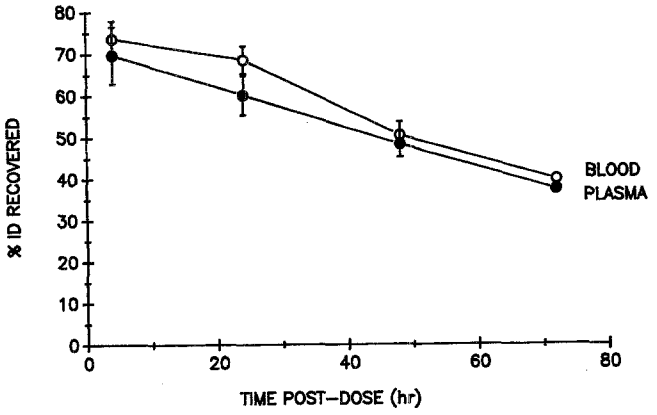

Fig. 1 Total amount of ^{131}I activity (expressed as percentage of injected dose; *% ID*) estimated to be present in the blood and plasma compartments of rats at various times following i. v. administration of about 2.22 mg/kg of ^{131}I-RG 83852. Each *point* represents mean of 4 determinations; *bars* ± 1 SD

radioactivity remaining in each organ, measured as % ID/organ, is shown in Table 2. The concentrations of radioactivity in all tissues except thyroid were much lower than those observed in the plasma. The concentration of ^{131}I activity in the thyroid was highest at 48 h, at which time almost 3% of the injected dose could be recovered from the tissue.

Among other tissues, lungs contained the highest concentration of radioactivity at 4 h (1.51 ID/g), followed by the kidney and the heart (Fig. 2). The concentrations of ^{131}I activity in all other tissues were less than 1% ID/g tissue. In most of the organs, including liver, kidney, heart, lung, gastrointestinal tract, testes, and spleen, the concentrations of radioactivity were lower at 24 h and showed further declines at 48 and 72 h. On the other hand, in the skin, skeletal muscle, fat, and some of the smaller tissues, including pancreas, urinary bladder, seminal vesicles, and prostate gland, the concentration of ^{131}I activity increased up to 24 h or 48 h but a decline was apparent in all tissues at 72 h. The concentrations of radioactivity detected in the brain were the lowest at all time points.

When the recovery of administered radioactivity was based on the measured or estimated total tissue weight, it was found that at 4 h, liver accounted for the highest percentage of administered radioactivity (9.69%), followed by skeletal muscle (9.44%), skin (7.04%), small intestine (4.60%), kidney (2.75%), and fat (2.71%) (Table 2). While

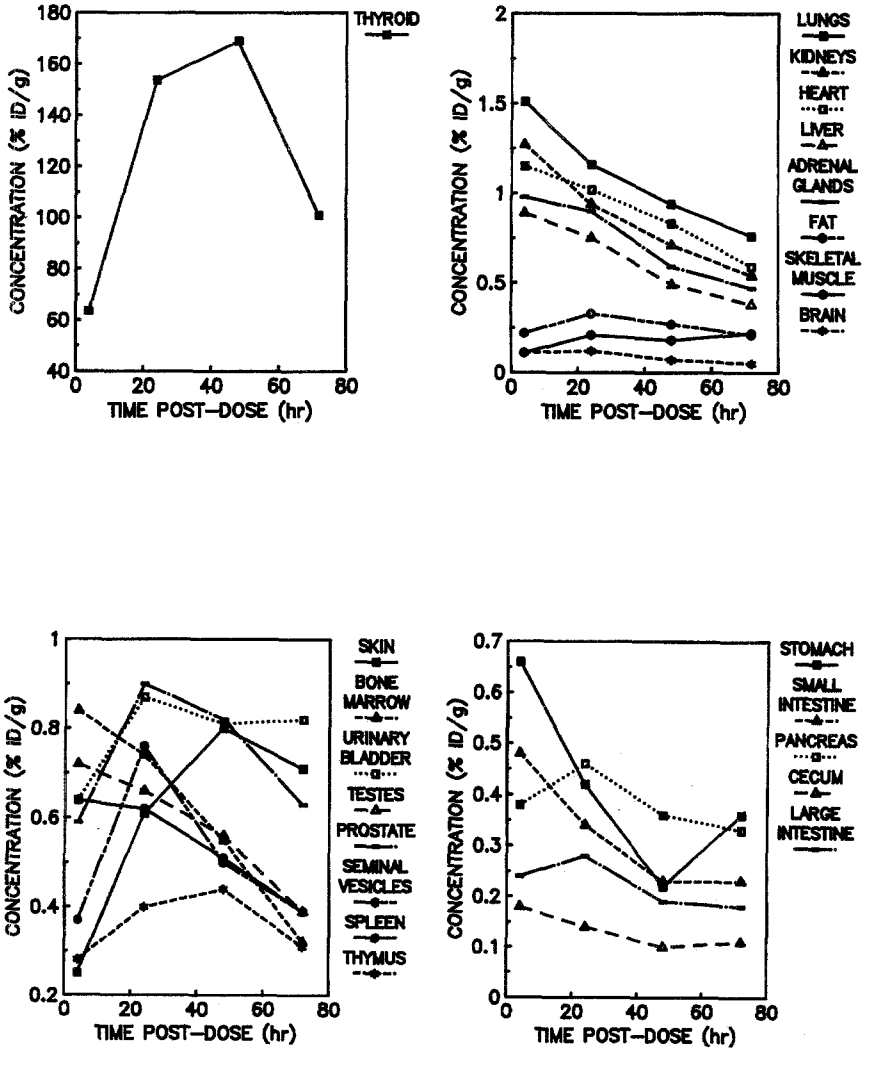

Fig. 2 Concentration of radioactivity (expressed as % ID/g) in various tissues of rats following i. v. administration of about 2.22 mg/kg of ^{131}I-RG 83852. Each *point* represents mean of 4 determinations. *Error bars* are not shown for the sake of clarity. Relative standard deviation was $<30\%$ in all cases

Table 2 Biodistribution of radioactivity as percentage of injected dose per organ after i.v. administration of ^{131}I-RG 83852 to rats (mean ± SD; $n = 4$)

Organ	% ID/organ			
	Time post-dose (h)			
	4	24	48	72
Liver	9.69±1.29	6.64±0.55	5.18±0.83	3.92±0.18
Kidney	2.75±0.45	1.77±0.25	1.45±0.17	1.10±0.03
Heart	1.07±0.23	0.80±0.10	0.72±0.04	0.51±0.03
Lungs	1.79±0.33	1.17±0.14	1.05±0.05	0.90±0.08
Thyroid	1.22±0.29	3.97±0.45	2.98±0.27	1.82±1.16
Stomach	2.28±0.48	1.43±0.28	0.84±0.11	0.94±0.22
Small intestine	4.60±1.14	3.07±0.23	2.36±0.09	2.00±0.04
Cecum	0.97±0.21	0.70±0.43	0.54±0.07	0.67±0.05
Large intestine	0.84±0.30	0.81±0.16	0.90±0.30	0.52±0.09
Testes	1.87±0.45	1.40±0.04	0.04±1.32	1.32±0.64
Spleen	0.58±0.12	0.40±0.05	0.32±0.04	0.24±0.04
Thymus	0.20±0.11	0.25±0.04	0.22±0.03	0.19±0.01
Pancreas	0.27±0.09	0.26±0.08	0.24±0.04	0.27±0.06
Urinary bladder	0.08±0.05	0.08±0.01	0.09±0.01	0.08±0.01
Seminal vesicles	0.11±0.02	0.11±0.03	0.11±0.02	0.09±0.01
Prostate gland	0.10±0.02	0.11±0.03	0.13±0.01	0.11±0.01
Brain	0.18±0.02	0.19±0.04	0.12±0.00	0.08±0.01
Adrenal glands	0.05±0.02	0.05±0.02	0.05±0.01	0.03±0.01
Skin	7.04±0.33	17.43±3.80	22.93±5.89	20.23±0.94
Skeletal muscle	9.44±1.21	18.52±3.05	15.87±1.34	19.68±1.08
Fat	2.71±0.99	4.17±1.50	3.38±0.53	2.62±0.02

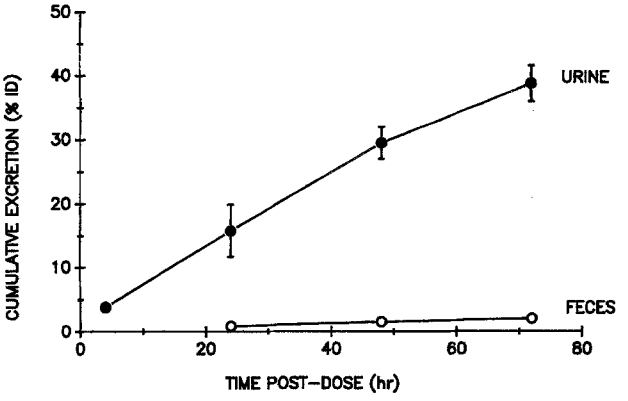

Fig. 3 Cumulative urinary and fecal excretion of radioactivity (expressed as % ID) in rats following an i.v. dose of about 2.22 mg/kg of ^{131}I-RG 83852. Each *point* represents mean of 4 determinations; *bars* ±1 SD

the dose percentage accounted for by most tissues decreased at 24 h and thereafter, the radioactivity measured in the skin and skeletal muscle accounted for a much higher fraction at later time points.

In almost all tissues, the tissue-to-plasma ratio was less than 0.1. Even when ^{131}I activity in the skin accounted for almost 23% of the administered radioactivity (48 h), the average ratio was determined to be 0.1.

The cumulative recoveries of ^{131}I activity in the urine and feces are shown in Fig. 3. The urinary excretion occurred linearly at a rate of about 0.52% ID/h. The excretion of ^{131}I activity in the feces accounted for a very small percentage of the administered dose and may, in fact, be due to contamination of the feces with urine sample.

Because of the presence of residual blood in tissues, an accurate assessment of mass balance could not be obtained. However, based on the amount of total radioactivity recovered in the sampled blood (5.6–6.2 ml), tissues and excreta, it was estimated that an average of 93% of the administered dose was accounted for at 4 h. At 24, 48, and 72 h, the proportion of the dose recovered averaged 118–120%.

Discussion

The results obtained in the present investigation indicate that following i.v. administration, RG 83852 is confined mostly to the plasma compartment. The concentration of ^{131}I activity in the plasma accounted for about 70% of the administered radioactivity at 4 h. Almost all of the ^{131}I activity measured in the blood could be accounted for by the plasma fraction, which indicates that the antibody was not taken up by the red blood cells. Although radioactivity measured in tissues accounted for a total of about 48% ID at this time, but it is suspected that a significant proportion of radioactivity was due to the residual blood remaining in these tissues. Based on the estimated blood content of tissues in exsanguinated rats [4], only 30% or less of the tissue radioactivity could be ascribed to the extravascular compartment. These data are consistent with the fact that RG 83852 is an immunoglobulin and, therefore, its diffusion into the extravascular space is expected to be limited [4].

A significant proportion of the radioactivity administered was found to be present in the thyroid gland. The concentration of ^{131}I activity was very high, indicating accumulation of the label in this tissue. The radioactivity measured in the thyroid, however, is not likely to represent intact antibody. It is suspected that ^{131}I label released from

the protein, as a result either of protein degradation or of instability of the label, was trapped by the gland, resulting in accumulation. Although not investigated in this study, dehalogenation has been shown to occur in vivo with a number of other iodinated monoclonal antibodies [5].

The antibody did not appear to concentrate in any tissue. This contention is based on the observation that tissue-to-plasma concentration ratios were less than 0.1. As discussed above, only about 30% of the measured ^{131}I activity in tissues could be attributed to diffusion of the antibody into the extravascular space. When the antibody concentration in the interstitial fluid of the tissues was computed by assuming this amount to be distributed into the extracellular fluid volume [19], it was found that the interstitial antibody concentration relative to plasma concentration ranged from only 0.2 to 0.5 in most tissues.

The concentration of radioactivity in richly perfused tissues seemed to decline in parallel with that of plasma. However, in the skin, and to a smaller extent in the skeletal muscle and fat, the proportion of administered radioactivity accounted for at 24 h was greater than that at 4 h. These results suggest slow diffusion of the labeled moiety into the extravascular space of these tissues. It is presumed that in the skin and skeletal muscle equilibrium occurs slowly, because the capillaries are less permeable to proteins and clearance by lymph is slower relative to other tissues [2]. A kinetic study in mice with ^{111}In-labeled monoclonal antibody against carcinoembryonic antigen also showed similar tissue distribution characteristics [6].

It is speculated that the excretion of ^{131}I label in the urine represents the degradation products of the antibody. The urinary excretion occurred at a constant rate of about 0.52% ID/h. The disappearance of radioactivity from plasma also occurred at a rate of 0.48% ID/h. Since the intact antibody is not likely to be excreted in the urine [12], the plasma and urinary data indicate that the antibody is degraded in the plasma and the iodinated product(s), excreted in the urine. The fecal excretion of iodinated product(s) was found to be minimal.

In conclusion, the data obtained in the present investigation show that following i.v. administration, RG 83852 is confined mostly to the plasma compartment. The antibody does not concentrate in any tissue. In the thyroid, accumulation of radioactivity most probably resulted from trapping of free ^{131}I released from the antibody. The results obtained are consistent with the fact that RG 83852 is a protein and therefore its diffusion into the extravascular space is expected to be limited. The elimination of RG 83852 follows zero-order kinetics, possibly due to either rate-limiting migration of the antibody to the site of catabolism or saturation of the metabolic processes. Based on the disposition data obtained in this study, it is conjectured that the antibody is first degraded, followed by appearance of the labeled product(s) in the urine. At a dose of about 2.22 mg/kg, the rate of degradation appeared to be about 0.5% ID/h.

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