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• LIVER CANCER •

Pharmacokinetics of radioimmunotherapeutic agent of direct labeling mAb ^{188}Re-HAb18

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Abstract

AIM: **To label anti-hepatoma monoclonal antibody (mAb) fragment HAb18 F(ab')$_2$ was labeled with ^{188}Re for the pharmacokinetic model of ^{188}Re-HAb18 F(ab')$_2$ and to evaluate its pharmacokinetic parameters in hepatoma-bearing nude mice.**

METHODS: **HAb18 F(ab')$_2$ was directly labeled with ^{188}Re using 2-mercaptoethanol (2-ME) as reducing agents. Labeling efficiency and immunoreactivity of ^{188}Re-HAb18 F(ab')$_2$ were evaluated by Whatman 3MM paper chromatography and live cell assay, respectively. Biodistribution analysis was also conducted in nude mice bearing human hepatoma in which animals were sacrificed at different time points (1, 4, 18, 24 and 24h) after ^{188}Re-HAb18 F (ab')$_2$ was injected through tail-vein into hepatoma-bearing nude mice. The blood and radioactivity of organs and mass were measured. The concentrations of ^{188}Re-HAb18 F(ab')$_2$ were evaluated with apharmacokinetic 3P97 software.**

RESULTS: **The optimum labeling efficiency and immunoreactive fraction were 91.7% and 0.78% respectively. The parameters of ^{188}Re-HAb18 F(ab')$_2$ were: $T_{1/2}$, 2.29h; Vd,1.49×10^{-9}L·Bq^{-1};AUC, 20.49×10^{9}Bq·h·L^{-1};CL, 0.45×10^{-3}L·h^{-1}. ^{188}Re-HAb18 F(ab')$_2$ could locate specially in hepatoma with high selective reactivity of HAb18 F(ab')$_2$. ^{188}Re-HAb18 F(ab')$_2$ was mainly eliminated by kidney. The maximal tumor to blood ratio was at 48h, and maximal tumor to liver ratio was at 18h.**

CONCLUTION: **The pharmacokinetics of ^{188}Re-HAb18 F (ab')$_2$ fit a l-compartment model.^{188}Re-HAb18 F(ab')$_2$ can be uptaken selectively at the hepatoma site.**

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INTRODUCTION

^{188}Re is a new radioisotope[1-16]. In the past,^{131}I was used as the main radioisotope for radioimmunotherapy(RAIT).^{131}I has its favour such as simple labeling, appropriate partical energy and path length,but the high energy of γ-ray produced harmness to the whole body, and β-energy(Emax,0.6MeV) was low[17-22]. So scientists have searched for more effective radioisotope. Rhenium-188 is of particular interest to this study as the ^{188}Re may be obtained from the ^{188}W/^{188}Re generator, and ^{188}Re decays by β- emission with energies (Emax=2.12MeV) similar to ^{90}Y and γ photons (Eγ=155keV; aboundance=15%) that are useful for dosimetry calculations and radioimmunoimaging, with a half-time of 17h. Furthermore, ^{188}Re has chemical properties similar to ^{99}Tc m, thus it can be conjugated to antibodies modeling on ^{99}Tc m labeling methods using direct or indirect method[23-29]. Direct methods require attaching the reduced form of Re to the endogenous thiols of antibodies, whereas indirect methods require the reduced Re to be complexed by a bifunctional chelator that is conjugated to the antibody[30-32]. There has been considerable interest in the direct labeling of mAb, which would result in the formation of an instant kit formulation for imaging or therapy.

^{188}Re can be provided at reasonable costs for routine preparation of radiopharmaceuticals for cancer treatment. ^{188}Re is an important therapeutic radioisotope which is obtained on demand as carrier-free sodium perrhentate by saline elution of the tungsten-188/rhenium-188 generator system.Because of its prominent physical characters, ^{188}Re will become a new therapeutic isotope.^{188}Re is a radioisotope currently under evaluation for a variety of therapeutic application, including that for metastatic bone pain and therapy in oncology.

The HAb18 antibody is a murine IgG_1 anti-hepatoma monoclonal antibody under investigation in our laboratory. It does not cross react with normal liver cells, and only rarely with other malignant tissues. Due to the smaller size, easier penetration into tumor tissues, rapid clearance from circulation, and less human anti-mouse antibody (HAMA) reaction, F(ab')$_2$ fragments showed that tumor localization is faster and better than the intact antibody. Previous studies of ^{99}Tcm labeled with HAb18 F(ab')$_2$ indicated that the conjugate is effective to detect hepatoma in the nude mice model[33]. The results encourage us to continue the radioimmunotherapy for hepatoma using ^{188}Re labeled with HAb18 F(ab')$_2$. we have studied the pharmacokinetics of ^{188}Re-HAb18 F(ab')$_2$ in hepatoma-bearing nude mice in order to prove if ^{188}Re-HAb18 F(ab')$_2$ was located specially in hepatoma, to establish the pharmacokinetical model and get the parameters of pharmacokinetics.

MATERIALS AND METHODS

Animals

Five-week Balb/c nude mice(derived from Experimental Animals Center of our university) were implated with 1×10^7(0.2mL) human hepatocellular carcinoma (HCC) cells in the right thigh. When the diameter of the tumors reached 1cm, the tumor bearing mice would be investigated further.

Monoclonal antibody fragment

HAb18 F(ab')$_2$ fragment was generated by pepsin digestion and phenyl-sepharose HP column purification with a relative molecular mass of 110,000. The solution containing the antibody fragment was concentrated by lypholization and reconstituted with distilled water.

Isotope

A 7.4GBq $^{188}W/^{188}Re$ generator was eluted with normal saline.

Radiolabeling

The antibody concentrated at 5g·L^{-1} was reduced by reaction with a molar excess of 2-ME at 4℃ for 20-30 min. The reduced antibody was isolated from reductant through a PD-10 column (Pharmacia) equilibrated with 0.05 mol·L^{-1} acetate-buffered saline.

For labeling, the reduced HAb18 F(ab')$_2$ was mixed with glucoheptonate (GH) solution, $SnCl_2$ solution, and 50-100μL perihenium solution for 2-3 h at 37℃ before it was analyzed by Whatman 3MM paper chromatography which was then developed in 100 g·L^{-1} trichloroacetic acid (TCA). R^a-f (distance of some composition moved/distance of extended reagent moved) values for 100 g·L^{-1} TCA are: mAb 0.0, ^{188}Re-GH 0.7, and ^{188}ReO-4 0.7. Labeled mAb was differentiated from ^{188}Re colloid by the method of Thrall *et al*[33]. The same strips impregnated with 10-20 g·L^{-1} human serum albumin before development with 5V:2V:1V; water: ethanol: 5 mol·L^{-1} NH_4OH(volum ratio). Colloid remained on the bottom of the strip while mAb-bound isotope migrated with the solvent front.

Immunoreactivity assessment

The *in vitro* immunoreactivity of the radiolabeled HAb18 F(ab')$_2$ was evaluated by a live cell assay[9]. Briefly, 5×10^9·L^{-1} HCC cells were centrifuged at 1000 r·min^{-1} for 5 min and washed twice with 1 g·L^{-1} bovine serum albumin (BSA) in PBS, then 5 serial 1:2 dilutions were made up in 10 g·L^{-1} BSA in Eppendorf tubes precoated with BSA. Radiolabeled HAb18 F(ab')$_2$ at a mass concentration of 40μg·L^{-1} in 10 g·L^{-1} BSA was added using a volume equal to half the volume of cell suspension. The total volume of cell-binding assay solution was 0.3 mL. After incubation for 2 h at 37℃, the total as well as the cell-bound radioactivity were counted in a gamma counter.

Study of biodistribution in nude mice

Fifteen hepatoma-bearing nude mice were divided into 5 groups randomly, the mice were tail-vein injected via tail vein with 1.85MBq^{188}Re-HAb18 F(ab')$_2$ in a volume of 0.1 mL and then they were sacrificed at 1, 4, 18, 24 and 48h (3 mice at each time). Samples of tumor, heart, liver, spleen, lung, kidney, large intestine, small intestine, muscle,bone were taken and weighed carefully. In addition, the blood sample was drawn from the heart. The radioactive concentrations in tissues were calculated and expressed as percent injected dose per gram(%ID·g^{-1}). The radioactivity of tumor/no tumor(T/NT) was also calculated.

Pharmacokinitics

The concentrations of blood and other organs were mounted by 3P97 software to get the parameters of pharmacokinetics and established the mode of pharmacokinetics was established.

RESULTS

Table 1 shows the biodistribution of ^{188}Re-HAb18 F(ab')$_2$. The blood concentration was measured by 3P97 software, which fits the 1-compartment model(Table 3). Figure 1 shows the curve of concentration-time in nude mice, and Table 2 shows the parameters of pharmacokinitics. The half-time(h) of each tissue was: tumor (32.99), blood (2.99), lung (5.67), bone (11.76), muscle (9.22), small intestine (7.47), large intestine (15.08), heart (2.29), liver (5.67), spleen (19.76),and kidney (11.53). Table 4 illustrates the influence of various concentrations of $SnCl_2$ and GH on the free $^{188}ReO_4^-$ amounts, colloid amounts and labeling efficiency. Optimal complexation with labeling efficiency of 91.7% was achieved in 0.8 mol·L^{-1} GH and 2 g·L^{-1} $SnCl_2$ solution. As shown in Figure 2, the immunoreactive fraction, 0.78 was determined by plotting the inverse of the bound fraction as compared with the inverse of the cell concentration, which is based on the assumption that the total antigen concentration (i.e., cell density) is a good approximation for the free antigen concentration.

Table 1 Biodistribution of ^{188}Re-HAb18 F(ab')$_2$ in hepatoma-bearing nude mice

Tissue	T(post-inj)/h	^{188}Re-HAb18 F(ab')$_2$	
		%ID·g-1($\bar{x}\pm s$)	T/NT ratio
Tumor	1	3.01±0.89	ND
	4	3.94±0.82	ND
	18	3.43±0.28	ND
	24	1.96±0.43	ND
	48	0.99±0.32	ND
Blood	1	4.58±0.63	0.66
	4	1.83±0.10	2.15
	18	0.21±0.04	16.30
	24	0.18±0.03	10.90
	48	0.05±0.01	19.80
Heart	1	1.60±0.38	1.88
	4	0.80±0.10	4.92
	18	0.36±0.03	9.53
	24	0.30±0.02	6.53
	48	0.21±0.03	4.71
Liver	1	2.07±0.40	1.45
	4	1.57±0.31	2.51
	18	0.77±0.12	4.45
	24	0.66±0.10	2.97
	48	0.47±0.13	2.11
Spleen	1	1.22±0.25	2.47
	4	0.91±0.22	4.33
	18	0.47±0.07	7.30
	24	0.45±0.08	4.36
	48	0.41±0.10	2.40
Lung	1	1.45±0.23	2.08
	4	0.86±0.29	4.58
	18	0.19±0.04	18.10
	24	0.18±0.04	10.90
	48	0.14±0.05	7.07
Kidney	1	59.81±14.52	0.05
	4	47.83±12.87	0.08
	18	18.72±4.94	0.18
	24	15.80±0.99	0.12
	48	7.31±2.10	0.13
Large intestine	1	1.36±0.38	2.21
	4	0.93±0.24	4.24
	18	0.57±0.06	6.02
	24	0.45±0.00	4.36
	48	0.18±0.03	5.50
Small intestine	1	1.61±0.43	1.87
	4	0.88±0.29	4.24
	18	0.33±0.05	10.40
	24	0.29±0.05	6.76
	48	0.13±0.05	7.62
Muscle	1	0.74±0.29	4.07
	4	0.44±0.12	8.95
	18	0.19±0.08	18.10
	24	0.16±0.06	12.25
	48	0.05±0.02	19.80
Bone	1	1.03±0.31	2.92
	4	0.68±0.12	5.79
	18	0.31±0.09	11.06
	24	0.27±0.02	7.26
	48	0.16±0.02	6.19

ND: not done

Table 2 Pharmacokinetic parameters of ^{188}Re-HAb18 F(ab')$_2$ in hepatoma-bearing nude mice

Parameter	Unit	Value	Standard error
C0	1×10^{9}Bq·L^{-1}	6.18	3.14E-01
Ke	h^{-1}	0.30	2.88E-02
Vd	1×10^{-9} L·Bq-1	1.49	
$T_{1/2}$(Ke)	h	2.29	
AUC	1×10^{9}Bq·h·L^{-1}	20.49	
CL	1×10^{-3}L·h^{-1}	0.45	

CO: Concentration at zero time Ke: Elimination rate constant
Vd: Apparent volume of distribution $T_{1/2}$: Half-life time
AUC: Area under the curve CL: Clearance

Table 3 Criteria for goodness of fitting for mean

REC No.	Mean No	WT	No. of camp	Weighted sum of squarers	R	R Squares	Goodness of fit	Max error C-CI	Max error %	AIC
1	1	1	1	0.672E-01	0.9993	0.9955	0.150	0.18	100.00	-9.499
2	1	1/c	1	0.329E+00	0.9856	0.9781	0.331	0.52	99.1	-1.560
3	1	1/cc	1	0.119E+01	0.9200	0.9207	0.630	3.58	78.2	4.877

Table 4 Effect of various concentration of $SnCl_2$ and GH on free $^{188}ReO^{-}_4$ amounts, colloid amounts and labeling efficiency

Concentration		$^{188}ReO^{-}_4$	Colloid	Labelingfficiency(%)
a$SnCl_2$ (g·L^{-1})	8	0.3	3.6	90.9
	4	0.4	2.8	90.1
	2	9.7	2.1	82.7
	1	21.8	1.2	71.2
bGH (mol·L^{-1})	0.8	1.1	1.1	91.7
	0.4	12.5	2.5	78.8
	0.2	16.6	2.8	72.7
	0.1	20.6	4.1	71.3

[a]Molar ratio of 2-ME: F(ab')$_2$ = 400:1,Concentration of GH=0.5 mol·L^{-1}
[b]Molar ratio of 2-ME: F(ab')$_2$ = 400:1,Concentration of $SnCl_2$=2 g·L^{-1}

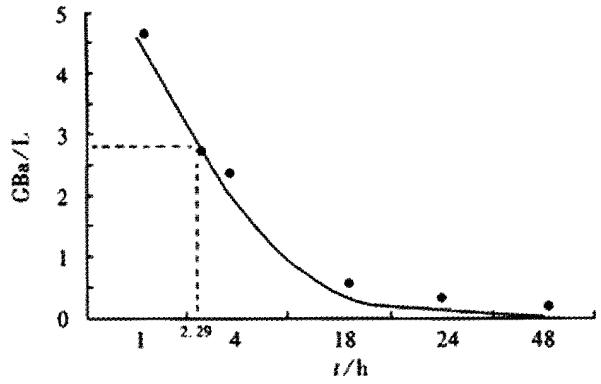

Figure 1 Concentration-time curve of ^{188}Re -HAb18 F(ab')$_2$ in nude mice

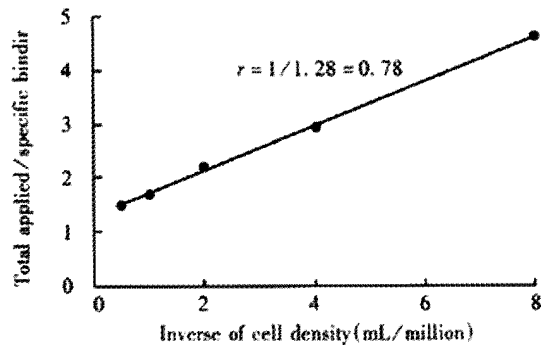

Figure 2 Binding assay for determination of immunoreactive fraction of ^{188}Re-labeled HAb18 F(ab')$_2$.

DISCUSSION

The occurrence of hepatoma is high in Southeast Asia, East Africa and Middle Africa. In China, hepatoma is one of the most three common cancers related death, but there is no effective treatment[34-45]. The therapy of hepatoma includes surgical operation, chemotherapy and radiotherapy. Targeting diagnosis and therapy of hepatoma with anti-hepatoma Mab have been developed quickly,giving a hopeful prospect to hepatoma treatment. Our reaserch focuses on the targeting therapy of hepatoma.[46-48]^{188}Re is a generator-produced radioistope which can be obtained. There were some studies on the biodistribution and pharmacokinetics of ^{188}Re-mAb. Safavy *et al*[49]have reported biodistribution of ^{188}Re-labelded trisuccin-HuCC49 and tisuccin-C49deltaCh2 conjugates in athymic nude mice bearing intraperitoneal coloncer xenografts.^{188}Re-labeled mAb was injected,and the mice were sacrificed 24h postinjection. Biodistribution of the radiolabeled mAb at 24h after injection showed median tumor uptake values of 23.5%ID·g^{-1} and 17. 6%ID·g^{-1} for the ^{188}Re-C49deltaCh2 and ^{188}Re-HuCC49, respectively. Yang *et al*[50] have prepared the conjugate of staphylococcal exterotoxin A(SEA) protein which is a bacterical Sag and the F(ab')$_2$ fragment of HAb18. The F(ab')$_2$ fragment of mAb HAb18 was prepared by papainic digestion method. The conjugate of mAb HAb18 F(ab')$_2$ fragment and SEA was prepared with chemical conjugating reagent N-succinimidy1-3-(2-pyirdyldithio) propionate (SPDP) and purified through chromatography column Superose 12 with FPLC system. The molecular mass was identified with SDS-PAGE assay,the antibody activity of in the conjugate was determined by indirect immunocytochemical ABC method. SEA is a protein, the method of labeling is indirect, SEA and antibody are conjugated by SPDP. ^{188}Re's labeling method is direct,it is more convienient and quicker than indirect method. In the animal experiment, ^{188}Re -HAb18F (ab')$_2$ can inhibit the growth of tumor, but the pharmacokinetics of ^{188}Re- HAb18F(ab')$_2$ in animal is seldom reported.

^{188}Re- HAb18F(ab')$_2$ can last a long time at a high level (Table 1). The maximal ratio of tumor: bLood was at 48h, and maximal ratio of tumor: liver was at 18h. From Table l, we can also find that after 1, 4, and 24 h(iv) injection, the radio percent of tumor is 3.83%, 6. 48%, and 9.74%, the liver is 1.64%,2.59% and 3.19%, the kidney is 76.24%, 78.8% and 76.3% respectively, showing that the antibody and its fragments were eliminated from kidney[51-52]. The half-time of ^{188}Re- HAb18 F(ab')$_2$ in the tumor was 32.99h, it was longer in tumor than that in other organs, this indicated that ^{188}Re- HAb18 F(ab')$_2$ was located in tumor, the rate of decay was low. It also showed that the mAb was specifically combined with tumor tissues and its harmness to normal tissues was low. Pharmacokinetic parameters (AUC, blood clearance, half-life, etc) were generated using the 3P97 software.

From 3P97 software, we can see the pharmacokinecs of conform to a 1-compartment model.Table 3 shows the criteria for goodness of fitting. we can judge the compartments from R squares, goodness of fit and AIC.1,1/C,1/C/C represented three weights.To the same weight,when the F test has marked significance (P<0.05 or P<0.01),we should choose the compartment of small AIC,and when the F test has not prominent significance (P>0.05),we should choose the small compartment. [53] From Table 3, it can be seen that the 1- compartment model is the best. ^{188}Re-HAb18 F(ab')$_2$ can distribute to the whole body instantly.The elimination rate was corresponded to the concentration of the drug. The higher the concentration was, the higher the speed of elimination was.

The half-time was 32.99h in tumor, being much longer than that in any other organs.It showed that ^{188}Re- HAb18 F(ab')$_2$ was located specifically in hepatoma and the elimination was low. It also showed the higher selective reactivity of HAb18 F(ab')$_2$ with hepatoma, the

harmness to other organs was small. The half-time was 2.29h in blood, and was 32.99h in tumor,the radioation of blood can decrease more rapidly than that of the tumor. The half-time of ^{188}Re was 17h, which was also lower than that in blood, so the ^{188}Re can be eliminated through the blood. It has excellent value in the clinical therapy.[54-62]

Carrier-free ^{188}Re is one of β emitting radionuclides recommended for RAIT because of suitable decay characteristics and availability from ^{188}W/^{188}Re generator. Some methods are reported in the literature for labeling mAb with ^{188}Re which imitate the labeling method of ^{99}Tcm. ^{188}Re eluted from generator will not bind to organic ligands without reduction to a lower oxidation state. We selected $SnCl_2$ as reductant and GH as transfer ligand and stablizer to avoid Sn- or Re-collide formation. Table 1 shows that the concentration of $SnCl_2$ and GH solutions is an important parameter to obtain good labeling results. The low percentage of free $^{188}ReO^-_4$ and radiocolloid shows that 0.8 mol·L^{-1} GH and 2g·L^{-1} $SnCl_2$ are the optimal values. Under these conditions, the labeled HAb18 F(ab')$_2$ keeps its immunoreactivity (Figure 2).

We believe that a variety of factors make ^{188}Re a potential alternative to other β-emitting radionuclides for RAIT. They include an efficient generator system and the direct labeling of IgG at high specific activity. The enchanced clearance of ^{188}Re-IgG from the circulation and the retention of immunoreactivity and tumortargeting of the Re-mAb conjugate are also important factors. In addition, the low-energy(155keV,15%) γ emission for imaging and the lack of accretion of metabolic products in nontarget tissues are important characteristics for further evaluation of ^{188}Re-labeled antibodies for tumor therapy.

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