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Tissue distribution of recombinant human tumor necrosis factor α derivative in mice

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KEY WORDS tumor necrosis factor; tissue distribution; receptor; lung; heart-lung perfusion

ABSTRACT

AIM: To study the tissue distribution and its mechanism of a new recombinant tumor necrosis factor alpha derivative (rhTNFαDa) in mice. **METHODS**: ^{125}I-rhTNFαDa was prepared by Iodogen method. Tissue distribution of ^{125}I-rhTNFαDa in mice was studied by determining radioactivity of tetrachloroacetic acid (TCA)- precipitable fraction in tissues. The isolated heart-lung perfusion study using ^{125}I-rhTNFαDa perfusate was carried out to study the distribution characteristics of ^{125}I-rhTNFαDa in lung. **RESULTS**: Except for thyroid, AUC of the TCA-precipitable ^{125}I-rhTNFαDa in tissues was highest in lung, which was 12.2-fold of that in serum, while concentrations in other tissues were all lower than that in serum. Perfusion study *in vitro* revealed that the concentration of radio-labeled peptide in lung was higher than that in perfusate. On the contrary, level in heart was much lower than that in perfusate. The overall distribution of ^{125}I-rhTNFαDa in lungs showed rapidly equilibratory, dose-dependent, saturable, competitive, and highly affinitive, with K_d 47.6 pmol·L^{-1} and B_{max} 348 fmol·g^{-1} (lung tissue). **CONCLUSION**: The specific distribution of rhTNFαDa in lungs was its distinctive characteristics.

INTRODUCTION

Tumor necrosis factor alpha (TNFα) showed anti-tumor effects in some tumors[1], but its systemic toxicity limited its application in human[2]. Recently, many studies focused on its structure-activity relationship and several TNF mutants showed some advantages over the wild-type TNFα[3]. A new rhTNFα derivative (rhTNFαDa) was produced with deletion of N-terminal 1 - 7 amino acids and replacement of Pro^8, Ser^9, Asp^{10}, Ala^{157} with Arg, Lys, Arg, Phe[4]. Its anti-tumor activities increased both *in vitro* and *in vivo*, and systemic toxicity decreased as compared with recombinant TNFα. The present study involved the tissue distribution of this mutant in mice. Most of the peptide and protein drugs were mainly distributed in blood and interstitial spaces[5]. The researches concerned with the specific distribution of peptide drugs were very limited. The underlying mechanism of distribution of cytokines was still unclear. The present experiment was to study the tissue distribution of rhTNFαDa and its mechanism.

MATERIALS AND METHODS

Materials [^{125}I]NaI (581 kBq·g^{-1} iodine) was from Amersham. Iodogen was synthesized by Prof LI De-You (Institute of Pharmacology and Toxicology, Beijing). All other reagents were of AR (Beijing Chemical Industry Factory). L929 cell line was got from the Chinese National Institute for the Control of Pharmaceuticals and Biological products. RhTNFαDa [purity > 95 %, specific activity 220 GU·g^{-1} (protein) assayed by L929 cell cytotoxicity] was provided by Wenzhou Fudan Biotechnology Co Ltd, Wenzhou, China. LACA mice (Certificate No 01-3025) were from Animal Center of Academy of Military Medical Sciences.

Radiolabeling and identification of ^{125}I-rhTNFαDa ^{125}I-rhTNFαDa was prepared by Iodogen method and purified by Sephacryl S-200HR gel

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(Pharmacia). The steps were similar to the preparation of ^{125}I-rhGCSF[6]. The radioactive purity of the purified ^{125}I-rhTNFαDa was identified by reversed phase high performanced liquid chromatography (RP-HPLC) and bioactivity was assayed by L929 cell bioassay[7]. The ^{125}I-rhTNFαDa with a specific activity of 577.2 GBq · g^{-1} (protein) and bioactivity similar to unlabeled peptide were used in the study.

Tissue distribution of ^{125}I-rhTNFαDa *in vivo* Forty-two mice were randomly assigned to 7 groups and each group contained 3 ♂ and 3 ♀. Each mouse was iv ^{125}I-rhTNFαDa 25 μg · kg^{-1} (204 MBq). Mice were bled at 0.17, 0.67, 2, 4, 8, 24, and 72 h. Serum was obtained after centrifugation at 10 000 × *g* for 5 min. Tissues were homogenized, weighed, and precipitated by adding equal volume 20 % (wt/vol) of tetrachloroacetic acid (TCA). The gamma radioactivity of TCA-precipitable fraction and total sample was counted separately.

Perfusion of heart-lung preparation *in vitro* Male mice weighing 24 – 26 g were heparinized and anesthetized by ketamine. The superior and inferior venae cava were ligated. A saline-filled cannula was inserted into aorta and ligated for outflow port. Another cannula was inserted and ligated at right auricle for inflow port. The isolated heart-lung preparation was washed by normal saline for 5 min at first, then perfused with saline perfusate containing 0.2 % bovine serum albumin and ^{125}I-rhTNFαDa (with or without 1000-fold unlabeled peptide) through the inflow port at flow rate of 0.5 mL · min^{-1}. Outflow was collected with 1 mL per tube. Gamma radioactivity in outflow was counted and standardized to percentage of inflow concentration. The lungs and heart were dissected and washed after 10-min perfusion. Uptake of ^{125}I-rhTNFαDa by heart and lungs was counted separately.

RESULTS

Distribution of TCA-preciptable ^{125}I-rhTNFαDa radioactivity in serum and tissues ^{125}I-rhTNFαDa identified by RP-HPLC in serum samples revealed that percentages of ^{125}I-hydrophilic degradation products in serum total radioactivity increased rapidly from 15 % to 74 % within 1 h. Comparison of serum TCA-soluble radioactivity with ^{125}I-degradated radioactivity demonstrated that the ^{125}I-hydrophilic degradation products were mainly in TCA-supernatant. The levels of TCA-preciptable radioactivity in most tissues after iv bolus injection were lower than that in serum. AUC_{tissue} to AUC_{serum} ratio in the last column was related to the partition coefficient between tissue and serum. Except for thyroid tissue, which had high affinity to iodine, the ratio for lungs was 12.2, which was the highest among other tissues observed. There was a specific high affinity of ^{125}I-rhTNFαDa to lungs (Tab 1).

Distribution of ^{125}I-rhTNFαDa after heart-lung perfusion *in vitro* The radioactivity levels in outflow rapidly dropped to 70 % at 2 min after perfusion, and gradually recovered to a stable level approximately to inflow level within 10 min (Fig 1).

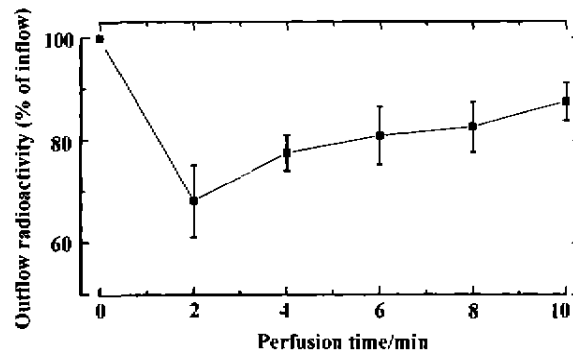

Fig 1. Mouse heart-lung preparation was perfused with perfusate containing ^{125}I-rhTNFαDa.

The results demonstrated a time-dependent binding of ^{125}I-rhTNFαDa by the preparation. The distribution of ^{125}I-rhTNFαDa in heart and lungs after various concentrations of ^{125}I-rhTNFαDa with or without 1000-fold unlabeled rhTNFαDa after perfusion was listed in Tab 2, respectively. The binding of ^{125}I-rhTNFαDa to lung and heart was remarkably different. The concentrations in lungs after perfusion with ^{125}I-rhTNFαDa 54, 177, and 502 pmol · L^{-1} were 7.4, 5.2, and 3.7-fold of those in inflow perfusate, respectively. On the contrary, concentrations in heart were only 0.50, 0.44, and 0.46-fold, respectively. The results were in accordance with those observed *in vivo*. The distribution of ^{125}I-rhTNFαDa in lungs with unlabeled rhTNFαDa was lower than that without unlabeled rhTNFαDa, while the uptake of ^{125}I-

Tab 1. Tissue distribution profiles of TCA-precipitable radioactivities [MBq·g^{-1}(wet tissue)] after iv bolus of ^{125}I-TNFαDa 25 μg·kg^{-1}. n = 6 mice (3♂, 3♀); at 72 h, n = 3. $\bar{x} \pm s$. $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* serum.

Tissues	10 min	40 min	2 h	4 h	8 h	24 h	72 h	AUC/ kBq·h^{-1}·g^{-1}(tissue)	AUC_{tissue}/ AUB_{serum}
Femur marrow	0.04±0.04	0.02±0.01	0.005±0.002	0.002±0.003	0.0006±0.001	0.0004±0.0006	0.00±0.00	-	-
Serum	25±14	16±5	4.4±1.8	2.5±1.2	1.0±0.6	0.6±0.5	-	51.4	1.00
Brain	0.5±0.5^{c}	0.3±0.1^{c}	0.12±0.06^{c}	0.1±0.1^{c}	0.03±0.02^{c}	0.1±0.1^{b}	0.06±0.02	2.0	0.03
Muscles	0.8±0.3^{c}	0.8±0.2^{c}	0.3±0.1^{c}	0.11±0.03^{c}	0.07±0.02^{c}	0.04±0.04^{b}	0.06±0.03	2.8	0.05
Thymus	0.9±0.5^{c}	0.9±0.5^{c}	0.6±0.4^{c}	0.2±0.1^{c}	0.06±0.03^{c}	0.04±0.05^{b}	0.01±0.02	3.5	0.06
Fat	1.0±0.6^{c}	1.0±0.4^{c}	0.5±0.2^{c}	0.3±0.2^{c}	0.1±0.1^{c}	0.05±0.02^{b}	0.06±0.05	4.4	0.08
Testes	0.5±0.4^{c}	1.1±0.2^{c}	0.8±0.3^{c}	0.4±0.1^{c}	0.08±0.04^{c}	0.05±0.05^{b}	0.05	4.9	0.09
Intestinal content	1.6±0.8^{c}	1.8±1.0^{c}	0.7±0.3^{c}	0.6±0.4^{c}	0.2±0.1^{c}	0.07±0.08^{b}	0.04±0.01	7.6	0.14
Heart	3.2±1.6^{c}	2.8±0.8^{c}	0.8±0.2^{c}	0.3±0.1^{c}	0.1±0.1^{c}	0.1±0.1^{b}	0.07±0.05	7.9	0.15
Duodenum	2.3±1.2^{c}	2.6±1.1^{c}	1.3±0.8^{c}	0.5±0.3^{c}	0.2±0.1^{c}	0.08±0.07^{b}	0.07±0.05	9.1	0.17
Ovaries	3.7±0.4^{c}	3.7±1.7^{c}	2.1±1.0^{b}	0.17±0.02^{c}	0.2±0.1^{c}	0.03±0.04^{b}	0.02±0.0	10.6	0.20
Mesenteric lymphnodes	1.7±0.8^{c}	4±6^{c}	1.1±0.6^{c}	0.5±0.2^{c}	0.2±0.1^{c}	0.10±0.07^{b}	0.05±0.02	10.3	0.20
Bladder	0.9±0.3^{c}	1.4±0.5^{c}	1.1±1.2^{c}	0.4±0.3^{c}	0.7±1.5	0.04±0.07^{b}	0.04±0.03	12.3	0.23
Gallbladder and bile	1.7±0.8^{c}	4.4±2.6^{c}	1.5±1.0^{c}	1.1±0.7^{b}	0.3±0.2^{b}	0.02±0.04^{b}	0.4±0.5	13.7	0.26
Adrenal glands	13±25	9±11	2.5±2.0	0.6±0.1^{c}	0.2±0.1^{c}	0.1±0.1^{b}	0.05±0.05	21.5	0.41
Feces in colon	0.2±0.2^{c}	0.8±0.4^{c}	0.9±0.5^{c}	1.6±0.5	1.3±0.8	0.4±0.3	0.2±0.2	23.1	0.44
Spleen	16±10	5.2±1.2^{c}	1.6±1.0^{c}	1.3±0.2^{b}	0.4±0.2	0.2±0.1	0.1±0.1	28.2	0.54
Kidneys	26±11	12.3±4.0	3.2±0.8	1.1±0.3^{b}	0.5±0.2	0.4±0.2	0.2±0.1	36.4	0.70
Liver	28±11	10.4±1.6^{b}	4.6±1.0	1.8±0.4	0.5±0.2	0.4±0.4	0.3±0.2	40.7	0.79
Lungs	41±25	100±32^{c}	60±47^{b}	44±25^{c}	11±65^{c}	22.4±7.3^{c}	11±17	628	12.21
Thyroid	18±10	119±72^{c}	942±901^{b}	1746±1424^{b}	1083±328^{c}	499±341^{c}	538±493	21758	423

rhTNFαDa in heart had no difference with or without unlabeled rhTNFαDa in perfusate. K_d 47.6 pmol·L^{-1} and B_{max} 348 fmol·g^{-1}(lung tissue) were obtained by Scatchard plot (Tab 2).

DISCUSSION

It is unclear about the distribution characteristics of cytokines when they were given pharmacologically. They have different specific biological activities and most of them are autocrine or paracrine, we infer that there must be some different distribution profiles for different bioactive proteins or peptides. In this paper we found that the concentration of rhTNFαDa in lungs was 12-fold of that in serum *in vivo*. The perfusion experiment of heart-lung preparation by various concentrations of ^{125}I-rhTNFαDa with or without 1000-fold unlabeled rhTNFαDa revealed that there was high affinity of ^{125}I-rhTNFαDa from the perfusate in lungs but not in heart. The distribution of ^{125}I-rhTNFαDa in lungs showed rapidly equilibratory, dose-dependent, saturable, competitive, and highly affinitive. The K_d was approximate to that reported (19 pmol·L^{-1})[8]. Therefore we assumed that there might involve a receptor-mediated mechanism in the distribution of this cytokine both *in vivo* and *in vitro*. The significantly high affinity with specific tissue might relate to the physiological function of the cytokine and the potential therapeutic application of the peptide in diseases. The specific distribution was also seen in other peptides. For example, the content of erythropoietin in bone marrow was 2-fold higher than that in serum[9] and high concentration of neuro-growth factor occurred in the sympathetic ganglia[10]. Also, The receptor-mediated mechanism might take a part role in the non-linear pharmacokinetics of TNFα. Our experiment showed

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Tab 2. Distribution of ^{125}I-rhTNFαDa in lungs and heart after 10-min heart-lung perfusion by ^{125}I-rhTNFαDa with or without 1000-fold unlabeled rhTNFαDa in the perfusate. $x \pm s$. $^{a}P < 0.01$ *vs* ^{125}I-rhTNFαDa 54 and 502 pmol · L^{-1}, respectively; $^{b}P < 0.01$ *vs* Col 1.

	^{125}I-rhTNFαDa in perfusate/pmol·L^{-1}		
	54	177	502
n	6	2	2
^{125}I-rhTNFαDa in lungs (pmol·g^{-1})			
Col 1 (without unlabeled)	0.40 ± 0.08	0.93 ± 0.04[a]	1.88 ± 0.02
Col 2 (with unlabeled)	0.21 ± 0.08[b]	0.66 ± 0.04[b]	1.55 ± 0.26[b]
Col 1 − Col 2 (competable)	0.19	0.27	0.32
^{125}I-rhTNFαDa in heart (pmol·g^{-1})			
Col 1 (without unlabeled)	0.027 ± 0.018	0.078 ± 0.016[a]	0.233 ± 0.011
Col 2 (with unlabeled)	0.019 ± 0.008	0.084 ± 0.025	0.239 ± 0.076
Col 1 − Col 2 (competable)	0.009	−0.006	−0.005

that the dosage ratio was 1.0∶2.7∶6.1, while the AUC ratio was 1.0∶4.4∶12.8, which was not increased proportionally with dose increasing. There were also similar results in other reports as well[11,12].

In summary, the present experiments showed that there was an extraordinary high concentration of ^{125}I-rhTNFαDa in lungs and a receptor-mediated mechanism might be involved in this process.

REFERENCES

1 Old LJ. Tumor necrosis factor (TNF). Science 1985; 230: 630 − 2.
2 Spriggs DR, Sherman ML, Michie H, Arthur KA, Imamura K, Wilmore D, *et al*. Recombinant human tumor necrosis factor administered as a 24-hour intravenous infusion. A phase Ⅰ and pharmacologic study. J Natl Cancer Inst 1988; 80: 1039 − 44.
3 Van Ostade X, Tavernier J, Fiers W. Strucure-activity studies of human tumor necrosis factors. Protein Eng 1994; 7: 5 − 22.
4 He XL, Chang JL, Cai WC, Zhao SY, Lu CL, Zhu HN. Genetic-engineered derivatives of the human tumor necrosis factor. Acta Biochim Biophys Sin 1995; 27: 67 − 73.
5 McMartin C. Pharmacokinetics of peptides and proteins: opportunities and challenges. In: Testa B, editor. Advances in drug research; v 22. London; Academic Press; 1992. p 54 − 75.
6 Liu XW, Tang ZM. Pharmacokinetics of recombinant human granulocyte colony-stimulating factor in rabbits and mice. Acta Phamacol Sin 1997; 18: 44 − 8.
7 Matthews N, Neale ML. Cytotoxicity assays for tumour necrosis factor and lymphotoxin. In: Clemens MJ, Morris AG, Gearing AJH, editors. Lymphokines and interferons: a practical approach. Oxford: IRL Press; 1987. p 221 − 3.
8 Grell M, Wajant H, Zimmermann G, Scheurich P. The type 1 receptor (CD120a) is the high-affinity receptor for soluble tumor necrosis factor. Proc Natl Acad Sci USA 1998; 95: 570 − 5.
9 Kinoshita H, Ohishi N, Tokura S, Okazaki A. Pharmacokinetics and distribution of recombinant human erythropoietin in rats with renal dysfunction. Arzneimittelforschung 1992; 42: 682 − 6.
10 Angeletti RH, Aneletti PU, Levi-Montalcini R. Selective accumulation of ^{125}I-labelled nerve growth factor in sympathetic ganglia. Brain Res 1972; 46: 421 − 5.
11 Zahn G, Greischel A. Pharmacokinetics of tumor necrosis factor alpha after intravenous administration in rats. Arzneimittelforschung 1989; 39: 1180 − 2.
12 Greischel A, Zahn G. Pharmacokinetics of recombinant human tumor necrosis factor alpha in rhesus monkeys after intravenous administration. J Pharmacol Exp Ther 1989; 251: 358 − 61.

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基因重组人肿瘤坏死因子α衍生物在小鼠组织中的分布

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关键词 肿瘤坏死因子; 组织分布; 受体; 肺; 心-肺灌流

目的: 研究基因重组人肿瘤坏死因子衍生物α (rhTNFαDa)的组织分布及其机制. **方法**: 用Iodogen法制备^{125}I-rhTNFαDa, 测定在小鼠全身组织的分布; 用离体心肺灌流研究^{125}I-rhTNFαDa在肺组织的分布机制. **结果**: 除甲状腺组织外, ^{125}I-rhTNFαDa的组织浓度-时间曲线下面积在肺组织中最高, 为血清的12.2倍; 离体心肺灌流显示^{125}I-rhTNFαDa在肺中的浓度高于灌流液3.7 − 7.4倍, 而心脏组织低于灌流液. ^{125}I-rhTNFαDa在肺中分布具有时间依赖性, 剂量依赖性, 可竞争性, 和高亲和性特征, K_d 为47.6 pmol·L^{-1}, B_{max} 为348 fmol·g^{-1}(肺组织). **结论**: ^{125}I-rhTNFαDa在肺组织中有特异性的高分布, 此过程可能有受体介导.

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