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Pharmacokinetics and tissue distribution of human recombinant interleukin-2 in mice

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ABSTRACT ^{125}I-labeled human recombinant interleukin-2 (^{125}I-rIL-2) was prepared by iodogen method with rIL-2 and Na^{125}I. Product was purified by Sephacryl S-200 gel filtration. Eluate fractions were identified by SDS-PAGE and compared with standard rIL-2. Radioactive 95 % purified ^{125}I-rIL-2 fractions were selected for pharmacokinetic study, with a specific activity of 56 PBq · mol^{-1}. Concentration-time curves after iv 75, 530, 603, and 6767 ng of ^{125}I-rIL-2/mouse were fitted to a 3-compartment model; with a fast distribution phase $T_{1/2}$ of 2 min, a slow distribution phase $T_{1/2}$ of 30—120 min, and a terminal elimination $T_{1/2}$ of 6 — 15 h. AUC was linearly related to the dosage (r = 0.9998). Systematic clearances were independent of the dosages. SDS-PAGE of plasma and urine samples showed that radioactivities due to ^{125}I-rIL-2 were 81±13 % (n=16) and 91±8 % (n=3, at 4 h), respectively. Levels of ^{125}I-rIL-2 after im were lower than those after iv, with bioavailability of 0.57. Time to peak concentration was about 1.1 h. The highest levels were seen at 15 min after iv in liver, bile and kidneys, the concentration gradients were blood > adrenals > plasma > lungs > thyroid > spleen > jejunum > mesenteric lymph nodes > jejunum contents > ovaries > heart > bladder > thymus > feces in colon > thigh skeletal muscle > testes > brain > fat. Peak concentration time in most tissues were found at 15 min, but at 4 h in the feces. About 80 % of the injected dose was excreted within the first 24 h, only 5 % excreted in the second day.

KEY WORDS interleukin-2; pharmacokinetics; tissue distribution; mice

Interleukin-2 (IL-2) is a 15-kDa glycoprotein produced by activated T cells. *In vivo* it represents the second signal in lymphocyte mitogenesis, being of prime importance in the immune response. IL-2 stimulates the lymphocyte transformation into lymphokine-activated killing or LAK cells, which lyse the tumor cells but not normal cells. Clinical treatment including IL-2 may become the therapy of choice for tumors[1,2] and human acquired immunodeficiency virus (HIV). In this paper pharmacokinetics and tissue distribution of recombinant IL-2 (rIL-2) were studied in mice, which were part of the preclinical studies of rIL-2 produced in our Academy.

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MATERIALS AND METHODS

Iodogen, synthesized by Prof LI De-Yu, Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences (AMMS); Na^{125}I, 0.74 GBq · ml^{-1} (Department of Isotopes, China Institute of Atomic Energy); rIL-2 produced by the Biotechnology Inst, AMMS[2], No 9003, purity > 98 %, 2 mg · ml^{-1}; Sephacryl S-200 (Pharmacia); other reagents, analytic grade (Beijing Chemical Industry Factory); γ counter (CLINIGAMMA, Pharmacia).

Iodogen 50 μg in 100 μl chloroform was transferred to a test tube and blown to dryness by N_2. After adding rIL-2 500 μg and Na^{125}I 0.37 GBq, shaking at 15 °C for 30 min, the product was applied on S-200 gel column maintained at 25 °C in a warer jacket. Gel filtration condition: column 10 mm × 470 mm, eluent: 0.05 % sodium dodecyl sulfate phosphate buffer solution 0.05 mol · L^{-1}, pH 7.0. Eluted fractions were detected by a γ counter. ^{125}I-rIL-2 was identified by comparing the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)[3] behavior of ^{125}I-rIL-2 with those of standard rIL-2 and molecular markers. After stained by Coomassie blue, cut out the ^{125}I-rIL-2 labeled lane and cut into 2.5-mm pieces for γ counting. The most purified fraction was selected for pharmacokinetic study.

LACA mice (the Animal Center of AMMS), age 70 — 90 d. ♂ 22 ± 3 g, ♀ 20 ± 2 g. Purified ^{125}I-rIL-2 was diluted by unlabeled rIL-2. 75, 530, 603, or 6767 ng/mouse was iv injected in 0.05 ml. Blood was obtained at 1, 5, 10, 15, 30 min, 1, 1.5, 2, 4, 8, and 24 h from post-ocular sinus venosus or femoral artery. Three samples were taken from each mouse, and each time consisted of samples from 5 to 6 mice. After centrifugation, plasma about 100 μl was detected by γ counter. Radioactivity of injected ^{125}I-rIL-2 was determined simultaneously for calibration. Pharmacokinetics after im 530 ng/mouse was compared with that at the same iv dose. Parameters were estimated by pharmacokinetic program 3P87.

Sixteen plasma samples collected at 15 min to 4 h and 3 urine samples collected at 4 h were analyzed by SDS-PAGE. The radioactivities corresponding to ^{125}I-rIL-2 were estimated according to electrophoresis behavior of rIL-2. Sample 7 — 10 μl was applied on gel for SDS-PAGE. Sensitivity for identification of ^{125}I-rIL-2 in plasma by SDS-PAGE was 40 ng·ml^{-1}.

Tissue distribution of ^{125}I-rIL-2 was studied after iv 603 ng/mouse. Each time consisted of 3 ♂ and 3 ♀ mice. At 0.25, 0.5, 2, 4, 8, 24, and 48 h the mice were killed by severing femoral artery. Samples from blood, urine, various organs and tissues were taken. Radioactivities were expressed as dpm/mg wet tissue. The levels in tissues were compared *vs* those in plasma by *t* test.

Mice ♂ and ♀ 3 each after iv ^{125}I-rIL-2 603 ng/mouse were kept individually in metabolic cages. Urine was absorbed on filter paper. At 8, 24, 32, and 48 h urine and faces were collected for γ counting.

RESULTS

Purity of prepared ^{125}I-rIL-2 Radioactive gel filtration profile indicated that complete separation of ^{125}I-rIL-2 with Na^{125}I, which were eluted at 18.7 — 22.8 ml and 109 — 217 ml, respectively. Commassie profile of ^{125}I-rIL-2 fraction appeared as a single band, its electrophoretic behavior and molecular weight were the same as those of standard rIL-2. SDS-PAGE radioactive profiles of fraction 13, 14, 15, and 16 indicated that radioactivities of rIL-2 were 87 %, 95 %, 95 %, and 91 %, respectively. 95 % purified fractions were selected for pharmacokinetic study. The estimated specific activity was 56 PBq·mol^{-1}.

Pharmacokinetics of iv ^{125}I-rIL-2 Concentration-time curves after iv of 75, 530, 603, and 6767 ng/mouse were best fitted with 3-compartment model. The fast and slow distribution phase $T_{1/2}$ were < 2 min and 0.5 — 2 h, respectively. The terminal elimination $T_{1/2}$ was 6 — 15 h. Area under curves (AUC) showed a linear relationship with the dosages ($r = 0.9998$, $n = 4$, $P < 0.01$). Systematic clearances (Cl) were similar in different doses.

Tab 1. Pharmacokinetic parameters of iv ^{125}I-rIL-2 in mice. $n = 5 — 6$.

Dose (ng/mouse)	75	530	603	6767
$T_{1/2\alpha}$/h	0.030	0.012	0.024	0.007
$T_{1/2\beta}$/h	2.05	0.114	0.55	0.43
$T_{1/2\beta}$/h	9.4	4.4	6.0	14.8
K_{12}/h^{-1}	17.8	32.3	20.0	78.5
K_{21}/h^{-1}	3.3	11.3	4.3	9.7
K_{13}/h^{-1}	0.63	17.2	3.8	12.0
K_{31}/h^{-1}	0.13	1.6	0.65	0.32
K_{10}/h^{-1}	1.27	2.48	1.49	2.59
V_c/ml	0.95	0.50	0.65	0.35
Cl/ml·h^{-1}	1.21	1.25	0.96	0.88
AUC/ ng·h·ml^{-1}	61.8	424.2	626.2	7 692.5

Results of SDS-PAGE analysis Plasma samples collected during 15 min to 4 h showed that ^{125}I-rIL-2 was 81 ± 13 % ($n = 16$) of

the total radioactivities of plasma. Urine was 91 ± 8 % (n = 3, collected at 4 h). Radioactivities of plasma samples collected longer than 4 h were too low to be detected in SDS-PAGE analysis.

Pharmacokinetics of im ^{125}I-rIL-2 Plasma levels after im were remarkably lower than those after iv. Concentration-time data were best fitted with first order absorption one-compartment model. Maximal concentration (C_{max}) was 19 ± 2 ng·ml^{-1}, time to peak (T_{peak}) was 1.1 h. Elimination $T_{1/2}$ was the same as that after iv. The estimated bioavailability (f) was 0.57 (Tab 2, Fig 1).

Tab 2. Pharmacokinetic parameters of im ^{125}I-rIL-2 530 ng/mouse in mice. n = 5 − 6.

Parameters	Values
$T_{1/2Ke}$/h	9.22
$T_{1/2Ka}$/h	0.21
T_{peak}/h	1.17
C_{max}/ng·ml^{-1}	16.8
AUC/ng·h·ml^{-1}	243.8
f*	0.57
V_c† /ml	16.7
Cl_t† /ml·h^{-1}	1.26

* *vs* AUC of iv 530 ng/mouse.
† after correction for bioavailability.

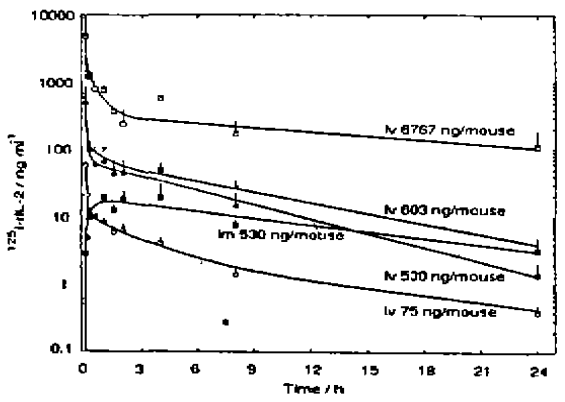

Fig 1. ^{125}I-rIL-2 concentrations in plasma after iv 75, 530, 603, and 6767 ng/mouse *vs* im 530 ng/mouse. n = 5 − 6. $\bar{x}\pm s$.

Tissue distribution of ^{125}I-rIL-2 Results were shown in Tab 3. At 15 min after iv, the highest levels were found in liver, bile, and kidneys, which were significantly higher than those in plasma ($P<0.01$). The concentration gradient of other tissues were blood > adrenals > plasma > lungs > thyroid > spleen > jejunum > mesenteric lymph nodes > jejunum contents > ovaries > heart > bladder > thymus > feces in colon > thigh skeletal muscle > testes > brain > fat. Levels in liver decreased more rapidly than those in plasma. At 4 h they were lower than that in plasma. On the contrary, levels in bile, which were higher than those in plasma at 2, 8, and 24 h, decreased much slower. Radioactivities in feces higher than those in plasma, reached the peak at 4 h. Levels in kidneys decreased more rapidly. Urine sample collected at 4 h was the highest among all. Levels in cerebral cortex and fat were the lowest, which were near the background level, indicating that there may be blood-brain barrier for ^{125}I-rIL-2, or the distribution in hydrophobic tissues had to be relatively low. Radioactivities in the lympho-immune tissues were not high, but decreased slowly and maintained for a relatively long time (Tab 3).

Radioactivities in thyroid gland at 15 min was lower than that in blood, equivalent to 0.6 ± 0.3 % of the injected dose. They were increased remarkably at 4 h. At 24 and 48 h the levels remained much higher than those in other tissues, being 16 ± 8 % and 18 ± 8 % of the injected dose, respectively.

Excretion of ^{125}I-rIL-2 in urine and feces A total of 80 ± 21 % of the injected dose was excreted in the first 24 h, in which 70 ± 19 % through urine and 10 ± 5 % through feces. Excretion in the next 24 h was 6 ± 2%, in which 4 ± 2 % through urine and 2 ±1 % through feces. Total recovery of injected radioactivities, including those retained

Tab 3. Distribution of radioactivities in tissues after iv ^{125}I-rIL-2 603 (1115×10^4 dpm)/mouse. Each time point ♂ n = 3, ♀ n = 3; $\bar{x}\pm s$; $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* plasma.

	Radioactivities in tissues (dpm/mg wet tissue) after iv						
	0.25 h	0.5 h	2 h	4 h	8 h	12 h	24 h
Plasma	2 455±1 140	1 852±477	1 058±580	1 133±595	547±333	82±53	25±7
Blood	3 315±370^a	2 083±680^a	1 398±340^a	1 045±317^a	525±197^a	137±52^a	27±20^a
Heart	1 008±205^b	928±177^c	503±53^b	402±92^b	213±73^b	25±8^b	10±3^c
Lungs	2 225±993^a	1 807±777^a	1 125±533^a	897±195^a	400±131^a	50±10^a	23±7^a
Spleen	1 895±365^a	1 470±248^a	665±117^a	680±357^b	402±315^a	52±23^a	47±57^a
Mesenteric lymph nodes	1 473±488^b	873±410^c	710±225^a	500±78^b	247±110^a	12±7^c	3±5^c
Thymus	855±167^c	913±250^c	498±188^b	538±197^b	247±102^a	10±7^c	10±8^c
Thigh muscle	382±40^c	307±92^c	200±48^b	185±88^c	113±67^b	7±2^c	3±1^c
Brain	162±48^c	105±20^c	52±12^c	43±20^c	23±12^c	3±2^c	7±3^c
Fat	158±42^c	93±58^c	101±108^c	172±38^c	87±68^c	10±3^c	3±2^c
Adrenals	2 807±1 182^a	1 070±780^a	600±367^a	387±50^b	370±335^a	23±2^b	7±8^c
Testes	262±105^c	340±258^c	308±167^c	462±63^a	167±152^c	17±7^b	7±5^c
Ovaries	1 053±237^b	728±258^c	432±88^b	302±142^a	168±40^a	8±5^b	12±17^c
Liver	6 720±2 097^a	3 007±900^b	820±217^a	500±180^b	327±88^a	78±12^a	60±13^c
Bile + gallbladder	6 475±3 681^b	5 817±5 870^a	4 450±2 850^b	1 865±1 276^a	1 190±465^b	70±27^a	97±52^c
Jejunum	1 482±387^a	1 103±140^c	978±755^a	665±267^a	333±157^a	22±7^b	20±13^a
Content in jejunum	1 403±370^a	1 118±378^b	1 458±1 683^a	1 227±1013^a	353±243^c	18±3^b	18±10^a
Feces in colon	680±370^c	700±342^c	820±265^a	2 077±863^b	1 637±1 393^a	148±165^a	68±42^b
Kidneys	4 453±1 388^b	2 243±808^a	977±337^a	790±242^a	432±148^a	70±15^a	53±5^a
Bladder	932±300^b	1 160±241^c	738±190^a	538±168^b	327±160^a	12±3^c	3±2^c
Urine	–	–	–	16 453±10 540^c	13 452±4 736^c	–	–
Thyroid	2 059±914^a	–	–	61 517±57 459^c	–	104 912±52 456^c	123 213±60 475^c

in thyroid, was 103 ± 24 %.

DISCUSSION

Pharmacokinetics and tissue distribution of bioactive protein become new research topics, having not yet been studied comprehensively[4]. Assay of low level of protein was difficult. Bioassay of rIL-2 had been used in pharmacokinetic study of rIL-2[3], a rapid drop immediately after an iv bolus could be detected, but the elimination phase was beyond the sensitivity of the assay[6]. Bioassay could not differentiate rIL-2 from endogenous IL-2 or other interferent. In this paper we used ^{125}I-labeled rIL-2 and SDS-PAGE analysis to overcome the difficulty, and the sensitivity and specificity of the assay were satisfactory.

Preparation of highly purified labeled rIL-2 is prerequisite for a reliable study. In a pilot study, there was an ^{125}I-labeled protein, whose electrophoresis behavior was different from rIL-2 in G-50 gel filtration eluate. In order to remove it, we used highly purified rIL-2, S-200 gel filtration, SDS-PAGE identification, and then the most purified fraction was selected. The resulting product was 95 % purified. The contaminated labeled protein was in the 13th fraction, indicating that S-200 gel filtration was an effective procedure for yielding a high quality labeled rIL-2.

SDS-PAGE analysis of plasma and urine samples was important for reliable studies. The sensitivity of SDS-PAGE for detection of ^{125}I-rIL-2 was lower than that of total radioactivities. Results demonstrated that 81 ± 13 % and 91 ± 8 % of the total radioactivities in samples were ^{125}I-rIL-2. At least within 4 h, the radioactivities of plasma and urine were mainly unchanged ^{125}I-rIL-2. Linear pharmacokinetics of rIL-2 and bioavailability after im were in accordance with those of Konrad *et al* studied in human being[6].

The tissue distribution and excretion experiments both demonstrated that the main excretion for rIL-2 was through urinary route in an unchanged form. The existence of hepatic-entero-feces excretion route was also remarkable. A small peak appeared at 4 h in concentration-time curves might be related to reabsorption of radioactivities in the intestine. We could not detect high levels of rIL-2 in lympho-immune tissues. It remains unclear whether the tissue distribution and excretion profile of rIL-2 were only general characteristics of low molecular protein or there may be some unique feature for rIL-2[4]. Obviously, it will be very interesting to make a comparative study with other recombinant bioactive protein growth factors. The lower levels and larger apparent volumes of distribution observed after im than those after iv indicated that the distribution pattern after two routes might be somewhat different.

The low detection rate of ^{125}I in thyroid gland might be a shortcoming of the present ^{125}I-labeled method, in which no pretreatment of unlabeled iodine was used. The results seemed not serious during early stage (only 0.6 ± 0.3 % of the injected dose at 15 min). It is not clear whether this phenomenon might somewhat be related to bio-degradation of the protein[4] or an artifact happened to the present ^{125}I-labeled protein method. Using radioactive tracing method in mice was easy to handle. The shortcomings were obvious, including couldn't follow the kinetics in same mouse, influence of blood sampling. The pharmacokinetic study of ^{125}I-rIL-2 in rabbits carried out in our lab showed that the results were similar to mice.

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基因重组人白细胞介素-2在小鼠的药物动力学和分布

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摘要 Iodogen 法制备^{125}I-rIL-2，放射纯度95 %。iv 后快速、慢速分布和消除 $T_{1/2}$分别为 < 2，30 — 120 和 6 — 15 h，AUC 与剂量呈正比。血尿原药占 81 ± 13 %。im 生物利用度0.57。iv 后15 min 浓度顺序为肝>胆汁>肾>血>肾上腺>血浆>肺>甲状腺>脾>小肠>肠系膜淋巴结>肠内容>卵巢>心>膀胱>胸腺>粪>肌肉>睾丸>脑>脂肪。24 h 排出 80 % 第 2 天 5 %。

关键词 白细胞介素-2；药物动力学；组织分布；小鼠

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Synergistic effect of probimane on anticancer cytotoxicity of doxorubicin *in vitro*

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ABSTRACT Using 3-(4, 5-dimethythiazole)-2, 5-diphenyltetrazolium bromide (MTT) method, the effect of probimane (Pro) on doxorubicin (Dox) cytotoxicity was studied. Pro 0.313, 0.625, and 1.25 $\mu g \cdot ml^{-1}$ potentiated cytotoxicity of Dox in Ehrlich ascites carcinoma (EAC) cells. Incubation of EAC cells with Dox 10 $\mu g \cdot ml^{-1}$ and Pro 116.5, 233, and 466 $\mu g \cdot ml^{-1}$ resulted in an increase in intracellular drug accumulation from 0.69 ± 0.06 to 1.08 ± 0.10 $\mu g/10^7$ cells. In S_{37}-bearing mice, Pro 23.3, 46.6, and 116.5 $\mu g \cdot ml^{-1}$ enhanced the malondialdehyde (MDA) formation in tumor and liver mitochondria and decreased MDA formation in liver mitochondria. These results suggested that the increases of Dox accumulation and MDA formation in tumor cells by Pro might be the reasons for synergistic effect of Pro on Dox cytotoxicity.

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KEY WORDS doxorubicin; probimane; malondiadehyde; cultured tumor cells; drug synergism

Formation of free radicals of doxorubicin (Dox) *in vitro* may play a significant role in killing tumor cell[1,2]. Dexrazoxane (bisdioxopiperazine compound) was cytotoxic to certain tumor cells and potentiated antitumor activity of Dox in murine L_{1210}, S180, and HL-60 cells[3,4]. Probimane (Pro), [*dl*-bis (4-morpholinmethyl 3, 5-dioxopiperazin-1-yl) propane] first synthesized in China, is also a dioxopiperazine compound with antitumor activity[5], reduced the cardiotoxicity of Dox by scavenging the Dox-semiquinone free radical and increasing the contents of SOD and GSH-Px in rat heart[6,7]. We also found that Pro