的影响. **方法**: 用流动血细胞计数仪测定肿瘤坏死因子(TNF-α)诱导人脐静脉内皮细胞 ICAM-1 和凝血酶诱导人血小板 P-选择素的表达. **结果**: HUVEC 经 TNF-α 处理后, 明显增加细胞表面 ICAM-1 的表达, 增加 HL-60 细胞粘附到内皮细胞表面达加入细胞总数的 30 % ± 6 % (对照组为 4.6 % ± 0.7 %). 在 TNF-α 处理前, 用 Tan (25 – 200 μmol·L^{-1}) 与 HUVEC 共孵育, 则 Tan 剂量依赖性地抑制 TNF-α 的作用. Tan (25 – 200 μmol·L^{-1}) 与人血小板孵育后, 可剂量依赖性地抑制凝血酶诱导人血小板表面 P-selectin 的表达. **结论**: Tan 可抑制内皮细胞和血小板表达粘附分子.

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Pharmacokinetics of recombinant human granulocyte macrophage colony-stimulating factor in *Macaca mulatta*

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KEY WORDS recombinant granulocyte macrophage colony-stimulating factor; enzyme-linked immunosorbent assay; pharmacokinetics; *Macaca mulatta*

AIM: To examine the pharmacokinetics of iv and sc recombinant human granulocyte macrophage colony-stimulating factor (rhGM-CSF) in *Macaca mulatta*. **METHODS**: Plasma levels of rhGM-CSF were detected with sandwich enzyme-linked immunosorbent assay. **RESULTS**: Plasma concentration-time curves after iv rhGM-CSF in monkeys were best fitted with 3-compartment model. The 1st, 2nd, and 3rd phase $T_{\frac{1}{2}}$ were 0.05 – 0.07, 0.14 – 0.58, and 1.4 – 4.1 h. Cl and K_{10} were similar between different doses, respectively. C_{max} was 0.93 ± 0.16 μg·L^{-1}, T_{max} was 2.65 ± 0.14 h, and elimination $T_{\frac{1}{2}}$ was 2.5 ± 0.3 h after sc rhGM-CSF. The bioavailability after sc rhGM-CSF was 0.61. **CONCLUSION**: Pharmacokinetics of rhGM-CSF in *Macaca mulatta* provided a useful index for clinical trial.

Human granulocyte macrophage colony-stimulating factor (hGM-CSF) is one of the hematopoietic growth factors which control the proliferation and survival of myeloid cells [1]. Recombinant hGM-CSF (rhGM-CSF) has been developed for treatment of several hematopoietic disorders, and has shown promise in the treatment of myelodysplastic syndromes, as an adjunct to autologous bone marrow transplant, and the treatment of bone marrow suppression induced by high dose chemotherapy[2-4]. In China, rhGM-CSF has been developed into therapeutic use. The present study, as part of the preclinical studies of rhGM-CSF, was initiated to investigate the pharmacokinetics of iv and sc injections of rhGM-CSF.

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MATERIALS AND METHODS

Chemicals Bacterial-derived rhGM-CSF (Lot No 95011, purity > 98 %, 300 μg/ampole, 6.67 × 10^{8} IU·μg^{-1}) was provided by Shanghai Huaxin Biological High Techniques Co Ltd, Shanghai, China. Standard GM-CSF was purchased from Schering-Plough. Monoclonal antibody against GM-CSF, biotinylated antibody against GM-CSF, and streptavidin conjugated horseradish peroxidase complex were purchased from GIF, Müster, Germany. 3,3,5,5-Tetramethylbenzidine (TMB), gelatin, and bovine serum albumin (BSA) were from Sigma. Ketamine hydrochloride (50 g·L^{-1}) was purchased from Shanghai Zhongxi Pharmaceutical Co Ltd, Shanghai, China.

Macaca mulatta *Macaca mulatta* (n = 9, ♂) weighing 4 – 5 kg were provided from Shanghai Institute of Physiology, Shanghai, China. Only monkeys that had not received rhGM-CSF previously and that did not demonstrate antibodies to rhGM-CSF were used for this study. Monkeys were randomly assigned to study groups

Pharmacokinetics of rhGM-CSF Monkeys were

anesthetized with im ketamine hydrochloride 10 mg·kg^{-1}. An indwelling catheter (B Braun Melsungen AG, Melsungen, Germany) was placed in the long saphenous vein for blood drawing. The catheter was capped with a closure stopper with injection port (B Braun Melsungen AG). The patency of the catheter was maintained by sodium heparin 10 kU·L^{-1} in saline. Monkeys were preconditioned to restraining chairs to minimize stress. rhGM-CSF was injected iv 5 or 50 μg·kg^{-1}, or sc 5 μg·kg^{-1}. Approximately 1 mL of blood was drawn each time from the venous catheter using a heparinized syringe. The void volume was removed from the catheter before collecting the blood. Blood samples were spun at 4000 × *g* for 5 min. Plasma was stored at −20 ℃ until assayed by sandwich enzyme-linked immunosorbent assay (ELISA).

Sandwich ELISA for detecting rhGM-CSF rhGM-CSF in plasma was measured by ELISA method[15] modified by us. The capture antibody (monoclonal antibody against GM-CSF) (1.5 g·L^{-1}, 100 μL/well) in phosphate-buffered saline (PBS) was coated overnight at 25 ℃ on 96-well microtiter plates (Sigma). Then the plates were blocked with PBS containing 1 % BSA at 25 ℃ for 6 h. The plates were washed 3 times with washing buffer (PBS with 0.02 % Tween 20). One hundred μL of dilutions of plasma containing rhGM-CSF diluted in diluting buffer (PBS with 0.02 % Tween 20 and 0.2 % gelatin) were added to the plates at 25 ℃ for 45 min. The plates were washed with washing buffer 3 times. Detection antibody (biotinylated antibody against GM-CSF) was added in wells and incubated at 25 ℃ for 45 min. After further washing, the plates were incubated with streptavidin conjugated horseradish peroxidase complex diluted 1∶1000 in diluting buffer (100 μL/well) for 30 min. The plates were washed, and 100 μL TMB 100 mg·L^{-1} with 0.003 % H_2O_2 in NaAc 0.11 mol·L^{-1} (pH 5.0) was added. The plates were covered with aluminum foils and incubated for 30 min. The reaction was stopped by adding HCl 2 mol·L^{-1} (50 μL/well). The optical density at 450 nm was measured with a DG3022 plate reader (Chinese Electron Tube Factory, Nanjing, China). The specificity of the assay was demonstrated by adding potential interfering substances into the plasma. Interlukine-1α (IL-1α) 10, IL-1β 10, IL-3 10, IL-4 1, IL-5 1, IL-6 1, IL-7 1, IL-8 1, IL-10 1, M-CSF 1, and G-CSF 1 μg·L^{-1} were not cross reacting. Background readings were determined from wells in which PBS containing 1 % normal monkey plasma without rhGM-CSF was incubated. The background reading was subtracted from all results. Assays were done in triplicate wells and were expressed as the mean levels. Plasma was analyzed from a pretreatment specimen and up to 24 h after rhGM-CSF injection. The endogenous GM-CSF level detected in the pretreatment specimen was subtracted from all plasma levels.

Standard curves and validation rhGM-CSF standard was diluted with the normal monkey plasma to get a standard curve in concentrations of 7.8, 15.6, 31.2, 62.5, 125, and 250 ng·L^{-1}. To test the inter- and intra-assay variations, 6 aliquots of each sample were assayed within 1 d and 1 wk. Accuracy and precision were evaluated by calculating the recovery values from the standard curves and coefficients of variation (CV) between the aliquots, respectively.

Pharmacokinetic analysis Pharmacokinetic data on blood elimination were modeled using the 3p87 Program.

RESULTS

Accuracy and reproducibility of the method The standard curve for plasma ranged from 7.8 to 250 ng·L^{-1} was linear ($r > 0.9993$). The inter-assay CV was ≤5.6 %, the intra-assay CV was ≤6.5 %. The mean recovery was 94.9 % ± 6.5 %.

Pharmacokinetics of iv rhGM-CSF The highest plasma levels of iv rhGM-CSF 5 or 50 μg·kg^{-1} measured at 2 min were 42 ± 3 and 450 ± 29 μg·L^{-1}, respectively. The curves after iv were best fitted with 3-compartment model: $C(t) = Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t}$ (Fig 1).

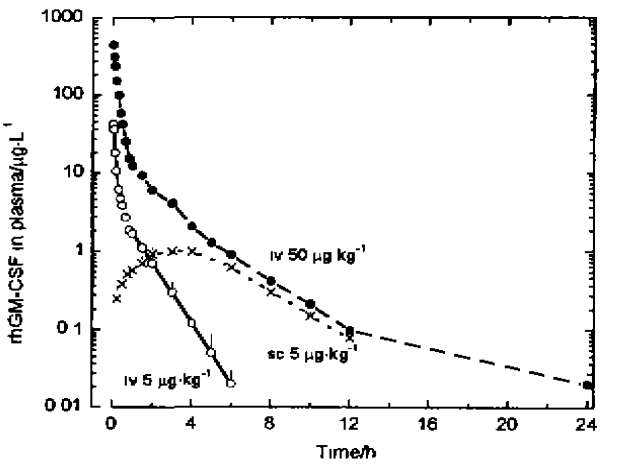

Fig 1. rhGM-CSF levels in plasma after iv 5, iv 50, or sc 5 μg·kg^{-1}. $n = 3$ monkeys, $\bar{x} \pm s$.

The first, second, and last phase $T_{\frac{1}{2}}$ were 0.05 − 0.07, 0.14 − 0.58, and 1.4 − 4.1 h, respectively. The areas under curves (AUC) were increased proportionally with doses. Clearance (Cl) and K_{10} were similar in different dosages (Tab 1).

Pharmacokinetics of sc rhGM-CSF Plasma levels of rhGM-CSF after sc were higher than those 2 h after iv 5 μg·kg^{-1} (Fig 1). Concentration-time curve was best fitted with 1-compartment model. Maximal concentration (C_{max}) was 0.93 ± 0.16 μg·kg^{-1}, time

Tab 1. Pharmacokinetic parameters of iv rhGM-CSF 5 and 50 μg·kg^{-1} in *Macaca mulatta*.

Parameters	Intravenous 5 μg·kg^{-1}				Intravenous 50 μg·kg^{-1}			
	Monkey A	Monkey B	Monkey C	$\bar{x} \pm s$	Monkey D	Monkey E	Monkey F	$\bar{x} \pm s$
P /μg·L^{-1}	53	61	34	49 ± 14	143	368	144	218 ± 130
A/ μg·L^{-1}	4.1	2.5	17.0	7.9 ± 8.0	373	240	422	345 ± 94
B/ μg·L^{-1}	1.74	0.05	4.00	1.93 ± 1.98	17	16	15	16.0 ± 1.0
π/h^{-1}	11	8	12	10.3 ± 2.1	14	12	17	14 ± 3
ω/h^{-1}	1.4	0.6	5.9	2.6 ± 2.8	5.2	4.2	5.3	4.9 ± 0.6
β/h^{-1}	0.78	0.06	0.74	0.53 ± 0.40	0.51	0.46	0.46	0.48 ± 0.03
$T_{\frac{1}{2}\pi}$/h	0.06	0.09	0.06	0.070 ± 0.017	0.05	0.06	0.04	0.050 ± 0.010
$T_{\frac{1}{2}\omega}$/h	0.51	1.12	0.12	0.58 ± 0.50	0.13	0.16	0.13	0.140 ± 0.017
$T_{\frac{1}{2}\beta}$/h	0.9	10.6	0.9	4.1 ± 5.6	1.2	1.5	1.5	1.40 ± 0.17
K_{12}/h^{-1}	3.7	2.1	1.1	2.3 ± 1.3	1.4	1.9	1.9	1.7 ± 0.3
K_{21} h^{-1}	2.2	0.9	8.0	3.7 ± 3.8	12	7	14	11 ± 4
K_{10}/h^{-1}	0.5	0.3	3.2	1.3 ± 1.6	1.4	1.6	1.4	1.47 ± 0.12
K_{31} h^{-1}	0.90	0.07	1.32	0.76 ± 0.64	0.69	0.62	0.62	0.64 ± 0.04
K_{10}/h^{-1}	5.8	5.0	4.9	5.3 ± 0.5	4.6	5.1	4.8	4.83 ± 0.25
V_c mL	474	384	473	444 ± 52	516	409	413	446 ± 61
Cl/ L·h^{-1}	2.8	1.9	2.3	2.3 ± 0.4	2.4	2.1	2.0	2.17 ± 0.21
AUC μg·L^{-1}·h	10.1	12.7	11.1	11.3 ± 1.3	115	123	121	120 ± 4

to peak (T_{max}) was 2.6 ± 0.1 h. The bioavailability (F) was 0.61 (Tab 2).

Tab 2. Pharmacolokinetic parameters of sc 5 μg·kg^{-1} rhGM-CSF in *Rhesus* monkeys. $n = 3$, $\bar{x} \pm s$. *vs* AUC of iv 5 μg·kg^{-1}. †after correction with F.

Parameters	Values
$T_{\frac{1}{2}ka}$/h	1.36 ± 0.11
$T_{\frac{1}{2}ke}$ h	2.5 ± 0.3
T_{max} h	2.65 ± 0.14
C_{max} μg·L^{-1}	0.93 ± 0.16
AUC μg·L^{-1}·h	6.9 ± 0.8
F	0.61
V_c^F L	6.1 ± 1.0
Cl^F L·h^{-1}	2.2 ± 0.3

DISCUSSION

In the present study, we have described a reliable ELISA method for detecting rhGM-CSF in plasma. The method is very sensitive and can quantitate rhGM-CSF at concentration as low as 10 ng·L^{-1}. This ELISA is not subject to major interference by other cytokines. With this method, we were able to determine the pharmacokinetics of rhGM-CSF iv or sc in *Macaca mulatta*. The highest level of rhGM-CSF after iv doses occurred immediately. The blood elimination pharmacokinetics of iv rhGM-CSF had been reported as a biphasic curve with a distribution phase $T_{\frac{1}{2}}$ of 0.14 – 0.17 h and an elimination phase $T_{\frac{1}{2}}$ of 3.7 – 4.7 h for ^{125}I-labeled rhGM-CSF in *Macaca mulatta*[10]. In our study, the plasma rhGM-CSF concentration-time curves were best fitted with 3-compartment model after iv administration with a first phase $T_{\frac{1}{2}}$ of 0.05 – 0.07 h, a second phase $T_{\frac{1}{2}}$ of 0.11 – 0.58 h, and a last phase $T_{\frac{1}{2}}$ of 1.4 – 4.1 h. One possible explanation for this finding is that the method for detecting plasma levels of rhGM-CSF in our study was different from the previous report[10].

The sc injection route for rhGM-CSF has been evaluated as a way to maintain drug blood levels at overall lower dose levels to minimize toxicity. The sc injection of Chinese hamster ovary (CHO) cell-derived rhGM-CSF has been compared to bacterial-derived rhGM-CSF in human, and it was found that bacterial-derived product reached maximal blood levels 2 – 4 h after sc injection of 3 – 5.5 μg·kg^{-1}, whereas CHO cell-derived material required 12 – 18 h after sc 8 μg·kg^{-1}. The latter result was similar to the observation in *Macaca mulatta* after sc injection of CHO cell-derived rhGM-CSF[10]. And the former

result was equivalent to our findings that a maximal blood level of the bacterial-derived rhGM-CSF in *Macaca mulatta* obtained 3 – 4 h after sc injection at dose of 5 $\mu g \cdot kg^{-1}$. The good correlation of the *Macaca mulatta* data and the human clinical data demonstrates the utility of the *Macaca mulatta* model.

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重组人粒细胞巨噬细胞集落刺激因子在恒河猴体内的药物动力学

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关键词 重组人粒细胞巨噬细胞集落刺激因子；酶连接免疫吸附测定；药物动力学；恒河猴

目的：研究重组人粒细胞巨噬细胞集落刺激因子（rhGM-CSF）在恒河猴体内的药物动力学。**方法**：用酶连接免疫吸附测定法检测血浆中 rhGM-CSF 的含量。**结果**：iv rhGM-CSF 后血药浓度时间曲线符合二房室模型。第 1，2 和 3 相的 $T_{\frac{1}{2}}$ 分别为 0.05 – 0.07 h，0.14 – 0.58 h 和 1.4 – 4.1 h。AUC 随剂量成比例增加，iv 高剂量和低剂量的 Cl 和 K_{10} 都相似。sc rhGM-CSF 后血药浓度的峰值为 0.93 ± 0.16 $\mu g \cdot L^{-1}$，达峰时间为 2.65 ± 0.14 h，生物利用度为 0.61。**结论**：恒河猴 rhGM-CSF 药物动力学数据为临床试验提供有用参考。