International Journal of Pharmaceutics 360 (2008) 225–227

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Note

Recombinant human interleukin-2 inhalation powders: Preparation and distribution in the alveolus

Tang Yue, Zhu Jia-bi*

School of Pharmacy, China Pharmaceutical University, Nanjing 210009, PR China

ARTICLE INFO

Article history:
Received 17 July 2007
Received in revised form 2 April 2008
Accepted 3 April 2008
Available online 22 April 2008

Keywords:
Recombinant human interleukin-2 (rhIL-2)
Inhalation powders
Preparation
In situ pharmacokinetic model

ABSTRACT

The aim of this work was to prepare recombinant human interleukin-2 (rhIL-2) inhalation powders, and the important parameters such as particle size, the remaining ratio of rhIL-2 were also studied. To elucidate the target effect of rhIL-2 inhalation powders, an in situ pharmacokinetic two-compartment model was used to explain their distribution characteristics in lung epithelial lining fluid and alveolar macrophage in the alveolus after the administration of the rhIL-2 inhalation powders in rats.

Interleukin-2 (IL-2) which is produced by activated T helper cells is an important mediator of the immune response. Inhalation therapy of IL-2 was proved to be an appropriate method of target immunological modulation (Ten and Anderson, 2002). Dry powder inhalers (DPIs) have become prominent alternatives to pressurized metered dose inhalers (MDIs) (Telko and Hickey, 2005) for pharmaceutical aerosol drug delivery. The purpose of the current study was to investigate the preparation of rhIL-2 inhalation powders.

Furthermore, in order to elucidate in vivo efficacy response, we attempted to investigate the distribution characteristics of rhIL-2 after endotracheal administration of rhIL-2 inhalation powders in the epithelial lining fluid which lies in pools on the inside surface of the alveolus and the alveolar macrophage (the target cells of rhIL-2 in the alveolus) which usually resides in the epithelial lining fluid in rats, and to clarify the mechanism underlying the distribution processes from a pharmacokinetic standpoint.

RhI-2 inhalation powders composed of rhIL-2 (Four Rings Biopharmaceutical Co., China), mannitol, threonine, human serum albumin (HSA, Jiangsu Chuangrui Biotechnology Co., China) in a weight ratio of 0.1/750/250/0.25 were prepared by spray drying with a Buchi 191 mini spray-drier. RhIL-2 was dissolved in 95% ethanol, and the other excipients were dissolved in distilled water. The two solutions were combined to form a 1% ethanol solution. The feed concentration was 1%. And the feed volume was 200 ml.

The particle size distributions of the dry powders were measured by Laser Diffraction Particle Size Analysis (LD) (Mastersizer 2000, Malvern Instruments, UK). And the spray-dried powders were reconstituted in deionized water to 1 mg/ml for bioactivity assays described elsewhere with MTT colorimetry (Cadee et al., 2002). The remaining ratio of rhIL-2 was calculated as the remaining activity percent after spray-dried.

Adult female Wistar rats weighing 200–250 g were purchased from Laboratory Animal Center, China Pharmaceutical University (Nanjing, China). They were maintained under normal conditions with free access to food and water and fasted for 12 h before the experiments. The rhIL-2 inhalation powders were administered by endotracheal route via a tubing-needle-syringe arrangement (Poyner et al., 1995) with some modification at a dose of 200 kU/kg (Tang et al., 2005) (Fig. 1).

At each predetermined time (5, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, 480 min) after administration, blood was colleted from the femoral arteries and then rats were immediately sacrificed by exsanguinations. The sedimentary alveolar macrophage and the diluent of the pulmonary epithelial lining fluid were gotten and separated (Sun et al., 2006). 0.75-ml 0.1 mol/l HCl was added in the alveolar macrophage to solve it with ultrasonic wave in the ice bath. The concentrations of rhIL-2 in the plasma, the diluent of the pulmonary epithelial lining fluid and the solution of alveolar macrophage were measured with ELISA (Choi and Yoo, 2002).

The content of total protein in the solution of alveolar macrophage was measured with Coomassie Brilliant Blue G-250 (Marshall and Williams, 2004). The volume of rat's alveolar macrophage is 4.2 μl/mg cell protein. The volume of the pulmonary

* Corresponding author. Tel.: +86 25 8533 8217; fax: +86 8328 1972.
E-mail addresses: tangyuetian@sina.com, jbzcpu@sina.com (Z. Jia-bi).

0378-5173/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2008.04.024

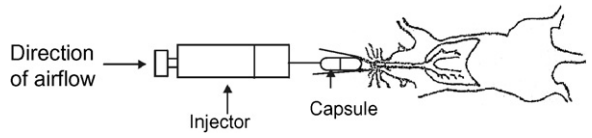

Fig. 1. Schematic diagram depicting the administration method to rat lungs.

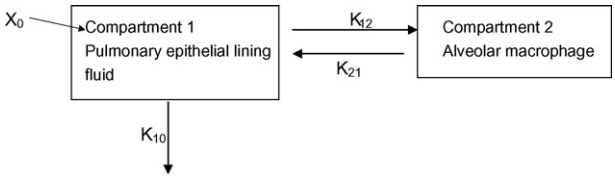

Fig. 2. In situ pulmonary pharmacokinetic model for describing the pulmonary distribution of rhIL-2 dry powder inhaler. k_{12} and k_{21} are the transportation rate constants between compartment 1 and compartment 2. k_{10} is the first-order elimination rate constant from compartment 1. X_0 is the dose delivered.

epithelial lining fluid is 395 μl/240 g rat (Sun et al., 2006). Total protein assay kits, human interleukin-2 ELISA assay kits, urea assay kits, were purchased from Nanjing Jiancheng Biotechnology Co. (China).

We employed the in situ pulmonary pharmacokinetic model in the alveolus (Fig. 2) to describe the pulmonary distribution after the administration of rhIL-2 inhalation powders in rats. After the rat inhaled rhIL-2 inhalation powders, rhIL-2 reached the pulmonary epithelial lining fluid firstly. Alveolar macrophage (the target cell of rhIL-2 in the alveolus) stays in the pulmonary epithelial lining fluid. During and after rhIL-2 therapy, the alveolar macrophage's ability of phagocytosis and secreting improves. And the cytokines such as IL-1, IL-10 are secreted. The production of IL-1 helps to the secretion of IL-2 of T helper cell (Bi, 2001).

Therefore, the rate process that the drug reaches the alveolar macrophage includes the additive kinetics process comparing to the pulmonary epithelial lining fluid. So the pulmonary epithelial lining fluid was regarded as compartment 1 and the alveolar macrophage was compartment 2.

$$\text{Compartment (1)} \quad \frac{dX_1}{dt} = k_{21}X_2 - k_{12}X_1 - k_{10}X_1,$$

$$V_1\frac{dC_1}{dt} = k_{21}V_2C_2 - (k_{12}+k_{10})V_1C_1$$

$$\text{Compartment (2)} \quad \frac{dX_2}{dt} = k_{12}X_1 - k_{21}X_2,$$

$$V_2\frac{dC_2}{dt} = k_{12}V_1C_1 - k_{21}C_2V_2$$

where k_{12} and k_{21} are the transportation rate constants between compartment 1 and compartment 2. k_{10} is the first-order elimination rate constant from compartment 1. C_1 and C_2 are the concentrations in the pulmonary epithelial lining fluid and the alveolar macrophage. V_1 and V_2 are the volumes of compartment 1 and compartment 2, respectively. X_1 and X_2 are the drug amounts in compartment 1 and compartment 2, respectively.

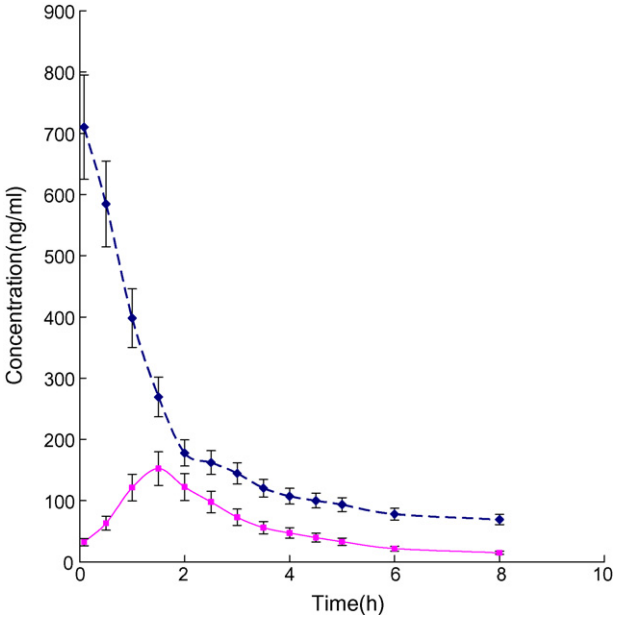

Fig. 3. Concentration–time profiles of rhIL-2 in pulmonary epithelial lining fluid (dashed line) and alveolar macrophage (solid line). The rhIL-2 inhalation powders were administered to rats at a dose 200 kU/kg ($n = 6$).

The pharmacokinetic modeling to obtain the "best" parameters was performed as judged by an nonlinear least-squares criterion with Akaike's information criterion (AIC) values using software program 3p97 and the Zhu-Koizumi method (Zhu and Koizumi, 1982).

The average size of the dry powders in all preparations was between 2 and 5 μm indicating dry powders with an appropriate particle size were obtained (Table 1). It was anticipated that spray drying would lead to the loss of rhIL-2 activity. The remaining ratio of rhIL-2 activity is an important parameter characterizing the effect of rhIL-2 inhalation powders after administration. The data obtained for the various processes indicate that the remaining ratio of rhIL-2 activity depends on the inlet temperature and aspirator setting: the lower the inlet temperature and the lower aspirator setting, the higher remaining ratio of rhIL-2 activity.

The concentration–time profiles of rhIL-2 in pulmonary epithelial lining fluid, alveolar macrophage after the administration of rhIL-2 inhalation powders are illustrated in Fig. 3. The concentration of rhIL-2 in pulmonary epithelial lining fluid decreased during the course. The concentration–time course of rhIL-2 in alveolar macrophage seemed different to that in pulmonary epithelial lining fluid, reaching the peak concentration at 1.5 h after administration, and then the concentration decreased.

Table 1
Experimental design used to evaluate the effect of process variables and formulation variables

Run	Airflow rate (ml/min)	Feed flow rate (ml/min)	Inlet temperature (°C)	Aspirator setting[a] (%)	Particle size (μm) d (0.5) by volume[b]	Remaining ratio of rhIL-2 (%)[b]
1	700	4	100	60	3.58 ± 0.12	98.75
2	700	4	100	90	3.27 ± 0.13	95.13
3	700	4	120	60	3.90 ± 0.18	92.46
4	700	4	120	90	2.70 ± 0.11	85.13
5	700	4	140	60	3.82 ± 0.20	78.65
6	700	4	140	90	2.71 ± 0.12	59.35

[a] Values are instrument settings (recorded values).
[b] Values are the mean ± S.D. ($n = 3$).

Table 2
Pharmacokinetic parameters of rhIL-2 in the in situ pulmonary pharmacokinetic model in rats after given rhIL-2 inhalation powders

Parameter	rhIL-2 dry powder inhaler
A (ng mL^{-1})	411.141
α (h^{-1})	1.315
B (ng mL^{-1})	378.89
β (h^{-1})	0.334
k_{21} (h^{-1})	0.804
k_{10} (h^{-1})	0.546
k_{12} (h^{-1})	0.299
AUC (ng mL^{-1} h)	1441.004
CL (ml h^{-1})	1.579
$t_{1/2}$ (h)	2.075

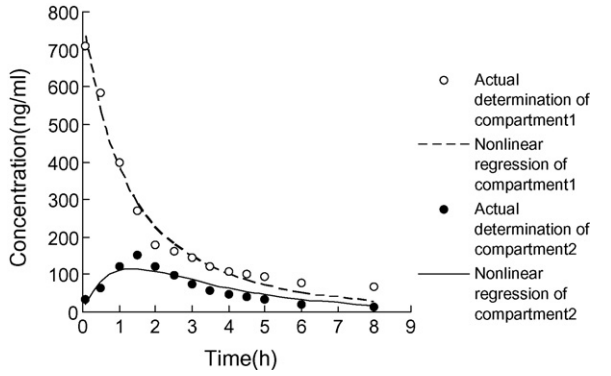

Fig. 4. Simulative curve of rhIL-2 in pulmonary epithelial lining fluid and alveolar macrophage after given rhIL-2 dry powder inhaler by inhalation.

The pharmacokinetic parameters were shown in Table 2. The result showed that open two-compartment model and one-order elimination were fitted to rhIL-2 inhalation powders in the pulmonary epithelial lining fluid and the alveolar macrophage concentration–time course in vivo. There was small deviation between the simulated data and the actual data (Fig. 4). The study indicated that rhIL-2 diffused to the alveolar macrophage rapidly and stayed for some time. Otherwise, the determination of the rhIL-2 in the plasma failed. The target effect of rhIL-2 inhalation powders was proved further.

In this paper, we demonstrated that the rhIl-2 inhalation powders could be prepared by spray drying. And the remaining ratio of rhIL-2 activity which is an important parameter characterizing the effect of rhIL-2 inhalation powders after administration depends on the inlet temperature and aspirator setting. A in situ pharmacokinetic two-compartment model was used to explain the distribution of rhIL-2 in the alveolus after the administration of the rhIL-2 inhalation powders in rats.

References

Bi, A.H., 2001. Cytokines. In: Medicinal Immunology. The People's Surgeon Press, Beijing, pp. 332–340.
Cadee, J.A., de Groot, C.J., Jiskoot, W., den Otter, W., Hennink, W.E., 2002. Release of recombinant human interleukin-2 from dextran-based hydrogels. J. Control. Release 78, 1–13.
Choi, I.S., Yoo, H.S., 2002. Expression of recombinant porcine interleukin-2 and application of its antibody to immunoassays. J. Vet. Sci. 3, 207–212.
Marshall, T., Williams, K.M., 2004. Interference in the Coomassie Brilliant Blue and Pyrogallol Red protein dye-binding assays is increased by the addition of sodium dodecyl sulfate to the dye reagents. Anal. Biochem. 331, 255–259.
Poyner, E.A., Alpar, H.O., Almeida, A.J., Gamble, M.D., Brown, M.R.W., 1995. A comparative study on the pulmonary delivery of tobramycin encapsulated into liposomes and PLA microspheres following intravenous and endotracheal delivery. J. Control. Release 35, 41–48.
Sun, J., Deguchi, Y., Tauchi, Y., He, Z., Cheng, G., Morimoto, K., 2006. Distribution characteristics of orally administered olamufloxacin, a newly synthesized fluoroquinolone antibacterial, in lung epithelial lining fluid and alveolar macrophage in rats. Eur. J. Pharm. Biopharm. 64, 238–245.
Tang, Y., Zhu, J.B., Cheng, D.S., Zhang, X.S., Zhou, B., Shen, H.Y., 2005. Effect of rhIL-2 in inhalant form on experimental respiratory tract infection with *Klebsiella pneumonia* in mice. Acta Pharm. Sin. 40, 17–21.
Telko, M.J., Hickey, A.J., 2005. Dry powder inhaler formulation. Respir. Care 50, 1209–1227.
Ten, R.M., Anderson, P.M., 2002. Interleukin-2 liposomes for primary immune deficiency using the aerosol route. Int. Immunopharmacol. 2, 333–344.
Zhu, J.B., Koizumi, T., 1982. An overture for the improvement of least square adjustment with mini-computer. Yakugaku Zasshi 102, 352–358.