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Administration Route Dependency of Distribution of PEGylated Recombinant Human Tumor Necrosis Factor Binding Protein (rhTNFbp-PEG20K dimer) following i.v. and s.c. Injection in Rats

Dong-Chool Kim and Duane C. Bloedow

Synergen, Inc. 1885 33rd Steet Boulder, CO 80301, USA

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Administration route dependency on the distribution of PEGylated recombinant human tumor necrosis factor binding protein (rhTNFbp-PEG20K dimer) was observed following a subcutaneous (sc) and an intravenous (iv) administrationin rats. rhTNFbp-PEG20K dimer is composed of two rhTNFbp molecules (molecular weight 18,278 daltons each) joined by polyethylene glycol 2000 (PEG20K). The steady state distribution volume of rhTNFbp-PEG20K was 55 ml/kg and 359 ml/kg following the i.v. and s.c. administrations, respectively. These results suggest that the distribution of rhTNFbp-PEG20K is limited within the capillary space after i.v. administration, while rhTNFbp-PEG20K can distribute into a space (35.9% of body weight) which is between extracellular space and total body water. A lymphatic absorption may play a role in the distribution of rhTNFbp-PEG20K dimer following the sc administration. The present study suggests that the administration route of a large protein molecule should be determined depending upon target sites.

Key words: Recombinant human tumor necrosis factor binding protein, Administration route dependency, Moment analysis, Polyethylene glycol, Steady state distribution volume

INTRODUCTION

Tumor necrosis factor (TNF) produced primarily by macrophages is a pleiotropic mediator of many inflammatory responses (Old, 1987). In natural form, TNF is comprised of trimers of 17,350 molecular weight and is unglycosylated (Jones *et al.*, 1989). Experimental and clinical evidence supports the hypothesis that TNF is a mediator of chronic autoimmune diseases (Tracey *et al.*, 1989). Therefore, attempts to control the adverse effects of TNF would be of clinical importance. An inhibitor of TNF acts by binding to either TNFα or TNFβ thereby preventing them from interacting with receptors on target cells and consequently inhibiting their activity (Engelmann *et al.*, 1989). The active part of this potential therapeutic agent is a modified form of the extracellular domain of the TNF receptors (Engelmann *et al.*, 1989).

In the present study, recombinant human tumor necrosis factor bindingprotein (rhTNFbp) is conjugated with polyethylene glycol 2000 to test the effect of administration route on the distribution of rhTNFbp-PEG20K dimer. Moment analysis is applied to plasma concentration time profiles of rhTNFbp-PEG20K dimer following a subcutaneous or an intravenous administration inrats.

Correspondence to: Dong-Chool Kim, Synergen, Inc., 1885 33rd Steet Boulder, CO 80301, USA

MATERIALS AND METHODS

rhTNFbp-PEG20K dimer was produced by Synergen Inc. (Boulder, CO). Allother reagents were commercially available and of analytical grade. Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used throughout the experiments. rhTNFbp PEG-20K dimer was bolus administered subcutaneously or intravenously (1 mg/kg s.c. & 4 mg/kg i.v). Then the ratswere returned to their cages. At designated sampling times, rats were anesthetized by isoflurane inhalation and blood samples were withdrawn fromtail vein. Rats were returned to the cage and allowed to recover. This procedure was repeated until the final sampling time point. rhTNFbp-PEG20K dimer in the plasma sample were determined by ELISA as

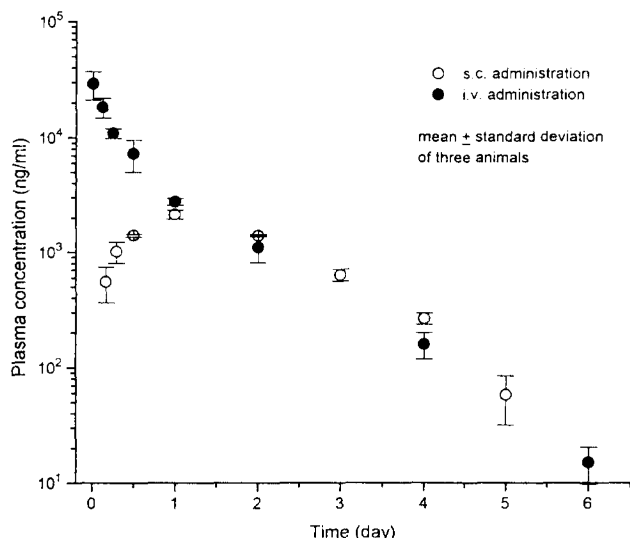

Fig. 1. Time courses of rhTNFbp-PEG20K dimer in plasma (normalized by 1 mg/kg dose) following iv and sc administration in rats.

follows; The antibody was raised against rhTNFbp-PEG 20K dimer in the goat and affinity-purified with rhTNFbp-PEG20K dimer. rhTNFbp-PEG20K dimer was used as the standard in the assay. Ninety-six-well plates were coated with 4 μg/ml of goat anti-rhTNFbp for 2 hours at 37℃. The plates were blocked with 2% bovine serum albumin (BSA) for two hours at room temperature. After washing, the samples and standards were placed on the plate. After washing, the biotinylated secondary antibody (goat anti-rhTNFbp) was added to the plate. After washing, streptavidin-horseradish peroxidase was added. The substrate solutions contained 2.5 mg/ml ABTS and 0.05% H_2O_2. The plates were read at405-490 nm on a Molecular Devices Vmax plate reader (Menlo Park, CA).

RESULTS AND DISCUSSION

Figure 1 shows time courses of rhTNFbp-PEG20K dimer in plasma (normalized by 1 mg/kg dose) following i.v. and s.c. administration in rats. Moment analysis was applied to the plasma concentration time courses of rhTNFbp-PEG20K dimer after s.c. and i.v. doses and is summarized in Table I. Following i.v. dose, the value of Vd_{ss} (55 ml/kg) was about 5.5% of body weight which is about the plasma volume, suggesting the distribution of rhTNFbp-PEG20K is restricted within capillary space. However, the distribution volume of rhTNFbp-PEG20K dimer was 359 ml/kg after s.c. injection which is larger than extracellular space (26% of body wt) and smaller than total body water (60%).

These results suggest the transport of rhTNFbp-PEG20K through the capillary membrane is highly inhibited by the PEGylation of rhTNFbp. In addition, a lymphatic absorption of rhTNFbp-PEG20K dimer may be responsible for the larger distribution volume (359 ml/kg, Table I) following s.c. administration. Supersaxo *et al.* (1988) reported that approximately 60% of recombinant human Interferon alpha-2 (Mw; 19,000) was recovered in lymph following s.c. administration in sheep, while molecules smaller than 16,000 daltons were mainly recovered from the systemic circulation after s.c. administration (Supersaxo *et al.*, 1990). The present study suggests that the administration route of large protein molecules should be carefully decided depending uponthe location of target site.

Table I. Summary of moment analysis following s.c. and i.v. administration of rhTNFbp-PEG20K dimer

	AUC [ng day/ml]	AUMC [ng day^2/ml]	MRT [hr]	Vd_{ss} [ml/kg]
s.c.	4652	7775	40.1	359
i.v.	12730	8890	16.8	55

The average value of plamsa concentration of rhTNFbp-PEG20K dimer from three animals was used to calculate AUC and AUMC using a trapezoidal rule. The elimination of rhTNFbp-PEG20K dimer is assumed to occur from central compartment following s.c. administration for the calculation of Vd_{ss}.

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REFERENCES CITED

Engelmann, H., Aderka, D., Rubinstein, M., Rotman, D., and Wallach, D. A tumor necrosis factor-binding protein purified to homogeneity from human urine protects cells from tumor necrosis factor toxicity. *J. Biol. Chem.*, 264, 11974-11980 (1989).

Jones, E. Y., Stuart, D. I. , and Walker, N. P. C. Structure of tumour necrosis factor. *Nature.*, 338, 225-228 (1989).

Old, L. J. Tumor necrosis factor. Polypeptide mediator network. *Nature (Lond.).*, 326, 330-331 (1987).

Tracey, K. J., Vlassara, H., and Cerami, A. Cachectin/tumour necrosis factor. *Lancet.*, 1 (8647), 1122-1126 (1987).

Supersaxo, A., Hein, W., Gallati, H., and Steffen, H. Recombinant interferon alpha-2a:Delivery to lymphoid tissue by selected modes of application. *Pharm. Res.*, 5(8), 472-476 (1988).

Supersaxo, A., Hein, W. R., and Steffen, H. Effect of molecular weight on the lymphatic absorption of water soluble compounds following subcutaneous administration. *Pharm. Res.*, 7(2), 167-169 (1990).