including such dose prediction in the protocol design of dose-ranging studies is that a smaller number of doses need to be tested before finding the final dose level. Interspecies dose predictions simply narrow the range of doses in the initial pharmacological efficacy studies, the animal toxicology studies, and the human safety and efficacy studies.

7. HETEROGENEITY OF PROTEIN THERAPEUTICS

The identity, purity, and potency of small synthetic drugs can be demonstrated analytically, and consequently, they are usually completely defined in terms of their chemical structure. Peptides, proteins, and other biotechnologically derived compounds are usually more complex compounds, and it is generally not possible to define them as discrete chemical entities with unique compositions. The physicochemical and biochemical characteristics of proteins are not only dependent on the amino acid sequence (primary structure), but also on the shape and folding (secondary and tertiary structures), and the relationship between the protein molecules themselves, such as the formation as aggregates (quaternary structure). Biotechnologically-derived and endogenous proteins may be heterogeneous at each structural level. For natural IFN-gamma, for example, six naturally occurring C-terminal sequences have been identified (78–80).

In addition post-translational modifications of proteins, such as the degree of glycosylation of amino acid residues, may be different. The secreted and membrane-associated proteins of almost all eukariotic cells are glycosylated (81,82), and different glycoproteins have also different carbohydrate contents, from $\sim$3% for serum IgG to $>$40% for erythropoietin (EPO). Erythropoietin has three *N*-linked and one *O*-linked sugar chains. The degree of glycosylation differs according to the cell line used for production. For example, GM-CSF and M-CSF are nonglycosylated in bacterial cell lines such as *E. coli*, moderately glycosylated in yeast, and heavily glycosylated in mammalian cell lines. Receptor binding studies with GM-CSF have shown that the receptor affinity decreases with an increase of the level of glycosylation (83).

Another classical example is recombinant human tissue-plasminogen activator (t-PA). Although the active enzyme was first derived from *E. coli* cultures, this cell line lacks several desirable biological activities, such as glycosylation ability and the ability to form the correct three-dimensional t-PA structure. Finally, recombinant t-PA was cloned into a Chinese hamster ovary (CHO) cell line. These mammalian cells carried out the glycosylation, disulfide bond formation, and proper folding similar to human cells (84).

Besides the importance of correct glycosylation for activity, differences in glycosylation may also have an influence on the pharmacokinetics. A typical example is that the removal of terminal sialic acid residues from