the sugar chains of EPO (asialo-EPO) causes complete loss of in vivo biological activity, but increases in vitro activity. The loss of in vivo activity of asialo-EPO was explained by a rapid removal from the systemic circulation, which resulted from hepatic uptake mediated by galactose-recognizing receptors.

8. CHEMICAL MODIFICATIONS OF PROTEIN THERAPEUTICS

Besides the mostly unwanted heterogeneity of protein drugs introduced by the manufacturing process, other chemical modifications of protein and peptide drugs are intentional to obtain molecules with specified characteristics. Variant proteins can be engineered that differ from natural proteins by exchange, deletion, or insertion of single amino acids, or longer sequences up to entire domains. Small changes in the chemical structure of proteins may cause differences in pharmacokinetics and pharmacodynamics. In addition, mutations may affect glycosylation patterns and conformational changes, which in turn may affect clearance and receptor interactions. A single amino acid mutation in t-PA or the removal of carbohydrate on a single amino acid in t-PA resulted in plasma concentration profiles that were very different from natural t-PA (Fig. 13) (85).

Modification of peptide and protein drugs with the aim of changing the pharmacological activity may at the same time affect the pharmacokinetic behavior of the molecules. In other instances, the increase of duration of response may be exclusively attributed to a change in the pharmacokinetics such as an increase in residence time. Such modifications include amino acid substitution, deletions and additions, cyclization, drug conjugation, glycosylation or deglycosylation, etc.

The elimination half-life of many peptide and protein drugs is rather small. Consequently, frequent dosing or continuous infusion is necessary to maintain efficacious plasma levels of the drug. Several approaches have been applied to decrease the elimination clearance of biotechnological drugs. One approach is chemical modification such as PEGylation, i.e., the attachment of monomethoxy polyethylene glycol polymer (PEG) to the protein. An example is PEG IL-2, which usually consists of a mixture of rhIL-2 molecules (Mw 15 kDa) with 1–5 or more PEG polymers attached to each molecule on the α-amino portions of the lysine residues. The production process determines the average number of PEG residues attached, but any process results in a mixture. With each PEG addition, the molecular weight increases with about 7 kDa, but because of the attraction of water molecules, the hydrodynamic size increases even more (95–250 kDa). Increasing the degree of PEGylation decreases the elimination clearance and the volume of distribution (Fig. 14). Since the elimination clearance usually decreases relatively more than the decrease in volume of distribution, the elimination half-life of PEG IL-2 is longer than for IL-2. Based