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당화 기술을 통해 개발된 인터페론 베타 변이체 (R27T)의 약물 동력학 및 생화학적 특성의 변화 연구 : 다발성 경화증의 바이오베터 단백질 치료제

>EFG년 E>월

서울대학교 대학원

약학과 병태생리학전공

이 세 형

Identification of Altered Pharmacokinetics and Biochemical Properties of Glycoengineered IFN-β Mutein (R27T): A Biobetter Protein Therapeutic for Multiple Sclerosis

당화기술을 통해 개발된 인터페론 베타 변이체 (R27T)의 약물 동력학 및 생화학적 특성의 변화 연구 : 다발성 경화증의 바이오베터 단백질 치료제

지도교수 신 영 기
이 논문을 약학박사 학위논문으로 제출함
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이세형의 박사학위논문을 인준함
2018년 12월

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Interferon-β-1a (IFN-β-1a) has an N-glycosylated at residue 80 exhibits higher biological activity (e.g., antiviral, antitumor and immune modulatory effects) than other type I IFNs and has been approved for the treatment of relapsing-remitting multiple sclerosis (RR-MS). However,

therapeutic proteins have their inherent limitations—such as physicochemical instability, immunogenicity and low half-life in serum. Recently, we glycoengineered a recombinant human IFN-β mutein (R27T) with an additional N-linked glycan at residue 25 to alter the pharmacokinetic properties of the protein. Glycoengineering has been used successfully to improve the biopharmaceutical potential of protein-based drugs because the oligosaccharide moiety of a glycan improves the structural stability and the physicochemical properties—such as pharmacokinetics, bioavailability, and a reduced dosing frequency—of the drug.

Preliminary characterization of R27T included evaluation of preclinical toxicity and the pharmacokinetics profile. The possibility of using R27T as a therapeutic agent for MS was confirmed using a relapsing-remitting experimental autoimmune encephalomyelitis (RR-EAE) model. In toxicity test, no systemically toxicity in Lewis rats were seen with either a single and repeated dose administration protocol. In both Sprague Dawley rats and cynomolgus monkeys, R27T administered by IM or SC delivery significantly increased the extent of systemic exposure—determined by PK parameters—relative to other IFN-β-1a drugs. Systemic clearance of R27T administered IV was significantly reduced relative to that of other drugs.

Clinical parameters of disease severity were also lower in the R27T-treated group than in the vehicle group. However, no significant difference was observed between R27T and IFN-β-1a treatments, possibly due to differences in the protein sequences of type I IFN receptors in humans and mice.

While IFN-β treatment reduces the severity of relapse and progression in MS patients, its therapeutic mechanism of action is not fully understood; however, its effect is believed to be due to known biological effects—such as antiviral, anti-proliferative, and immunomodulatory effects. Next, cellular and biological responses following R27T treatment were confirmed in a cell line expressing the human type I IFN receptor. Several published studies have determined a direct relationship between N-linked glycosylation and biochemical properties to alter biological activities. Depending on where they are attached to a protein, N-linked glycans may influence the interaction of the protein with its binding partner, leading to changes to biological activities. Type I IFN receptors—IFNAR1 and IFNAR2—have different binding affinities for IFN and sequentially bind to IFN in the dominant direction, leading to signal activation and induction of a diverse range of biological activities. Receptor binding kinetics for each state of binary and ternary complex formation between IFN-β-1a and R27T were

evaluated with heterodimeric Fc-fusion technology, then biological responses were compared.

N-glycosylation at residue 25 of R27T not only improves pharmacokinetics but also induces sustained cellular activation and enhanced biological activity by altering receptor binding kinetics. These altered properties of the R27T molecule could be clinically useful to patients with MS.

L!4 M(+ 0B preclinical, toxicology, pharmacokinetics, multiple sclerosis, glycoengineering, Fc-fusion proteins, interferon-β-1a

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