Effect of Molecular Structure of PEG on the Biological and Therapeutic Potentials of PEGylated Human Growth Hormone Derivatives

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Statement of Purpose:

The growth hormone therapy is frequently used to treat children's growth-related problems [1]. However, due to the short in vivo biological half-life, the frequent and daily injections were required to achieve proper therapeutic efficacy [2]. Therefore, there are continuous demands for the efficient long-acting human growth hormone (hGH). For this purpose, the PEGylated hGH with different PEG structures (branched or trimerc form) were prepared and their biological and therapeutic potentials were explored *in vitro* and *in vivo*.

Methods:

Preparation and characterization of PEG-hGH

The N-terminal PEGylated hGHs were synthesized by reductive amination by using aldehyde-terminated mPEGs (20 kDa branched and 23kDa trimeric PEG (3 kDa PEG spacer arm and branched PEG 20 kDa) aldehyde). Then, the mono-PEGylated hGH was purified ion-exchange chromatography (Figure 1). The N-terminal PEGylation was confirmed by peptide mapping after trypsin digestion.

In vitro bioactivity characterizations

The biological activities of PEGylated hGHs were evaluated by cell proliferation assay of Nb2 cell. The receptor binding assay was also performed by using Nb2 cell debris.

In vivo bioacitivity and pharmacokinetics

The pharmacokinetics of hGH and PEG-hGHs were evaluated after s.c. injections and following measurements of plasma concentrations of hGH. In vivo biological activity of hGH or PEG-hGHs were evaluated by using hypophysectomized rats after daily (hGH) or once weekly (PEG-hGHs) injections. The weight gains of the animals were continuously monitored as criteria of the bioactivities of hGH derivatives. After 2 weeks drug treatments, the experimental animals were sacrificed and their tibias were harvested, fixed, sectioned, and trained with toluidine blue to evaluate another therapeutic efficacy of hGH derivatives.

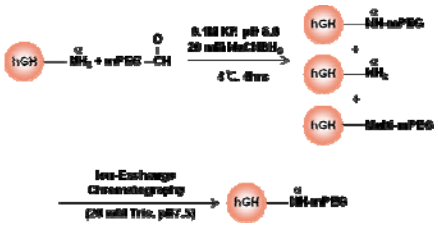

Figure 1. Synthetic Scheme of PEG-hGH derivatives

Results:

The branched or trimeric PEG conjugated hGH derivatives were successfully prepared by the coupling reaction and ion exchange chromatography (data not shown). In addition, the peptide mapping revealed that the conjugation reactions were occurred on the N-terminal of hGH. The in vitro bioactivity assay revealed that PEGylation of trimeric PEG showed well-preserved biological activity than branched PEG conjugation presumably owing to the improved hGH receptor recognition by additional PEG spacer arm (data not shown). Pharmacokinetic evaluations revealed that the plasma half-lives were increased 5.3 and 7.7 times by PEGyaltion with 20 kDa and 23kDa PEG, respectively. Furthermore, the single bolus injection of 23kDa PEGylated hGH successfully achieved the comparable weight gain of daily injection of hGH (Figure 2). However, 20kDa branched PEG conjugation failed to achieve similar therapeutic effect of hGH daily injection. In addition, the dose dependent therapeutic evaluations revealed that 23kDa PEGylation showed 3 times enhanced therapeutic effect than 20kDa PEGylation (Figure 3)

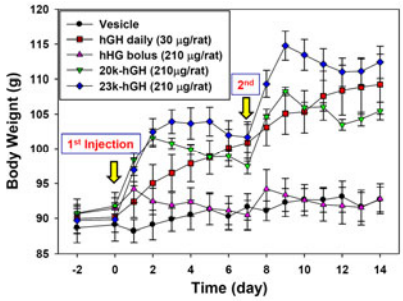

Figure 2. *In vivo* bioactivity of PEG-hGH conjugates.

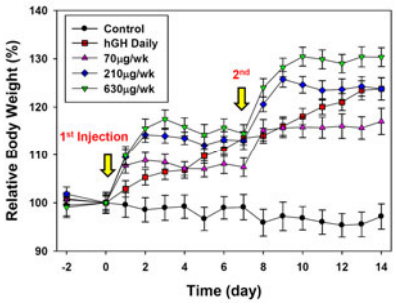

Figure 3. Dose dependent *in vivo* bioactivity of PEG-hGH conjugates

Conclusions

The present researches demonstrated that the PEGylation of hGH is one of the most successful approachs for the long-acting hGH derivatives. In addition, the introduction of additional spacer arm structure of PEG might be a more effective strategy for the hGH PEGylation.

References

[1] Clark R, et al. J Biol Chem 1996;271;21969-21977
[2] Cox GN, et al. Endocrinology 2007;148;1590-1597