

NEW LONG LASTING PEGylated-hGH

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Recombinant human Growth Hormone (h-GH) is a 191 aminoacid (22 kDa) protein drug which is used to treat short height in children due to h-GH deficiency, Turner's syndrome and chronic renal failure. Currently, h-GH is administered by daily difficult and painful injections. Several approaches have been attempted to optimise dosage.

PEGylation is one of the most investigated strategies to provide greater safety and efficacy and improve patient compliance because of less frequent administrations.

In this work a new PEGylating agent discovered by Bio-Ker in collaboration with Padua University, namely N-[(succinimidoxy)carbonyl]-β-alanine N-PEG (mPEG-NH-CO-βAla-NH-CO-NHS) was conjugated to the amine groups of h-GH lysines.

The reactivity of this PEGylating reagent was significantly lower than that of PEG-O-CH₂-CO-NHS. This means that the new PEG is more selective and reacts preferably with the most nucleophilic and exposed protein amino group; a fact that can be useful in reducing the number of PEG-protein isomers obtained by PEGylation reactions.

The new PEGylating agent showed the formation mainly of mono- and bi- PEGylated species; these conjugates demonstrated the ability to release, at least in part, linked PEG chains when incubated in PBS over 3-5 days. This is interesting because it could offer the possibility to restore *in vivo* the native protein.

The pharmacokinetic profile of conjugated h-GH with PEG-NH-CO-βAla-NH-CO-NHS displayed a great increase of the blood circulating half-life of the conjugate form with respect to the native protein. In particular, the protein half-life is increased from 0,8 to 8,3 h in rats and from 3,1 to 20,8 in monkeys.

In vivo activity in hypophysectomised rat model (body weight gain) indicated that PEGylated h-GH was bioactive and that, if administered once a week, it showed the same pharmacological activity of the native protein administered daily.

In conclusion, this new PEGylated h-GH has a prolonged permanence *in vivo* and maintains the pharmacological activity for more than 72h after a single subcutaneous administration in rats.

We believe that the new PEGylated h-GH will be active in humans for 7 – 15 days, as demonstrated by the PK data in monkeys, where significant protein levels are detected 6 days after administration.