

PROLONGING THE ACTION OF RECOMBINANT HUMAN GROWTH HORMONE BY MONOPEGYLATION OF MUTEINS

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The development of long-acting and “user-friendly” therapeutic proteins has been received with increasing interest in recent years. Proteins, unlike conventional small molecule drugs, are not readily absorbed by the body; moreover, they are digested if taken orally. After injection, most proteins undergo rapid kidney clearance because of their low stability.

Human Growth Hormone (h-GH), a polypeptide hormone synthesized by the h-GH-N gene in the somatotropic cells of the anterior pituitary gland, is currently used for the treatment of pediatric hypo-pituitary dwarfism and to treat children showing short height due to h-GH deficiency, Turner’s syndrome, or chronic renal failure. H-GH is currently administered by daily injections for several years.

Studies have been performed to develop a long lasting form of h-GH which maintains efficacy and improves patient compliance by reducing the number of injections to once a week or even longer intervals. The experimental approach was based on construction, expression and purification of h-GH mutants having a free cystein thiol, since no free thiol groups are present in native h-GH wherein the four cystein residues form two intrachain disulfide bonds. The reaction between the cystein thiol group and a suitable polyethylene glycol reagent (e.g. PEG-maleimide) was used to obtain specific monoPEGylation. A fair number of the analyzed mutants showed the same in vitro potency when compared to the native h-GH. The in vivo biological activity has been evaluated on the basis of weight increase in hypophysectomized rats after treatment with the PEGylated mutants. Results showed that a single administration of three times equivalent of the protein dose for two selected PEGylated mutants had the same effects as the daily h-GH administration, after 3 and 6 days from treatment, demonstrating their longer persistence in the bloodstream.