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RESEARCH ARTICLE

Human growth hormone and insulin, from tissue extracts to recombinant DNA technology, their biosimilars and analogues in treatment of Endocrine Disorders

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ABSTRACT:
Forty years ago, recombinant DNA technology caused a revolution in the way growth disorders and diabetes could be treated. Bacteria or human cells were modified so as to be able to produce human hormones instead of relying on cadaveric material in the case of growth disorders and porcine insulin in the case of diabetics. The new technology also removed the risk of contracted hepatitis or fatal Creutzfeldt Jacob disease.
A signal peptide was used to transport the new protein into the periplasmic space giving rise to an exact, correctly folded version of monomeric human growth hormone.
More eligible patients could be treated, resulting not only in a large number of clinical trials. Daily tiny subcutaneous injections were found to be much more effective than twice or thrice weekly in the treatment of children and adults,
The progress for recombinant human insulin turned out to be somewhat different. Insulin is a more complicated protein, with an Alpha and Beta chain joined by two disulphide bridges, but is produced in the human pancreas as a single chain, proinsulin, the C peptide is removed to make the active molecule.
Recombinant insulin development was in a way the opposite to growth hormone since human insulin acts faster than porcine. Short fast and long-acting analogues have been developed all to improve the treatment regimens for both type 1 and type 2 diabetics,
A similar development is now taking place in the growth hormone field. Depot preparations for weekly administration are under development to achieve better compliance. One recently approved depot uses an analogue, modified in one amino acid of the peptide chain to permit acylation and albumin binding. In a way this is a retrograde step and it remains to be seen if the patient's growth and metabolic responses are comparable to the daily administration of tiny amount of recombinant natural sequence hormone over several years of treatment.

Introduction:

Forty years ago, recombinant DNA technology caused a revolution in the way that human proteins could be produced without using human tissues, organs or blood or other species. Human Growth Hormone[1] (hGH (Fryklund[1], Gellerfors et al[2]) and human insulin (Riggs[3]) were the first proteins to be produced on an industrial scale. This review is not intended to have a clinical focus but more to explore the rationale for the choice of these two hormones as a starters for this new technology and to examine the divergent ways two human hormones could be developed into safe and stable pharmaceutical preparations and also to provide some insight into possible directions in the future.

What were the issues with the use of human or animal tissues for chronic therapy in human beings? The risk of the contamination of the products with virus and bacteria was understood and avoided by better use of new purification techniques and in analysis of the final product to the best standards of the day. Human plasma from outdated human transfusion blood (Farrugia et al[4]) has been used for a very long time to treat human coagulation disorders or to provide a blood substitute in acute situations. The risk of the contamination of the products with virus and bacteria was understood and avoided by analysis of the final product to the best standards of the day. However, the identification of hepatitis C and HIV[2] infections in patients forced manufacturers and the medical professions to reassess risk benefits, see Isfordink[5] et al for a recent review. The isolation and purification of human growth hormone from human pituitary glands from cadavers was not only ethically questionable but apart from the virus borne contamination there was a risk of contamination with so called CJD[3] prions which were an unknown cause of fatal neurological disease.

The restricted supply of human pituitaries meant that patients had to be selected by very strict criteria, most countries had a committee who decided, see Collett-Solberger et al[6] for a recent review.

Apart from the contamination issues poor extraction procedures led to the formation of polymers which proved antigenic in patients and meant the response was suboptimal or even totally blocked, see Roos et al[7] for the milder and more efficient extraction methods and Holmström and Fhölenhag[8] for a critical review of the different pituitary derived hGH products on the market using procedures available in the early eighties, and how improvements could be made, for example extracting the pituitary proteins from thawed frozen pituitaries and using modern gel filtration methods and analysis to monitor the removal of unwanted components and contaminants.

Sadly the perceived risk of contamination with prions was realized when a considerable number of patients treated with growth hormone isolated from human pituitaries were found to be infected with the CJD prion in France. Billette de Villemeur & Pradel[9] describe the thirty patients in France.

"All patients were treated between January 1984 and July 1985. The risk to transmit CJD with treatments of human origin (pituitary derived treatment, blood, placentas and corneal and dura mater graft) is analyzed. The selection of donors and techniques of purification on the one hand, the rigor of the indication and the quality of the followup on the other hand, are the only guarantees to reduce the risks secondary to utilization of products of human origin".

Rudge et al [10] describe the situation in the UK. No definitive CDJ tests were available at the time.

Most of these patients were treated with hGH preparations from university laboratories rather than from pharmaceutical companies who had naturally been subjected to more rigorous approval requirements. See (Roos et al[7] for the milder and more efficient extraction methods and Holmström and Fhölenhag[8] for a comparative review of the different pituitary derived hGH products on the market) Probably the use of gel filtration separated out both the hepatitis C virus, the polymers of hGH and the CJD prions. Brostedt and Roos[11] describe isolating dimers of hGH. Purello et al [12] describe neutralizing antibodies in subjects treated in Italy.

The advent of recombinant DNA technology could not have been developed at a better time.

CH Li[13] summarized how human pituitary hormones, (hGH) and prolactin (hPRL), regulate a large variety of physiological processes, among which are growth and differentiation of muscle, bone and cartilage cells, and lactation published the primary structure of hGH in 1969 showing that hGH consisted of a single polypeptide chain of 191 amino acids The disulphide bonds are between C53-C165 and C182-C189 De Vos et al [14] *describe hGH as a "4 helix bundle with some unusual topology*

[1] Human Growth Hormone hGH, pituitary derived hGH somatotropin
Recombinant derived hGH somatropin

[2] HIV human immunodeficiency virus

[3] CJD Creutzfeldt Jacob disease

similar to that of cytokines" Richi and Brems [15] describe the comparison with other cytokines.

Somers et al[16] described the three-dimensional structure, which was similar to that found by X ray crystallography of hGH bound to the dimer receptor by hormone–receptor binding. The HGH and hPRL receptors (hGH*R* and hPHL*R*, respectively) are single-pass transmembrane receptors from class 1 of the haematopoietic receptor superfamily. This "*In the case of hGH, activation involves receptor homodimerization in a sequential process: the active ternary complex containing one ligand and two receptor molecules is formed by association of a receptor molecule to an intermediate 1:1 complex. hPRL does not bind to the hGH receptor, but hGH binds to both the hGHR and hPRLR, and mutagenesis studies halve shown that the receptor-binding sites on hGH overlap*".

hGH is transported in plasma bound to a specific binding protein as described by Baumann[17] which protects hGH on its way to the target organs. The presence of a binding protein was suspected for many years but was only identified much later when receptor binding assays were developed.

Insulin was isolated from bovine and porcine pancreas tissue, and though lifesaving was problematical since the product was not identical to human insulin and antigenic blocking antibodies could prevent effective treatment for the diabetic patient.

Diabetics treated with porcine insulin could develop neutralizing antibodies which could possibly lead to worsening of their diabetes (Rubenstein et al [18], Markussen et al[19] ,Rançon et al[20] Virgili et al[21], Schernthaner et al[22])

As is well known proinsulin is not the storage form in the pancreas but removal of the C peptide by specific enzymes leads to the formation of monomeric insulin, which however is stored in hexamers stablilized by zinc ions as described by Mukerjee et al[23] before being released into the circulation.

The Insulin hexamer is such a self-assembled structure and the most stable of the possible oligomers. The monomer is the biologically active form consisting of two chains, A and B, after the C peptide is removed by enzymatic action in the pancreas. Association of two monomers leads to the formation of an insulin dimer. Insulin is stored in the Zn2+ rich vesicles in the β-cells of the pancreas. Six histidine residues from chain-B (His-10) of each monomer get coordinated to two Zn2+ ions, giving insulin hexamer its unique torus shape. The stable hexamer not only acts as a storage unit but also prevents aggregation which is a major biomedical problem. Insulin is released on a short term basis into the blood stream in response to increased blood glucose levels. In order to simplify therapy long-lasting preparations of insulin were developed for both bovine and porcine insulin.

Recombinant hGH somatropin

As with all new technology there were surprises along the way. New contaminants of bacterial or mammalian cell derivation were found, as well as the find that for some reason E. coli bacteria did not remove the amino terminal methionine as expected. The N terminal methionine stabilized the hGH molecule prolonging its half-life to some extent but was also found to be antigenic; however, no evidence of neutralizing antibodies as in the case with pituitary hGH were found. New developments using a signal peptide to transport the new protein into the periplasmic space were developed instead. (Fryklund[1]). Production methods refining the removal of bacterial, or mammalian cell contaminants could also be developed, as well as new formulations and pen devices for subcutaneous injection with small volumes.

See Gellerfors et al[24]. Karlsson et al[25] and Andersson et al[26] for other hGH modifications isolated from E. coli, including deamidation, oxidation and trisulphide bond formation

At last there were no supply limitations for the recombinant product; this led to several major findings

- The classical group of growth hormone deficient children could be treated adequately and for as long as necessary, no rationing was needed
- New groups of patients with growth syndromes could be tested and treated.
- Adults with idiopathic or acquired hGH deficiency could also be treated
- Proper dose response studies, pharmacokinetics could be performed
- New and more stable low volume formulations of both freeze dried and solution could be developed in new administration devices
- The structure and function of hGH both in its monomeric and bound form could be investigated properly

Once the supply of recombinant human growth hormone of high purity, quality and stability was established the evaluation of new patient groups could be carried out. For example, reviews for children with classical GH deficiency, Collett-Solberg et al[6] and Boguszewski[27] , Ranke and Wit[28], Deodati and Cianfarani[29] Richmond and Rogol[30] . and Boguzewski et al[31] for children and

adults with acquired hGH deficiency after cancer treatment, De Zegher et al[32] published a metaanalysis for children with short stature, born small for date. A systematic review and metaanalysis for hGH treatment in cystic fibrosis patients by Thaker et al[33] a systematic review for hGH treatment for adult patients by Dutta et al [34].

Ranke and Saenger[35], and Steiner and Sanger[36] provide updates on Turners syndrome, Rosenberg et al[37] a metaanalysis on Prader Willi syndrome, Atkinson et al[38] for a systematic review and metaanlysis in osteoporosis treatment with hGH.

Development of hGH biosimilars

The success with recombinant human growth hormone (Somatropin) prompted the development of several products independently as well as biosimilars. The latter are an interesting case for pharmaceutical development, the manufacturers of the originator product seldom publish data on their processes, preferring that knowledge to be knowhow rather that published in patent applications. Some sort of reverse engineering can be carried out by the biosimilar producer, but only comparative studies are available. See Fryklund et al[39] for a systematic and critical review of the scientific literature regarding biosimilars for hGH . The principles described here are applicable to any biosimilar including insulin.

The authors looked specifically at the possible development of neutralising antibodies developed in patients treated with growth hormone biosimilars as compared to the reference drug, and found two major issues

Namely the poor quality of the comparative clinical trials and the poor quality of the antibody assays used during the trials, out of more than 1500 articles reviewed only 6 were of good standard, with good quality antibody assays and with good analysis of the biosimilar quality.

Matar[40] points out that two products that were initially deemed biosimilar or interchangeable could each undergo unique patterns of drift and evolution in their manufacturing processes (divergence), ultimately resulting in two products that would be no longer biosimilar.In cases where divergence in potency, safety and immunogenicity may be present, care should be taken with multiple switches between reference and biosimilar products: each time a switch occurs, the difference between products could be greater. Taking into account that post-marketing comparative bio similarity validation is not required, drift, evolution and divergence may present greater challenges when assessing biosimilar. In a marketplace with multiple biosimilars of a given reference product and in the context of interchangeability with drift and divergence, pharmacovigilance systems should be strengthened.

New guidelines have been issued. (See EMEA guidelines[41])

Parallel with the biosimilar developments the originator companies have pursued the refining of their products, both by injector developments and by clinical databases (Graves[42], Maghnie et al[43], Blethen & MacGillivray[44])

Recombinant human insulin

It transpired that rather than being a new gold standard in treatment of diabetes, recombinant human insulin acted faster than the porcine and bovine equivalents and that some patients did not recognize the onset of hypoglycaemia. That necessitated the development of a whole series of new analogues with modified insulin action, derived from modifying the DNA used to produce the parent molecule produced by recombinant DNA technology. See Mergulhão et al[45] for some information about the production system.

See systematic reviews by Siebenhofer et al[46], Fullerton et al[47] and metaanalyses by Nicolucci et al[48] and Monami et al[49]. Rosetti et al[50] have written an expert opinion on the superiority of analogues rather than human insulin itself.

Tibaldi[51] in his paper discusses the evolution of human insulin to analogues. Perhaps the ultimate test is in pregnancy. Treatment of diabetic pregnant mothers is perhaps the most important use of the right glucose lowering therapy since the complications of macrosomia, difficult delivery and so on are not so threatening for the mother but all the more for offspring, these issues are explored by Toledano et al[52] in their expert opinon. See also Rodbard and Rodbard[53]

"Biosynthetic human insulins and analogues have replaced animal insulins and permitted structural modifications to alter the rate of absorption, duration of action, improve reproducibility of effects, and modulate relative efficacy in various target tissues. Several forms of rapidly acting insulins nearly achieve rapid pharmacokinetics and pharmacodynamics similar to first-phase insulin release. There is need for even faster-acting analogs to mimic normal physiology and improve control of postprandial glycemic excursions. Two biosynthetic insulin analogs have sufficiently long duration of action for use as once-daily basal insulins; controversy persists regarding their respective risks of hypoglycemia and relative glycemic variability." and Mayer et al[54].

Advancements in patient care have been paced by breakthroughs in core technologies, such as semisynthesis, high performance chromatography, rDNA-biosynthesis and formulation sciences. How the structural and conformational dynamics of this endocrine hormone elicit its biological response remains a vigorous area of study. Numerous insulin analogs have served to coordinate structural biology and biochemical signaling to provide a first level understanding of insulin action. The goals of continued investigation remain the delivery of insulin therapy where glycaemic control is more precise and hypoglycaemic liability is minimized. Additional objectives for medicinal chemists are the identification of super agonists and insulins more suitable for non-injectable delivery. The historical advancements in the synthesis of insulin analogues by multiple methods is reviewed with the specific structural elements of critical importance being highlighted.

Development of insulin biosimilars

Some biosimilars have been developed to human insulins as produced by Lilly and NovoNordisk, but their use is more restricted given the problems encountered by the use of human insulin, see Dolinar et al[55] for a recent review of the field. Franzè et al[56] and Peters et al[57] have examined the effect of biosimilars and analogues. Monnier et al[58] review the use of basal insulin analogues.

Long lasting hGH

A similar development is now taking place in the growth hormone deficiency field. Several companies have developed or are developing depot preparations that can be administered one a week instead of once a day.

Yuen et al[59] have examined the usefulness and pitfalls of long-acting Growth Hormone analogues and NovoNordisk published the data on a long-acting depot, (somapacitan) of a randomized clinical trial See Højby-Rasmussen et al[60] for a description of the results in a group of Growth Hormone deficient adults.

See also analysis of preclinical and phase 1 data, (See also Helleberg et al[61] and Thygessen et al[62] on pharmacokinetics and pharmacodynamics of somapacitan)

Miller et al[63] present data where at least similar results are obtained from the first year of therapy in Growth Hormone deficient children in a randomized trial. They do not present any data on compliance.

Its not clear what the compliance gain would be. NovoNordisk have utilized the successful technology from their diabetes treatment programmes, viz using an amino acid change from Leucine to Cysteine at position 101, allowing an acylated chain to be attached as a structure which forms a non-covalent association to albumin. Human growth hormone normally is transported in plasma by a dimer of a binding protein which has lower affinity than the receptor.

Since the initial recombinant hGH forms had an extra amino terminal methionine (somatrem) which was recognized by the immune system we are back to the issues of antigenicity and growth affecting antibodies that hindered progress before the recombinant revolution. Approval has been granted for somapacitan recently but no long-term studies have been possible given the short time period the product has been available. Presumably Phase IV studies have been started. See Miller et al[63] who describe the product NNC0195-0092 C *Somapacitan is a novel analogue, a reversible, albumin-binding human GH (hGH) derivative, intended for once-weekly subcutaneous administration with the aim of improving convenience for patients by reducing injection frequency from 365 to 52 injections per year and potentially improving treatment adherence. In NNC0195-0092, fatty acids with noncovalent, albumin-binding properties have been attached by acylation as previously described for insulin detemir, a long-acting insulin analogue.*

The significance of non-pulsatile Growth Hormone and relatively high levels of circulating IGF-1 (insulin like growth factor 1) levels on metabolic function in children in the long term is not clear. (see Kjaer et al[64]) No data has been presented on binding affinity to either the circulating Growth hormone binding proteins or the receptor, or antibody analysis.

Conclusions:

In both Growth hormone deficiency and diabetes, the treatment is chronic, but the clinical endpoints are somewhat different. The clinical effect of Growth hormone therapy in children can be best measured on an annual basis and compared with predicted adult height whereas failure to lower blood sugar can be seen immediately in insulin or insulin analogue therapy. In the case of children height not achieved can never been regained since with increasing age the natural growth rate slows and a normal growth spurt during puberty may not be achieved. Treatment of diabetic pregnant mothers is perhaps the most important use of the right glucose lowering therapy since the complications of macrosomia, difficult delivery and so on create problems both for the mother but all the more for the offspring.

As concluded by Rodbard and Rodbard[53] in their comprehensive review biosynthetic human insulins have radically revolutionized management of both type 1 and type 2 diabetes worldwide. The ability to manipulate the structure and formulation of insulin provides for more physiologic pharmacokinetics and pharmacodynamics, enabling improved glycaemic control, reduced risk of hypoglycaemia, and reduced rates of long-term complications. The plethora of insulin analogues makes direct long-term comparison difficult; given the issues of antigenicity encountered with porcine and bovine insulins. I would be surprised if some of these novel analogues did not come into difficulty later on. Insulin in high doses is capable of having growth factor like activities such as those of IGF-1 and IGF-2.

Using an analogue of human growth hormone as a once weekly regimen to treat growth hormone deficiency in order to improve convenience in administration is perhaps a retrograde step, risking the patient's possibility to achieve an appropriate result from years of treatment, time will tell.

Or perhaps the risk of the use of analogues instead of the natural sequence has been exaggerated. Given the creative successes in diabetes treatment inspired by the unexpected poor properties of human insulin, perhaps it is time to review the classical treatment options for growth failure? Perhaps the new approach lies in genetics, diagnosis of which genes are involved in failure of growth and whether the new pharmaceuticals of the future should be applied to modifications at the genome level? (see Van der Kaay et al[65], De Graaf et al[66] Binder et al[67] and Johnston et al[68])

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