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A Recombinant DNA Technique in The Genetic Engineering of Insulin from Bacteria

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ABSTRAK

Pertumbuhan Manusia dan insulin manusia adalah protein pertama yang diproduksi dalam skala industri. Metode penelitian yang digunakan dalam penelitian ini adalah tinjauan literatur klinis mengenai teknik DNA rekombinan dari insulin pada bakteri dari tinjauan literatur yang diambil dari Pubmed dan SCopus, untuk selanjutnya dicari dan dianalisis dengan penelitian lain yang sudah ada. Teknologi DNA rekombinan terdiri dari pengubahan materi genetik di luar suatu organisme untuk memperoleh karakteristik yang ditingkatkan dan diinginkan pada organisme hidup atau sebagai produknya. Dua analog insulin biosintetik mempunyai durasi kerja yang cukup lama untuk digunakan sebagai insulin basal sekali sehari. Beberapa biosimilar telah dikembangkan untuk insulin manusia seperti yang diproduksi oleh Lilly dan NovoNordisk, namun penggunaannya lebih dibatasi mengingat permasalahan yang dihadapi dalam penggunaan insulin manusia, lihat Dolinar dkk untuk tinjauan terbaru di lapangan. Beberapa perusahaan telah mengembangkan atau sedang mengembangkan persiapan depot yang dapat diberikan satu kali dalam seminggu, bukan sekali sehari. Teknologi DNA rekombinan merupakan perkembangan penting dalam ilmu pengetahuan yang telah membuat hidup manusia lebih mudah. Efek klinis terapi hormon pertumbuhan pada anak-anak paling baik diukur setiap tahun dan dibandingkan dengan prediksi tinggi badan orang dewasa, sedangkan kegagalan menurunkan gula darah dapat langsung terlihat pada terapi insulin atau analog insulin.

Kata Kunci : DNA Rekombinan; Rekayasa Genetika; Insulin dari Bakteri.

ABSTRACT

Human Growth and human insulin were the first proteins to be produced on an industrial scale. Methods: The research method used in this study was a clinical literature review on recombinant DNA techniques from insulin in bacteria from the review literature taken from Pubmed and SCopus, for further search and analysis with other existing research. Recombinant DNA technology comprises altering genetic material outside an organism to obtain enhanced and desired characteristics in living organisms or as their products. Two biosynthetic insulin analogs have sufficiently long duration of action for use as once-daily basal insulins. 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 for a recent review of the field. Several companies have developed or are developing depot preparations that can be administered one a week instead of once a day. Recombinant DNA technology is an important development in science that has made the human life much easier. 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.

Keywords : Recombinant DNA; Genetic Engineering; Insulin from Bacteria.

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A. Backgrounds

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 and human insulin 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 starter 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 [1].

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 al4) 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 infections in patients forced manufacturers and the medical professions to reassess risk benefits, see Isfordine 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 prions which were an unknown cause of fatal neurological disease. criteria, most countries had a committee who decided, see Collett-Solberger et al for a recent review [2], [3].

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 for the milder and more efficient extraction methods and Holmström and Fhölenhag 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 [4].

Recombinant DNA technology is playing a vital role in improving health conditions by developing new vaccines and pharmaceuticals. The treatment strategies are also improved by developing diagnostic kits, monitoring devices, and new therapeutic approaches. Synthesis of synthetic human insulin and erythropoietin by genetically modified bacteria and production of new types of experimental mutant mice for research purposes are one of the leading examples of genetic engineering in health. Likewise, genetic engineering strategies have been employed to tackle the environmental issues such as converting wastes into biofuels and bioethanol, cleaning the oil spills, carbon, and other toxic wastes, and detecting arsenic and other contaminants in drinking water. The genetically modified microbes are also effectively used in biomining and bioremediation [5].

The advent of recombinant DNA technology revolutionized the development in biology and led to a series of dramatic changes. It offered new opportunities for innovations to produce a wide range of therapeutic products with immediate effect in the medical genetics and biomedicine by modifying microorganisms, animals, and plants to yield medically useful substances. Most biotechnology pharmaceuticals are recombinant in nature which plays a key role against human lethal diseases. The pharmaceutical products synthesized through recombinant DNA technology, completely changed the human life in such a way that the U.S. Food and Drug Administration (FDA) approved more recombinant drugs in 1997 than in the previous several years combined, which includes anemia, AIDS, cancers (Kaposi's sarcoma, leukemia, and colorectal, kidney, and ovarian cancers), hereditary disorders (cystic fibrosis, familial hypercholes- terolemia, Gaucher's disease, hemophilia A, severe combined immunodeficiency disease, and Turnor's syndrome), diabetic foot ulcers, diphtheria, genital warts, hepatitis B, hepatitis C, human growth hormone deficiency, and multiple sclerosis. Considering the plants develop multigene transfer, site- specific integration and specifically regulated gene expression are crucial advanced approaches. Transcriptional regulation of endogenous genes, their effectiveness in the new locations, and the precise control of transgene expression are major challenges in plant biotechnology which need further developments for them to be used successfully [6].

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 [7]. Diabetics treated with porcine insulin could develop neutralizing antibodies which could possibly lead to worsening of their diabetes [8].

As is well known proinsulin being 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 before being released into the circulation [9].

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 [10].

B. Methods

The research method used in this study was a clinical literature review on recombinant DNA techniques from insulin in bacteria from the review literature taken from Pubmed and Scopus, for further search and analysis with other existing research.

C. Results and Discussion

Recombinant DNA Technology

Recombinant DNA technology comprises altering genetic material outside an organism to obtain enhanced and desired characteristics in living organisms or as their products. This technology involves the insertion of DNA fragments from a variety of sources, having a desirable gene sequence via appropriate vector. Manipulation in organism's genome is carried out either through the introduction of one or several new genes and regulatory elements or by decreasing or blocking the expression of endogenous genes through recombining genes and elements. Enzymatic cleavage is applied to obtain different DNA fragments using restriction endo- nucleases for specific target sequence DNA sites fol- lowed by DNA ligase activity to join the fragments to fix the desired gene in vector. The vector is then introduced into a host organism, which is grown to produce multiple copies of the incorporated DNA fragment in culture, and finally clones containing a relevant DNA fragment are selected and harvested. The first recombinant DNA (rDNA) molecules were generated in 1973 by Paul Berg, Herbert Boyer, Annie Chang, and Stanley Cohen of Stanford Uni- versity and University of California San Francisco. In 1975, during "The Asilomar Conference" regulation and safe use of rDNA technology was discussed. Paradoxically to the view of scientists at the time of Asilomar, the recombinant DNA methods to foster agriculture and drug developments took longer than anticipated because of unexpected difficulties and barriers to achieve the satisfactory results. However, since the mid-1980s, the number of products like hormones, vaccines, therapeutic agents, and diagnostic tools has been developed continually to improve health [11].

A quick approach is offered by recombinant DNA tech- nology to scrutinize the genetic expression of the mutations that were introduced into eukaryote genes through cloned insulin genes insertion inside a simian virus fragment. In a similar way, tumor growth was inhibited by adenoviral vector that encodes endostain human secretory form through antiangiogenic effects. Antiangiogenic effect can be enhanced by dl 1520 through rescuing replication of Ad-Endo Targeted gene disruption has been used to roduce antitumor derivatives in other hosts which were structurally similar for the production pathways. Besides, longer acting therapeutic proteins have been developed through recombinant DNA technologies; for example, sequences containing additional glycosylation site are one of the most followed approaches. A new chimeric gene has been devel- oped through this technique which contains the FSH β- subunit coding sequences and the C-

terminal peptide of the hCG β-subunit coding sequences. Researchers have also developed vectors and combined vectors for gene therapy and genetic modification approaches. Presently, viral vectors have received immense consideration in clinical settings, some of which have also been commercialized. In principle, viruses are modified to be safe for clinical purposes. They have several applications including treatment of severe diseases including cancer either through in vivo or gene therapy (ex vivo), vaccination, and protein transduction approaches [12].

The production of clinical grade viral vectors improvement has become possible due to advance manufacturing technologies. At present, due to the severe adverse effects, retroviral vectors are losing their importance although the viral entities transfer genes quickly and correctly into a number of species. The simplest nonviral gene delivery system uses "naked" DNA, when injected directly into certain tissues, particularly muscles, produces significant levels of gene expression with least side effects. More recently, a P1 vector has been designed to introduce the recombinant DNA into E. coli through electroporation procedures. This new cloning system is used for establishing 15,000 clone library initially averagely 130−150 kb pairs insert size. PAC cloning system is considered useful for complex genome analysis and in mapping. The construction of low copy number vectors, for example, pWSK29, pWKS30, pWSK129, and pWKS130, was carried out using PCR and recombinant DNA technology. These vectors can also be used for generating unidirectional deletions with exonuclease, complementation analysis, DNA sequencing, and run-off transcription [12], [13].

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 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 [14].

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[15].

Matar 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. Parallel with the biosimilar developments the originator companies have pursued the refining of their products, both by injector developments and by clinical databases [16], [17].

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 for some information about the production system [17]–[19].

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 in their expert opinon. "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 [20], [21].

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 for a recent review of the field. 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 [22], [23].

Yuen et a 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 for a description of the results in a group of Growth Hormone deficient adults [24].

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 [25].

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 [26]–[28].

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. No data has been presented on binding affinity to either the circulating Growth hormone binding proteins or the receptor, or antibody analysis.

D. Conclusion

Recombinant DNA technology is an important development in science that has made the human life much easier. In recent years, it has advanced strategies for biomedical applications such as cancer treatment, genetic diseases, diabetes, and several plants disorders especially viral and fungal resistance. The role of recombinant DNA technology in making environ- ment clean (phytoremediation and microbial remediation) and enhanced resistace of plants to different adverse acting factors (drought, pests, and salt) has been recognized widely. The improvements it brought not only in humans but also in plants and microorganisms are very significant. The challenges in improving the products at gene level sometimes face serious difficulties which are needed to be dealt for the betterment of the recombinant DNA technology future. In pharmaceuticals, especially, there are serious issues to produce good quality products as the change brought into a gene is not accepted by the body. Moreover, in case of increasing product it is not always positive because different factors may interfere to prevent it from being successful. Considering health issues, the recombinant technology is helping in treating several diseases which cannot be treated in normal conditions, although the immune responses hinder achieving good results.

Several difficulties are encountered by the genetic engineering strategies which needed to be overcome by more specific gene enhancement according to the organism's genome. The integration of incoming single-stranded DNA into the bacterial chromosome would be carried out by a RecA-dependent process. This requires sequence homol- ogy between both entities, the bacterial chromosome and incoming DNA. Stable maintenance and reconstitution of plasmid could be made easy. The introduction of genetic material from one source into the other is a disaster for safety and biodiversity. There are several concerns over development of genetically engineered plants and other prod- ucts. For example, it is obvious that genetically engineered plants can cross-breed with wild plants, thus spreading their "engineered" genes into the environment, contaminating our biodiversity. Further, concerns exist that genetic engineering has dangerous health implications. Thus, further extensive research is required in this field to overcome such issues and resolve the concerns of common people.

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.

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