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Continuing Education — Review

HUMAN INSULIN

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Abstract:

Two methods of synthesis of human insulin have been developed to the stage of commercial production. One entails synthesis of human insulin by bacteria ("biosynthetic" human insulin) and one entails the conversion of pork insulin into human insulin by an amino acid substitution ("semisynthetic" human insulin). Both forms of human insulin have been shown to be safe, and to have similar efficacy and pharmacokinetics to purified pork insulin. Human insulin given by subcutaneous injection has been shown to elicit antibody formation in man, although the extent of this may be slightly less than in the case of purified pork insulin, and is certainly less than in the case of beef insulin and beef-pork combinations. The clinical significance of these differences in immunogenicity is doubtful. However there are rare defined situations, such as allergy to pork insulin and antibody-mediated insulin resistance where human insulin has been shown to have advantages over purified pork insulin.

Bacterial synthesis of insulin provides an assured supply for the world's future needs, and it is conceivable that further technological refinement may make bacterial synthesis cheaper than extraction and purification of animal insulins. However, current evidence provides no basis for recommending human rather than purified animal insulin for the routine management of insulin dependent diabetes. (Aust NZ J Med 1983; 13: 647-651.)

Key words: Human insulin, insulin antibodies, bacterial insulin, insulin allergy.

1. INTRODUCTION

The isolation of an extract of pancreatic islets which was effective in reversing diabetic ketoacidosis in dogs in 1921¹ heralded a new era in the management of diabetes in man. Commercial production of insulin from beef pancreases followed shortly after, and the very high mortality associated with diabetic ketoacidosis was greatly reduced. Initial preparations of insulin were relatively impure, and were administered up to four times per day as a short-acting preparation. Various methods of delaying absorption by complexing with zinc and/or protamine were devised. These allowed symptomatic control on one injection of insulin per day. Allergic responses to the insulin preparations and fat atrophy at the sites of insulin injection were not uncommon. With the development of radioimmunoassay technology, it became possible to

measure circulating antibodies to injected insulin. It was shown that with prolonged insulin use, virtually all diabetics developed high concentrations of insulin antibodies.² These were usually of relatively low affinity and did not interfere with insulin action, but occasionally high affinity antibodies produced the phenomenon of insulin resistance.³

Pork insulin was first used shortly after the introduction of beef insulin. Pork insulin elicits less antibody formation than beef or beef-pork mixtures,⁴ although the purity and form of the insulin as well as its species of origin are important in determining its antigenicity.⁵ Highly purified pork insulins were introduced to clinical medicine in the 1970s.⁶ Antibody-mediated insulin resistance is virtually unknown with purified pork insulins, insulin allergy is extremely uncommon and fat

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atrophy does not occur. Commercially available beef insulins in routine use in Australia are much more pure than previously, and highly purified preparations of these are now also produced. Although beef and combined beef-pork insulins may be associated with some degree of antibody-mediated resistance, this phenomenon is not now a major clinical problem.

2. THEORETICAL ADVANTAGES OF HUMAN INSULIN

(a) Lessened antigenicity

Recent evidence using purified pork and human insulin suggests that the physical form, pH and contamination by insulin aggregates and noninsulin peptide fragments are major determinants of its immunogenicity, regardless of its species of origin.³ Thus preparations of homologous insulin can be immunogenic.⁴ As human insulin differs only by one amino acid from pork insulin, it is by no means certain, on theoretical grounds, that human insulin will be less antigenic than purified pork insulin in human subjects. Preliminary clinical evidence in relation to this point is reviewed later.

If it is shown that human insulin is less antigenic than purified pork insulin, the question of whether lowered insulin antibody production is an advantage in clinical practice must be answered. If the syndrome of antibody-mediated resistance is excluded on the basis that it is not seen with purified pork insulin, there is little convincing evidence that circulating insulin antibodies are harmful.^{9,10} Certainly the pharmacokinetics of injected insulin are altered by antibodies to insulin, affecting the time course of action of injected insulin,¹¹ but the total dose requirement does not correlate closely with the titre of insulin binding antibody.¹² It has been suggested that insulin antibodies¹³ or immune complexes involving insulin and its antibody¹⁴⁻¹⁶ may be important in the pathogenesis of diabetic vascular complications. However, there is little convincing evidence to support this.^{5,17} Similarly, the possibility that circulating insulin antibodies may accelerate pancreatic beta cell failure must be regarded as theoretical rather than substantiated. Thus, although high levels of insulin antibodies previously found with impure beef insulin preparations were of occasional clinical significance, there is no evidence that the low titre antibodies found in subjects treated with purified pork insulin are of any clinical significance.

(b) Guaranteed supply

The ability to manufacture insulin independent of the availability of animal pancreases has some potential advantages. Although increased efficiency

of pancreas collection from slaughtered cattle would more than accommodate the world's need for insulin in the foreseeable future, the supply of pork insulin is more dubious, particularly if fact or fashion directs a widespread conversion into the less antigenic pork rather than beef insulin. Widespread livestock disease could also affect supply. Thus, although current sources can more than adequately cope with demand for insulin, it can be argued that this may not always be so. Obviously, preparation of human insulin from pork insulin by chemical transformation ("semisynthetic"—see below) would not lessen the requirement for animal insulin.

(c) Cost

At present, production of human insulin by recombinant DNA technology, conversion from pork insulin, or total chemical synthesis is more expensive than extraction from animal sources. It is conceivable that with technological advances the bacterial synthesis of insulin could become less expensive than its extraction from animal sources, but this does not seem likely in the immediate future, because of the extensive purification required after bacterial synthesis.

3. METHODS OF PREPARATION OF HUMAN INSULIN

(a) Extraction from human pancreases¹⁸

Early studies were performed with insulin extracted from human cadaver pancreases.⁴ The limited supplies and low yield prevent this being a practical source for therapeutic purposes.

(b) Chemical synthesis

Human insulin has been prepared by total chemical synthesis.¹⁹ However, because of its relatively high molecular weight, and the inefficiency of the method of joining the A and B chains by disulphide bridges, this method is cumbersome and prohibitively expensive for therapeutic purposes.

(c) Chemical transformation of pork into human insulin ("semisynthetic")

Pork and human insulin are identical in structure except for the amino acid in position 30 of the B chain (threonine in human, alanine in porcine) (Fig. 1). The enzymic and chemical conversion of pork into human insulin has become a major commercial method of preparation of human insulin.²⁰

(d) Recombinant DNA technology and bacterial synthesis ("biosynthetic")

Two techniques have been described in which recombinant DNA technology has been used to allow bacterial synthesis of insulin. The technique

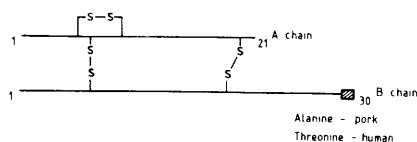


Figure 1: Diagrammatic representation of primary structure of pork and human insulin, showing that the only difference is the amino acid at position B30. Beef insulin has two additional amino acid differences to human insulin, both in the A-chain in the region of the intrachain disulphide bridge.

which has been developed commercially is shown in outline in Figure 2.²¹ Briefly, the DNA sequences coding for the A chain and B chain of insulin are chemically synthesised. Each is inserted into separate *E. coli* plasmids, in association with the β -galactosidase gene. *E. coli* colonies are formed, and translation is induced. This is achieved by exposure to galactose, which leads to the production of the insulin chain in association with

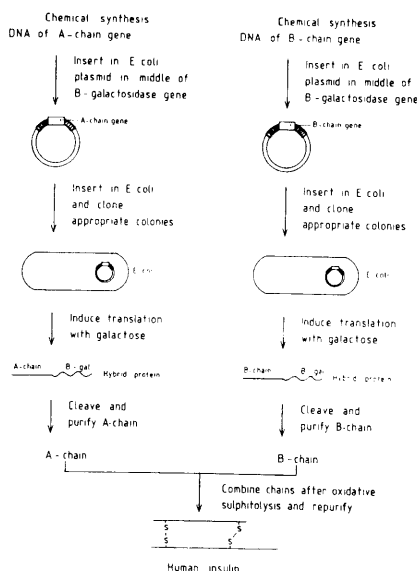


Figure 2: Diagrammatic representation of the bacterial synthesis of human insulin currently being used. The DNA sequences of the A and B chains are synthesised, and inserted separately into the plasmids of *E. coli*. After cloning and induction of protein synthesis, the individual chains are isolated, purified and chemically combined.

β -galactosidase. The separate A chains and B chains are cleaved from β -galactosidase and purified. The two chains are then combined. The last step remains relatively inefficient.

The other method entails isolation of insulin precursor mRNA from islets or insulinoma tissue, synthesis of the cDNA for the insulin precursor using the enzyme reverse transcriptase, and insertion of the DNA coding for the insulin precursor into the *E. coli* plasmid. The *E. coli* containing the insulin DNA sequence are identified and cloned, transcription and translation induced, and proinsulin purified. Proinsulin is readily converted into insulin with proteolytic enzymes. This approach has been demonstrated using rat islet mRNA as the starting material²² but has not yet been developed commercially. A variant of this technique may form the basis for a more efficient process for bacterial synthesis of insulin than that currently in use.

4. CURRENT EVIDENCE IN RELATION TO SAFETY, EFFICACY AND ANTIGEN OF HUMAN INSULIN

In view of the minor difference in amino acid structure between human and pork insulin, and since the one variant amino acid is well-removed from the portion of the molecule concerned with biological activity, it would be surprising if major differences in potency between human and pork insulin were observed. Moreover, as insulin dose is calculated on the basis of units which are calibrated by bioassay in mouse or rabbit, the two insulins are, by definition, equipotent on a unit for unit basis in the species used for bioassay. However, it is possible that differences in pharmacokinetics or differences in activity in man could exist.

In vitro studies of biosynthetic human insulin have revealed no significant differences in binding characteristics or biological activity of human compared with pork insulin.²³ Early studies of the comparative potency, and pharmacokinetics, of biosynthetic²⁴ and semisynthetic²⁵ human insulin in normal subjects have now been complemented by many further acute and short-term studies of efficacy, antigenicity and safety in diabetic subjects. These have recently been reviewed.^{17,26} The major conclusions will be summarised.

(a) Safety of human insulin

Extensive analysis of purified human insulin prepared by the recombinant DNA technique has shown it to be equivalent to semisynthetic human insulin, and free from contamination by bacterial pyrogens or other antigenic proteins.²⁷ Semi-

synthetic human insulin is also free of any unexpected harmful effects.^{17,28} Thus the preparations of human insulin currently being evaluated for human use have been demonstrated to be safe.

(b) Efficacy of human insulin

It appears that human insulin is identical to pork insulin in its pharmacokinetics and potency when given by intravenous injection. There is a suggestion in some studies but not in others that after subcutaneous administration, absorption of human insulin is slightly more rapid than pork, and that in the NPH form in particular, the onset of maximal action is earlier, and its duration of action shorter. The differences are minor.^{26,29}

In three substantial clinical trials of biosynthetic human insulin³⁰⁻³² and two using semisynthetic human insulin^{28,33} major differences in efficacy of human and purified pork insulin have not emerged. In three of these studies,^{30,31,33} control was slightly worse with regard to some parameters when patients were in the human insulin phase of crossover studies. The differences were interpretable on the basis of the minor pharmacokinetic differences between pork and human insulin described above, and were of doubtful clinical significance.

(c) Antigenicity of human insulin

Several studies have shown that human insulin can elicit insulin-specific antibodies in human subjects.^{8,34,35} These may be of either IgG or IgE species.^{34,35} Subjects transferred from purified pork to human insulin showed a fall in bound insulin levels,³⁶ although major differences in insulin antibody levels compared with subjects on treatment with purified pork insulin have not yet been established.^{33,36} The antibodies in sera of insulin-resistant diabetic subjects previously treated with animal insulins show a lower affinity for human insulin compared with pork insulin, explaining the benefit of human insulin in the rare setting of antibody-mediated insulin resistance.^{37,38} Beneficial effects of transfer to human insulin have also been seen in the rare situation of allergy to pork insulin.³⁹ Thus, although it is established that human insulin is immunogenic in man, it shares the low immunogenicity of purified pork insulin, and may have immunologic advantages over purified pork insulin, in certain defined situations. It is also possible that the immunogenicity of human insulin is due to its administration in large complexes in the intermediate acting forms. Thus it is conceivable that when it is administered as soluble insulin by continuous infusion, it may be totally nonimmunogenic.

5. CONCLUSIONS

The development of the techniques required for the production of human insulin by bacteria and the chemical conversion of pork into human insulin represent major technological advances. Biosynthetic and semisynthetic human insulin have been shown to be safe and effective. However, in the clinical setting, clear advantages of human insulin over purified animal insulins have not been demonstrated, except in the rare circumstances of insulin allergy and antibody-mediated insulin resistance. The latter phenomenon has virtually disappeared since more purified insulins have been used widely in clinical practice, so this will not be a major indication for the use of human insulin. There may prove to be minor advantages of human insulin over purified pork insulin with respect to immunogenicity, but the clinical significance of this difference is debatable. If techniques for the bacterial production of human insulin are further refined, it is possible that there will be advantages in terms of cost and a guaranteed supply compared with purified insulins of animal origin.

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The next Annual Scientific Meeting of the Australian and New Zealand Society of Nuclear Medicine will be held at the Hilton International, Adelaide from May 7 to 9, 1984. The Convenor will be Dr I. H. Butterfield, Department of Nuclear Medicine, The Queen Elizabeth Hospital, Woodville, 5011.