Human Insulin of Recombinant DNA Origin: Clinical Potential

JAY S. SKYLER

Insulin was first isolated in 1921 and became available for clinical use the following year. Since that time commercial insulin preparations have been derived from bovine and porcine pancreata. There are known differences in amino acid sequence between these animal insulins and human insulin (Table 1). These differences in primary structure, as well as impurities in commercial insulin preparations, have been held responsible for the appearance of anti-insulin antibodies in patients treated with insulin.[1-3] The immunogenicity of commercial insulin also may lead to local or systemic insulin allergy, immunologic insulin resistance (IIR), and lipoatrophy at injection sites. The use of insulin of homologous sequence to native human insulin might be expected to obviate some of the immunogenicity of commercial preparations.

Human insulin, i.e., insulin homologous in sequence to native insulin produced by human beings, can be prepared in four ways. One potential source is extraction from pancreases of human cadavers. Although some insulin has been produced in this manner, there are two problems: obtaining human pancreases, and postmortem autodigestion by enzymes derived from the exocrine pancreas. Although the latter problem could be resolved by obtaining pancreases from "heart-beating" cadavers (i.e., individuals who have sustained brain death but are maintained on life-support systems), the numbers of such pancreases are limited and would best be directed to basic research or for potential use in transplantation.

A second potential source is by full chemical synthesis, since insulin is a relatively small protein. Although some insulin has been produced in this manner,[4] the yield is relatively low due to the production of a number of error peptides. As a consequence, this a very expensive process not suitable for widescale clinical application.

A third source is the enzymatic conversion of porcine insulin to human insulin. Porcine insulin differs from human insulin only by a single amino acid difference at the carboxy-terminal of the B-chain. Therefore, it has been possible to develop enzymatic methods that remove the alanine residue at that position in porcine insulin and replace it with a threonine residue, with a resultant semisynthetic human insulin.[5-6] This method is suitable for commercial preparation and is being used for such by several manufacturers.

A fourth source is the production of human insulin by recombinant DNA technology.[7-9] In fact, human insulin of recombinant DNA origin is the first product of this technology to come to potential usefulness. Production of human insulin by recombinant DNA technology assures unlimited insulin availability in the future, thus obviating any potential shortages of insulin, which have been projected for the future.[10,11]

As reviewed by Johnson,[9] there are two general schemes which can be used for the production of human insulin by recombinant DNA technology. One involves the separate production of A and B chains, involving two fermentations, with the resulting chains then chemically linked to form human insulin. Alternatively, a single fermentation can be used to make proinsulin, and this subsequently cleaved to yield human insulin and human C-peptide. This latter process also permits the availability of abundant quantities of human proinsulin and human C-peptide, sufficient for use in both in vitro and human studies designed to definitively determine their possible biologic actions. Such studies are now in progress.

Human insulin (recombinant DNA), produced in *Escherichia coli* by recombinant DNA technology, involving separate production of A and B chains with subsequent chemical linkage, has been shown to be chemically, physically, and immunologically equivalent to human insulin derived by extraction from cadaveric pancreases.[8,9] This insulin has been exhaustively studied in terms of its receptor binding, biologic actions in vitro, in animals, and in human subjects.[12] It has also been shown to be free of contamination with *E. coli* peptides.[9,13] Because only the insulin sequence is produced, human insulin of recombinant DNA origin is free of the pancreatic peptides that are present as impurities in insulin preparations derived from pancreatic extraction. Such peptides include proinsulin and proinsulin intermediates, glucagon, somatostatin, pancreatic polypeptide, and vasoactive intestinal peptide (VIP).

TABLE 1
Differences in the amino acid structure of insulin species

	A-chain		B-chain
	A 8	A 10	B 30
Bovine	Alanine	Valine	Alanine
Porcine	Threonine	Isoleucine	Alanine
Human	Threonine	Isoleucine	Threonine

Further studies using human insulin of recombinant DNA origin are reported in this symposium. These studies have been grouped into five categories: pharmacology, biologic actions, immunologic effects, clinical trials, and case reports and clinical experience. This paper summarizes these reports and projects their clinical significance.

Pharmacologic studies. The pharmacology of human insulin (recombinant DNA origin) has been examined in a number of studies using several formulations.[14-35] These pharmaceutical formulations (galenic preparations) have included short-acting regular insulin, intermediate-acting NPH and lente insulins, and a variety of mixtures of regular and NPH insulin (10:90, 15:85, 20:80, 25:75, and 30:70 mixtures).

Almost all of these studies have found human insulin (recombinant DNA origin) and purified pork insulin to be equivalent. There are a few exceptions to this. For example, Kemmer et al. found slightly accelerated absorption of regular human insulin compared to pork insulin.[25] Pickup et al. found slightly higher plasma insulin levels at some time points after administration of one dose of regular human insulin subcutaneously, although the total area under the curve was not different.[26] Several studies have suggested more rapid absorption of NPH human insulin than NPH pork insulin.[29,32,33] In addition, some studies suggest that NPH human insulin may have a shorter duration of action than NPH pork insulin.[32,33] It is still too early to assess the overall clinical significance of these subtle differences, if they are in fact real. The early clinical trials with NPH human insulin suggest that the shorter duration of action may be clinically evidenced by higher fasting plasma glucose levels in patients switched to this insulin.[36,37] This will be discussed in greater detail later in this article.

Interestingly, there appeared to be very little difference in the time courses of action of several of the regular:NPH mixtures.[28,30,34] For example, in one study, no differences were detected when 20:80, 25:75, and 30:70 mixtures were compared, or when 10:90, 15:85, and 20:80 mixtures were compared.[34] Although at first glance this may seem surprising, it must be appreciated that only 20 U of insulin were used in the test, thus making these mixtures differ by 1–2 U of insulin. Moreover, the magnitude of such differences, 10%, is less than the coefficient of variation of either the rabbit hypoglycemia test by which insulin is quantified, or the intrasubject coefficient of variation of insulin absorption from day to day.[38] Indeed, such inherent variation coupled with the small number of subjects in any of these studies, leaves open to question the interpretation of the significance of any subtle changes observed.

Biologic effects. The careful study by Howey et al. demonstrates that the dose response curves for insulin action, in terms of both suppression of hepatic glucose output and stimulation of peripheral glucose utilization, are identical in diabetic subjects using either purified porcine insulin or human insulin (recombinant DNA origin).[39] This is an important addition to the previously reported studies of insulin action in vitro[12] and in nondiabetic human subjects.[16]

Studies of counterregulatory hormone responsiveness to insulin-induced hypoglycemia have yielded some potentially interesting although sometimes conflicting results.[15,18,22,40-43] These studies are difficult to interpret as the number of subjects studied is small, the differences in response usually are minimal, and full comparable statistical analysis is lacking. Therefore, it seems to me that it is not yet possible to give credence to these studies. I will not comment further.

Two in vitro studies are also reported in this symposium. One demonstrates that human insulin (recombinant DNA origin) and pork insulin are equipotent in stimulating lipogenesis in human adipocytes.[44] The other examines binding of human insulin (recombinant DNA origin) and human proinsulin (recombinant DNA origin) to mononuclear cells.[45] Human proinsulin was shown to have a 100-fold lower average affinity for the receptor. In addition, it was found to displace human insulin from its receptor only at very high concentrations not likely to have physiologic relevance.

Immunologic effects. The immunologic studies with human insulin (recombinant DNA origin) are the most interesting. Fineberg et al. studied a number of parameters related to circulating antibodies in patients transferred either from mixed beef-pork insulin (MBP) or purified pork insulin (PPI) to human insulin (recombinant DNA origin).[46] They found that on transfer from either MBP or PPI bound insulin in the circulation (calculated as the difference between total insulin and free insulin, and presumably reflecting insulin bound to circulating antibodies) decreased while on human insulin. Those patients transferred from MBP, but not from PPI, also showed decreases in species specific binding of beef, pork, and human insulin; and decreases in both high- and low-affinity binding capacities of circulating anti-insulin antibodies. There also was an increase in the affinity constant of the high-affinity binding. These observations suggest that overall circulating antibody levels are likely to decrease on transfer of patients from MBP to human insulin, with only minimal changes occurring on transfer from PPI to human insulin.

Schernthaner et al. studied the affinity of preformed IgG anti-insulin antibodies for pork and human insulin.[47] In 29 patients, they found the affinities to be equivalent. On the other hand, in four patients with immunologic insulin resistance (IIR) and high circulating levels of anti-insulin antibodies, they found the affinity for human insulin to be significantly less than that for pork insulin. Interestingly, all four patients were HLA-DR4 positive and HLA-DR3 neg-

ative, consistent with the hypothesis that such an HLA pattern is associated with greater likelihood of significant insulin antibody formation.[48]

Velcovsky and Federlin report the formation of both IgG and IgE insulin-specific antibodies in seven newly diagnosed diabetic patients treated only with human insulin.[49] Fireman et al. studied 31 newly diagnosed diabetic patients also treated only with human insulin and followed for 1 yr.[50] These investigators found that 13 of their subjects showed increased total immunoglobulin binding of insulin, and that two of these also showed formation of IgE antibodies. In another study, reported elsewhere, Fineberg et al. examined antibody formation in 101 patients treated only with human insulin and compared their findings with a comparable group of 51 patients treated only with purified pork insulin.[51] In that report they found similar rates of antibody formation in both groups, i.e., in about 50% of subjects after 6 mo of treatment.

Thus, it is clear that human insulin (recombinant DNA origin), despite being of homologous sequence as native human insulin, is immunogenic in human beings when administered subcutaneously in repository form. This may not be surprising since it is possible for many homologous substances to be immunogenic when given subcutaneously in the proper adjuvant. One could speculate that such immunogenicity might be obviated if newly diagnosed patients were treated from the onset of their disease only with unmodified (i.e., regular) human insulin either by continuous subcutaneous insulin infusion (CSII), which has but a minimal subcutaneous depot and no real repository, or ideally by continuous intravenous infusion, thus avoiding the subcutaneous route and any repository. The inherent difficulties with the latter approach preclude its being tested, but such use of CSII might be possible. One doesn't know the contribution to antibody formation of other additives in commercial insulin preparations.

It is important to note that although much attention is paid to the immunogenicity of insulin preparations, there is no convincing evidence of adverse effects of circulating insulin antibodies, except in the rare cases of immunologic insulin resistance. Indeed, some authors have argued that since circulating antibodies can serve as a reservoir for insulin, this may serve as a stabilizing factor facilitating glycemic control.[52] Nevertheless, it would seem desirable to avoid the confounding influence of circulating insulin antibodies, since inappropriate release of insulin from such a reservoir may create unwanted hypoglycemia.

Clinical trials. One extensive clinical trial involved the administration of human insulin (recombinant DNA origin) to patients who had never previously been treated with insulin.[37] Some of the findings of this trial, in terms of antibody formation, have been discussed above and reported separately.[50,51] In these patients, human insulin was found to be relatively effective and apparently safe. These patients remained free of local or systemic allergy and free of lipoatrophy, although 2 of 101 patients developed lipohypertrophy during the first 6 mo of human insulin use. Patients did not show intradermal sensitivity to either human insulin or *E. coli* peptides, and they remained free of antibodies to *E. coli* peptides. As noted, anti-insulin antibodies were detected in about 50% of subjects by 6 mo of treatment with human insulin.[51]

There have been three major clinical trials in which subjects previously treated with mixed beef-pork (MBP), beef, or pork insulins were transferred to human insulin (recombinant DNA origin).[36,37,53] These studies, conducted in the United Kingdom, United States, and Germany, were all multi-center double-blind studies in which half of the subjects enrolled were maintained on their old insulin and the other half were transferred to human insulin (recombinant DNA origin). In the British and German studies, patients treated with beef and pork insulins were separately randomized, whereas in the U.S. study, patients had previously been treated with either MBP or purified pork insulin and were separately randomized. All three studies found human insulin to be safe and relatively effective. In comparison with beef insulin in both the British and German studies, and in comparison with pork insulin in the German study, no differences in diabetes management were noted on transfer to human insulin.[36,53] On the other hand, in comparison with pork insulin in the British and American studies, and in comparison with MBP in the American study, there was some evidence of deterioration of glycemic control, particularly in the fasting state, on transfer to human insulin.[36,37] This is consistent with the pharmacologic studies, discussed earlier, which suggested a shorter duration of action of NPH human insulin.

As noted by Galloway, it is possible that human insulin crystals and animal insulin crystals differ in their interaction with protamine.[24] This may account for a difference in the duration of action of these insulins. Even intermediate-acting insulins of animal origin have little effect beyond 18–20 h when given at low doses.[54] If there is even less effect at this time period for human intermediate-acting insulin, then perhaps physicians will be forced to abandon their futile attempts to attain glycemic control with a single morning injection of intermediate-acting insulin. Such an approach is clearly inadequate in type I diabetes (except perhaps during the honeymoon period) if excellent control is desired,[55] and is probably not the best insulin regimen for type II diabetic patients treated with insulin.[56,57] Yet, some of the trial patients were treated with two daily injections. The data have not been separately analyzed to determine whether the deterioration in control could be accounted for by those treated with one daily injection. If there was deterioration in control on twice daily regimens as well, this may indicate the need to develop new insulin formulations with differing time courses of action, or new strategies for insulin use. Such might also provide further support for the administration of evening intermediate acting insulin at bedtime rather than prior to supper.[56,58,59]

There may be a sobering lesson buried in the clinical trial data. If one examines the degree of glycemic control, measured by blood glucose or glycosylated hemoglobin, at any stage of the U.S. study,[37] one discovers that this is really a report of poorly controlled, inadequately treated diabetes.

Apart from decreasing the clinical relevance of the study and making it more difficult to interpret, this is a sad commentary on the state of diabetes management in the United States. These patients were being treated at some of the best centers for diabetes and were participants in a research trial, generally a motivating factor. Such an outcome emphasizes the difficulty of successfully controlling diabetes and perhaps indicates that our overall treatment approaches for diabetes should be re-examined.

Case reports and clinical experience. Human insulin has been used in the treatment of diabetic ketoacidosis and hyperosmolar coma,[60] in infusion pumps,[61] and in a variety of special types of patients. Although these have included patients with insulin allergy[62,63] and immunologic insulin resistance,[64] the number of such patients reported is so small that no conclusion can yet be reached as to the efficacy of human insulin in these circumstances, despite the fact that these types of patients might be the ones in whom human insulin would be expected to be particularly beneficial.

Additional reports describe use of human insulin in newly diagnosed patients,[65] patients with receptor abnormalities,[66] and a vegetarian patient who refused animal insulin.[67]

Conclusion. Human insulin produced by recombinant DNA technology has been extensively evaluated in diabetic patients in a series of studies reported in this symposium. An earlier symposium[12] focused primarily on studies of receptor binding, in vitro insulin action, and pharmacologic studies in nondiabetic individuals.

With few exceptions, human insulin has been found to be equivalent to pork insulin. Regular human insulin may be more rapidly absorbed than pork insulin. NPH human insulin may have a shorter duration of action than animal insulins. These differences may have clinical significance. Other differences, such as potency in suppressing C-peptide release, stimulating counterregulatory hormones, or inhibiting lipolysis, are less certain and in need of further statistical evaluation of existing data as well as further studies.

Human insulin is not nonimmunogenic. On the other hand, anti-insulin antibody levels appear to decrease when patients are transferred from animal insulins to human insulin. Studies of patients with immunologic complications of insulin therapy (immunologic insulin resistance, insulin allergy, lipoatrophy) are too few to permit a conclusion as to the efficacy of human insulin in these circumstances. On a theoretical basis, these patients might be considered particularly desirable candidates for therapy with human insulin.

Thus, human insulin produced by recombinant DNA technology appears to be safe and effective in the management of diabetes mellitus. In comparison with purified pork insulin, it may offer no unique advantages. On the other hand, in comparison with beef insulin or mixed beef-pork insulin, human insulin is less immunogenic, and less antigenic in that levels of preformed anti-insulin antibodies decrease on transfer to human insulin. In this regard, it should be noted that most insulin used worldwide is beef insulin that is not highly purified.

Due to their favorable immunogenicity, human insulin or purified pork insulin would seem preferable to beef insulin or mixed been-pork insulin in patients being exposed to insulin for the first time or in whom insulin use is anticipated to be temporary (e.g. type II patients undergoing surgery, or gestational diabetes).

There are no obvious circumstances in which human insulin would appear to be contraindicated. On the other hand, there are no clear indications for switching patients with established diabetes to human insulin, except in the presence of immunologic complications of insulin therapy, as noted above.

The real advantage of human insulin production by recombinant DNA technology is that it provides an inexhaustible resource of a highly purified product. The real excitement of human insulin is that it is the first product of recombinant DNA technology to come to fruition. It marks the first major step in the biotechnology revolution. This technology also offers the theoretical possibility of intentionally altering the amino acid sequence of human insulin to create a series of analogues with different biologic characteristics, as well as for the production and clinical evaluation of human proinsulin and human C-peptide as discussed above.

From the University of Miami School of Medicine, 1475 N.W. 12th Avenue, Miami, Florida 33136.

Address reprint requests to Jay S. Skyler at the above address.

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