Which insulin to use? Human or animal?

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The introduction of insulin was a breakthrough in the treatment of diabetes and it produced a remarkable increase in the life expectancy of diabetic patients. Animal-derived insulins have been used to treat people with diabetes since insulin was first discovered and continuously subjected to various purification technologies. Genetically engineered human insulin was introduced in 1982 and now the vast majority of people requiring insulin treatment worldwide are prescribed synthetic human insulin. Although there exists a debate on which insulin to use, the decision of choosing a particular insulin ultimately falls upon the physician who should make the right choice depending on the diagnosis, expected clinical outcome and the affordability of the patients. This brief document provides an account of historical review of human versus animal insulins and discusses their relative advantages and disadvantages. The choice of insulin selection has to be thoroughly weighed by the physician focusing on the patient's clinical and economic status. However in developing countries like India, there is still a role for continuing animal insulins.

INSULIN injections are needed in all patients with Type 1 (insulin dependent) diabetes right from the time of diagnosis of diabetes. In Type 2 (non-insulin dependent) diabetic patients, insulin is needed in those with primary or secondary failure to oral hypoglycemic agents and at times of stress like infection, myocardial infarction, etc. Insulin is also indicated in diabetes complicating pregnancy and gestational diabetes. The primary goal of treatment of diabetes is maintenance of near-normoglycemia to significantly reduce the risks for long-term microvascular complications of diabetes[1].

Today physicians have the choice of at least 10 to 12 different insulin preparations which are in the Indian market. The newer insulins available today differ from older insulins in purity, homogenicity and price. While conventional insulin preparations, though recrystallized, are often heavily contaminated with other proteins, the new insulins were highly purified or monocomponent, which means that contamination with proinsulin is less than 1 part per million (ppm). Again while the old insulins are mostly mixtures of beef and pork insulins, the new insulins are purified monospecies insulins, either beef, pork, or human structure. With the emergence of recombinant technology, biosynthetic human insulin has become the preferred choice of insulin for many physicians. The issue of whether to use animal or human insulin has been hotly debated in various parts of the country.

A number of attempts world over have been made to answer this question, including a recent Cochrane review[2,3]. This article is an attempt to analyse the scientific data available for various insulin species available in the country including the Cochrane review in order to understand their advantages and disadvantages and to provide clarity on which species to choose when it comes to choosing an insulin.

Origin and preparation

Insulin has been available for the treatment of diabetes mellitus for the past 80 years following its momentous discovery by Banting and Best in 1922. Insulin is effective in restoring normoglycaemia, suppressing ketogenesis and in delaying or arresting diabetic complications[4]. The introduction of insulin was a breakthrough in the treatment of diabetes and it produced a remarkable increase in the life expectancy of diabetic patients. The earliest insulin preparations were obtained from beef pancreas. They were unstable in neutral solution and were provided to patients in powder or tablet form, which was suspended in water or saline immediately before injection. Stable amorphous preparations in acid solution were then developed. The effects of these lasted for only a few hours when injected subcutaneously. Intensive efforts were made to obtain longer acting preparations. Conventional insulins are significantly contaminated with other pancreatic hormones and Insulin precursors (Proinsulin, etc.) and this led to investigations that provided stimulus for further purification of insulin by gel filtration and ion exchange chromatography that yielded highly purified bovine insulins.

Insulins from porcine source have been available from 1923, though most patients were treated with bovine or bovine–porcine mixtures. Arrival of the purer porcine insulins in the Danish market seems to have abolished insulin resistance in Denmark by 1970, but the highly purified porcine insulins did not appear until 1972 (ref. 5). The lack of equivalence of porcine and bovine insulin preparations has always made it difficult to test formally the clinical impression of greater antigenicity of bovine insulins, but comparisons of lente insulins of equivalent

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purity have recently shown this to be the case. The highly purified bovine and porcine insulins available today may not claim to be non-immunogenic, probably because of the formation of zinc aggregates and insulin polymers when they are stored. This is further exacerbated when zinc is complexed with insulin in the preparation of lente insulins and these considerations are likely to apply to insulins of any species.

Around 1976, there was a scare that the demand for insulins was rising so rapidly that it would outstrip the supply and by 1992 there was an acute shortage of insulins worldwide even if measures were taken to raise pigs exclusively for this purpose. Thus it was clear that an alternative to animal insulins was urgently needed. By 1980, human insulin produced by recombinant DNA technology had been introduced, thus ensuring that the world would never run short of insulin supplies.

Two methods of synthesis of human insulin have been developed to the stage of commercial production. Human insulin was produced by recombinant DNA technology by using *E. coli* and yeast ('biosynthetic' human insulin) and the other way by conversion of pork insulin into human insulin by an amino acid substitution ('semisynthetic' human insulin)[6,7]. The semi-synthetic human insulin was initially produced by converting the porcine insulin into human insulin by replacing alanine with threonine at the B30 position (Table 1). 'Human' insulin is so called because structurally and chromotographically it is identical to the insulin produced by the human body and not because it is extracted from the human body!

Purification process

Since 1922, diabetic patients have been treated with insulin preparations extracted from the pancreas of pigs and cattle. Purity of insulin preparations is generally reflected by the amount of non-insulin-pancreatic proteins in the preparation. Proinsulin content is usually used to reflect purity. Insulins are defined as purified when they contain 10 parts per million (ppm) of proinsulin. Clinical problems associated with impurity of insulin preparations include: (1) local and systemic insulin allergies, (2) lipodystrophy at injection sites, (3) immunologic insulin resistance, and (4) altered time course of action due to antibodies[8]. Improved purification in the past decades has resulted in marked improvement in purity of commercially available insulin.

Table 1. Amino acid sequence of human, porcine and bovine insulins

Insulin	A8	A10	B30
Human	Threonine	Isoleucine	Threonine
Porcine	Threonine	Isoleucine	Alanine
Bovine	Alanine	Valine	Alanine

Insulin formulated by recrystallization was only 92% pure, while chromatographically purified 'single-peak' insulin contained up to 10,000 ppm of proinsulin. Further improvements in the production process progressively lowered impurity level of 100 ppm and later this was further reduced to 10 ppm. Simultaneously the purified 'monocomponent' or 'single component' insulins have undergone remarkable improvement such that the purity of these preparations now is less than 1 ppm of proinsulin. In the early years, insulin allergy was common, but during the last five decades, production techniques have become progressively more sophisticated, ultimately leading to the development of highly purified insulins containing less than 1 ppm proinsulin and virtually no other pancreatic peptides. When these preparations are used, especially those of porcine origin, local or systemic insulin allergy, lipodystrophy or immunological insulin resistance occur extremely infrequently[9].

The chemical nature and properties

In 1955, insulin became the first protein to be fully sequenced. That work resulted in a 1959 Nobel Prize for Frederick Sanger. Many proteins have more than one chain, joined together in specific ways. Human insulin has two peptides. The A chain has 21 amino acids and B chain has 30 amino acids. The two chains are connected by two disulphide bridges, bonds formed between the sulphur atoms in the amino acid cystine. The A chain also has a third internal disulphide bridge. The disulphide bridges hold the molecule together. Although the amino acid sequence of insulin varies among species, certain segments of the molecule are highly conserved, including the positions of the three disulphide bonds, both ends of the A chain and the C-terminal residues of the B chain[10].

These similarities in the amino acid sequence of insulin lead to a three-dimensional conformation of insulin that is very similar among species and insulin from one animal is very likely biologically active in other species. Looking at the enzyme in more detail, the sequence of porcine (pig) insulin and human insulin is almost identical, but not exactly – it differs by one amino acid. Bovine (beef) insulin is different by three amino acids from human.

It is of interest to point out that the newly developed insulin analogs like Lispro, Aspart and Glargine; also differ from 'human insulin' in two or more aminoacids as shown in Table 2.

Table 2. Amino acid sequence of human and insulin analogs

Insulin	A21	B28	B29	B31	B32
Human	Proline	Lysine	–	–	Aspargine
Lispro	Lysine	Proline	–	–	Aspargine
Aspart	Aspartic acid	Lysine	–	–	Aspargine
Glargine	Proline	Lysine	Arginine*	Arginine*	Glycine

*The two arginines are added to the C terminus of 15C B chain.

Clinically relevant points

Structure

Due to the fact that the animal insulins (both bovine and porcine) are different from the natural human insulin in their amino acid sequence, it is often argued that this could affect their clinical efficacy. But it appears that none of the amino acid changes are at sites crucial to the binding affinity or action of insulins. Hence there is no significant difference between insulin species in their ability to bind to the receptors and their action. Theoretically changes in amino acid could affect the solubility and diffusion properties of insulin molecules[11].

Indeed, receptor studies have shown that there is complete identity between porcine and human insulins employing equal potency. The parameters measured include receptor number, affinity, association and dissociation kinetics, down regulation negative, comparative and internalization[12].

Purity

The purer the insulin the better it is for clinical efficacy and safety profile. It is generally accepted that purity of insulin preparations is more important for immunogenicity and allergenicity than the species specificity[13]. Purity abolishes and overcomes insulin resistance. Insulin impurities, not the insulin itself, was responsible for the immunogenicity of recrystallized insulin in patients. The relentless efforts made in the past five to six decades have resulted in purification techniques that could yield insulin with less than 1 ppm purity (the monocomponent insulins). Thus today the purity is hardly an issue and in most clinical studies, human insulin was shown to be indistinguishable from porcine insulin of comparable purity with respect to plasma glucose and glycosylated hemoglobin levels and insulin dose requirements[14].

Antigenicity

Essentially all patients who receive insulin for long periods develop antibodies to insulin (even antibodies to endogenous insulin is known). Hence antigenicity will exist even if completely purified insulins are made available, as it is a fundamental property of polypeptide hormones.

Antigenicity is largely related to purity and site of action. Indeed even human insulin preparations made by biotechnological or chemical techniques could be less contaminated by such derivatives (desamido insulin, arginyl insulin, insulin ethylesters). These may well be more difficult to separate from the insulin than the contaminants found in pancreatic extracts. In a clinical study performed by Larkins *et al.*, they found that the human insulin was no less antigenic than porcine insulin; significant IgG associated insulin binding activity was detected in six of the ten patients in the human insulin-treated group and four of ten patients in the porcine insulin-treated group[14].

Clinical efficacy

Clinical efficacy of insulins clearly does not depend on the species of insulin used. A number of clinical trials have clearly shown that animal insulins and human insulins are comparable in their clinical efficacy. It is also claimed that the doses of human insulin required was also less compared to animal insulin, but this was only true when the animal insulins were impure. The duration of action of human insulin is slightly shorter than animal insulins. This is a slight disadvantage as patients on twice daily insulin tend to have higher late evening sugars if on human insulin unless a noon dose is also introduced.

The Cochrane review compared the effects of synthetic human insulin and natural animal insulins in diabetic patients from 1966 to May 2002 (ref. 2). The objective of the review was to assess the effects of different insulin species and evaluating their efficacy (in particular glycemic control) and adverse effects profile (mainly hypoglycemia). For which a highly sensitive search for randomized controlled trials combined with key terms for identifying studies on human versus animal insulin was performed using Cochrane Library (issue 2, 2002), *Medline* (1966 to May 2002) and *Embase* (1974 to Feb 2002) including the reference lists and databases of ongoing trials. They included randomized controlled trials with diabetic patients of all ages that compared human to animal (for the most part purified porcine) insulin. Altogether 2156 participants took part in 45 randomized controlled studies that were discovered through extensive search efforts. At the end of the review, the reviewers made the following conclusions:

(1) A comparison of the effects of human and animal insulin as well as of the adverse reaction profile did not show clinically relevant differences.
(2) No differences were found in metabolic control, and no differences in HbA1c between 'human' and 'animal' insulins.
(3) Many patient-oriented outcomes like health-related quality of life or diabetes complications and mortality were never investigated in high quality randomized clinical trials.
(4) Most of the studies comparing the two insulins were of poor methodological quality.
(5) None of the studies assessed the costs or socio-economic effects.

It can be concluded that both human and animal insulins are equally good and the decision to use one or other of the insulin rests entirely with the physician.

Hypoglycemic events

Switching from one source of insulin to another can cause difficulties in controlling blood sugar level and subsequently patients should have their doses readjusted. It has been reported that some diabetic subjects when switching from animal to human insulin, lose their usual warning symptoms like sweating, tiredness, etc. associated with low blood sugar or 'hypoglycemia. This has been termed 'hypoglycemia unawareness'. It has been claimed by some authors that the clinical symptoms of hypoglycemia while taking human insulin are significantly less pronounced when compared to porcine insulin[15,16]. However, surprisingly these studies were mainly from UK and despite human insulin being exclusively used in USA, Australia and other countries there have been very few reports of hypoglycemia unawareness in these countries. In the author's view also there does not appear to be any hypoglycemic unawareness issue with human insulin given that thousands of patients have been treated with human insulin.

Conclusion

Since the production of recombinant human insulin, numerous studies have been performed to compare its efficacy against that of porcine insulin in treating diabetes. Many of these studies showed that there were no differences in ability to transport glucose, and that neither insulin is less reactive than the other with insulin antibodies. It is unlikely that an improvement in diabetic control can be achieved merely by changing to human insulin. In any case, whether animal or human insulin, insulin therapy should aim to normalize not only blood glucose levels but also the HbA1c (glycosylated haemoglobin), which is an index of blood glucose control for 2–3 months and thus prevent long-term complications of diabetes. Therefore, the decision of choosing a particular insulin ultimately falls upon the physician who should make the right choice depending on the need of the hour and the affordability of the patients.

Currently human insulins are almost twice as expensive as porcine insulin. Hence in developing countries like India, animal insulin should continue to be available. It would be a great pity if patients could not afford the insulins and die merely due to socio-economic reasons. There are moves to produce human insulins in India with the promise of delivering high quality human insulins at low cost. Until this becomes a reality there is place for both human and animal insulins and the choice is entirely left to the physicians and perhaps more importantly the patient. In situations where insulin therapy is only for a short period, e.g. pregnancy, infections, etc. it may be better to use human insulin as cost implications are not that serious. Whenever long-term insulin therapy is encouraged, it is better to check the affordability of the patient and decide which insulin to use.

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