Insulin Dependent Diabetes Trust

The safety of insulin analogues - should patients be concerned?

As a patient-centred, independent charity the Trust has a responsibility to provide people with diabetes with information. The global insulin manufacturers are withdrawing some of the most widely used GM 'human' insulins and recommending treatment with insulin analogues. After very careful consideration, this Supplement has been produced to help inform people with diabetes about the risks and benefits of following this recommendation.

Insulin analogues are new biotechnology products and as such, are likely to have different patterns of toxicity with unknown consequences. The long-term effects and safety of insulin analogues have not been established.

The clinical benefits of insulin analogues have been extensively studied and have proved to be negligible in terms of glycaemic control but the biological effects have *not* been systematically studied despite their carcinogenic potential being recognised by the scientific community.

People with diabetes are no exception to the principle that they should have an informed choice of treatment including risks and benefits but the majority of them are not aware of the carcinogenic potential of insulin analogues or that their long-term safety has yet to be established.

Some patients may consider even a minimal carcinogenic risk with insulin analogues is unacceptable when there is little or no benefit in day to day blood glucose control.

"The carcinogenic potential of insulins is recognised by the scientific community."

Gupta K, et al
Am J Med Sci. 2002; 323(3)

"Recent publications concerning the assessment of carcinogenic potential of specific human insulin analogues are scarce"

Stammberger I, et al
Int J Toxico. 2002;21(3)

"People are being prescribed too many drugs, before the full consequences of adverse side effects are known."

"Tighter controls on the promotion of new drugs should be introduced until more is known of their potential side effects."

"Post-marketing surveillance in the UK is inadequate. This has several causes: lack of investigation of a drug's benefits and harms in real life situations and institutional indifference to the experience and reports of medicines users."

House of Commons
Health Committee Report, April 5th 2005
The Influence of the Pharmaceutical Industry

What are insulin analogues?

Insulin analogues are artificial derivatives of the natural hormone insulin and are designed to have different absorption profiles compared to GM 'human' insulins. Short-acting insulin analogues [eg Humalog and NovoRapid] are absorbed more quickly and are of shorter duration than GM 'human' insulin and long-acting analogues [Lantus, Levemir] are designed to have a longer action with a more consistent release during the day.

National Institute of Clinical Excellence [NICE] Guidance on the use of long-acting insulin analogues - insulin glargine [Lantus] December 2002

1. Insulin glargine [Lantus] is recommended as a treatment option for people with Type 1 diabetes
2. Insulin glargine is not recommended for routine use for people with Type 2 diabetes who require insulin therapy. It should be considered only for those people with Type 2 diabetes who require insulin therapy and who fall into the following categories:
    - Those who require assistance from a carer or healthcare professional to administer their insulin.
    - Those whose lifestyle is significantly restricted by recurrent symptomatic hypoglycaemic episodes.
    - Those who would otherwise need twice daily basal insulin injections in combination with oral anti-diabetic drugs.

Cost

NICE estimates that 137,000 people in the UK would be eligible for insulin glargine treatment. The extra cost of glargine per annum for Type 1 diabetes is £101 compared to NPH [long-acting human insulin] and £162 for Type 2 diabetes compared to NPH. If all the potentially eligible people were changed to glargine, then this would cost the NHS around £16million per annum. These costs are based on vial costs and so would be increased with use of the more expensive cartridges.
Note: Determir [Levemir] is a similar cost to glargine.

GLOSSARY OF TERMS

Apoptosis - normal self-induced termination of a cell's life, to become replaced by a new one.
Carcinogenic - a substance that has cancer forming properties.
Carcinoma - a type of cancer.
Co-morbidity - the presence of several diseases/conditions.
Endpoints - a research term that defines what is being measured in the study to show the outcomes of a treatment.
Hexamers - the bonding together of insulin molecules forms a six-pack [hexamer] but only individual insulin molecules are biologically active so the body must first break the six-pack.
IGF-1 or insulin-like growth factor - a hormone which has a broad range of effects including promotion of cell survival, cell proliferation of cells, inhibition of apoptosis, stimulation of metabolism.
Insulin receptors - insulin receptors are the chemical structures on cells, where insulin binds to the cell and where insulin can get its messages inside the cell.
In-vitro testing - literally means 'in glass' and is a research term for observations made outside the body eg the action of drugs on bacteria, in-vitro fertilisation means the fertilisation of the egg outside the body

In-vivo testing - studying something in living creatures [human beings and animals]
Monomers - single insulin molecules
Mitogenicity - promotion of the division and proliferation of any cell, including malignant and non-malignant tumour cells.
Neoplasm - another word for a tumour that literally means 'new formation'
Sprague-Dawley rats - a type of rat used in research into the possible development of breast tumours because of its high spontaneous incidence rate of breast cancer ie the type of rat used is the one that is most likely to produce breast tumours if this risk is present.
Subcutaneous injection - injection into the tissue beneath the skin.
Thrombocytes - blood platelets involved in coagulation to stop bleeding
Toxicity - the poisonous effects of a substance.

Use of terminology when referring to insulins

Genetically engineered, genetically modified and GM are used interchangeably throughout this document. The same applies to the names of insulin and their brand names:

Name of insulin	**Brand Name**
Insulin glargine	Lantus
Insulin aspart	NovoRapid [NovoLog in the US]
Insulin lispro	Humalog
Insulin determir	Levermir

NPH - **N**eutral **P**rotamine **H**agedorn also referred to as isophane insulin and is the most commonly used long-acting insulin in the UK.

This Supplement contains our concerns in two versions. This first version uses layman's language and is less technical but it is based on the more technical version that follows which provides more detail and the supporting references.

Regulatory requirements for insulin analogues: weighing therapeutic benefits against potential carcinogenicity

Authors:
Prof. Dr. med. Ernst Chantelau
Department of Endocrinology, Diabetes and Rheumatology
Diabetesambulanz MNR-Klinik
PO Box 10 10 07
D- 40001 Dusseldorf
Germany

Mrs Jenny Hirst
Co-Chairman
Insulin Dependent Diabetes Trust
PO Box 294
Northampton
UK

Background

In September 2004, the commonly used arthritis drug Vioxx was withdrawn from the market after it became publicly known that patients taking it were at twice the relative risk of heart attack and stroke as those taking a placebo or dummy pill. During the five years that it was on the market, it is likely that many people suffered heart attack, even death, and stroke. Questions have been raised about the effectiveness of the regulatory system and how long this information was known prior to drug's subsequent withdrawal.
Adverse events associated with the effects of the anti-depressant, paroxetine [Seroxat] highlighted in the BBC programme, Panorama, resulted in the Committee on Safety of Medicines [CSM] issuing a safety statement about the recommended dose. However, this statement was not based on new evidence but *"on a review of the original dose finding studies carried out for the licensing of paroxetine".* In other words, information the CSM had before the drug reached to market and once again lives were unnecessarily harmed or lost as a result of this system failure.
These situations necessarily raise questions about the effectiveness of drug regulatory authorities, their speed of response and their vigilance. They demonstrate the need for more effective research prior to a drug reaching the market and of equal importance, the need for improved post-marketing surveillance when new drugs are used on the wider population. Patients are rightly concerned about the safety of the drugs they take and the systems in place to ensure their short and long-term safety and effectiveness. These needs are especially important with the introduction of new biotechnology products, such as insulin analogues, which can have unpredictable adverse effects.

Drug toxicity

There is a now a wide range of drugs available and the pattern of drug toxicity is likely to change with the introduction of new biotechnology products.
Drug toxicity:

- may vary
- may affect any organ system
- the different genetic make up leads to different drug responses between people
- the clinical consequences of individual drug response can be great
- new biotechnology products are likely to have different patterns of toxicity compared to the more predictable ones of chemically produced drugs.

An early example of this was genetically engineered tryptophan introduced in 1988 and withdrawn within months because it was associated with 37 deaths and 1500 people being permanently disabled. Genetic engineering was implicated because the toxin responsible has never been shown to be present in the non-genetically engineered tryptophan that was used for many years without these adverse effects.

Adverse reactions
Adverse reactions can occur immediately or within weeks, months or years after starting to take a drug. Reporting suspected adverse reactions [ADRs] is not a mandatory requirement for physicians and health professionals. So spontaneous reporting schemes of suspected ADRs, such as the Yellow Card Scheme in the UK, are the cornerstone of post-marketing surveillance and are still the only way of monitoring the safety of a drug throughout its life. The problem with spontaneous reporting is that less than 10% of all serious and only 2-4% of non-serious suspected ADRs are actually reported.
Thus with 90% of serious adverse drug reactions going unreported, it is unsurprising that patients are concerned about the safety of drugs and the systems in place for monitoring them.

Introduction of genetically engineered insulin

In 1982, genetically engineered insulin, misleadingly named 'human' insulin, was the first drug genetically engineered drug to be marketed. In common with tryptophan, there was a failure to recognise that drugs produced by biotechnology could have different patterns of toxicity and it appears that when giving marketing approval, the regulatory authorities considered the method of manufacture to be unimportant. It is possible that assumptions about the safety of GM 'human' insulin could have been made because its predecessor, natural animal insulin, had an excellent and long history of safety.
During the 1980s in the UK over 80% of people with insulin-requiring diabetes were changed to genetically engineered 'human' insulin for no clinical reasons but on *assumptions* of its superiority, and not on *evidence* of its superiority, over natural animal insulin previously used. An estimated 10% of people reported, and continue to report, adverse effects when using GM insulin but patients' reports of adverse reaction reports were largely ignored and GM 'human' insulin became first line treatment for people with insulin requiring diabetes.
No long-term, large-scale studies to compare GM 'human' and animal insulin have ever been carried out and in 2002 a Cochrane Review showed that the vast majority of the studies that have been carried out are of methodologically poor quality. It also showed that many of the important issues for patients such rates of diabetes complications and mortality and quality of life issues were never investigated in high quality randomised clinical trials. So 'human' insulin was given marketing authorisation:

- without consideration of the method of manufacture and possible unexpected adverse reactions
- with little attention paid to the quality of the post-marketing studies

- without any long-term studies to compare complication and mortality rates or long-term safety.

However patients were not made aware of these facts or their rights to a choice of insulin treatment, so they are understandably concerned about the quality and validity of information they receive and the drug monitoring systems in place, supposedly for their protection.

Introduction of insulin analogues

Insulin analogues are the most recent biotechnology products used in the treatment of diabetes. They are artificial derivatives of the natural hormone human insulin. They were designed to have absorption profiles that more nearly mimic the action of normal insulin production by the body compared to artificial 'human' insulin. In short-acting 'human' insulin the individual insulin molecules clump together [aggregate], six at a time, to form a hexamer. Only individual insulin molecules are biologically active, so the body must first break the six pack into individual molecules [monomers]. But in analogues the hexamers bind together so weakly that they break apart much faster making the insulin molecules biologically active immediately.

However analogues also differ in their biological effects with unknown consequences, such as their effects on:

- mitogenicity [promotion of division and proliferation of any cell, including tumour cells]
- apoptosis [see glossary]
- glucose and lipid metabolism
- thrombocyte function
- protein degradation

The therapeutic effects of analogues have been extensively investigated and have shown negligible clinical benefit for patients but the biological effects have not been systematically studied. It is of special concern that the carcinogenic potential of insulin analogues remains to be determined on human carcinoma tissue in accordance with the recommendations issued by the European Agency for the Evaluation of Medical Products [EMEA] in their document, Points to consider CPMP/SWP/372/01.

While scientists have used analogues to study the insulin molecule, insulin manufacturers were more interested in their commercial potential and in 1988 Novo Nordisk announced the development of their prototype analogue, B10Asp. By virtue of a slight modification of the human insulin molecule B10Asp did not aggregate as much as regular 'human' insulin and was absorbed from the subcutaneous tissue 15minutes earlier. B10Asp was absorbed into the circulation significantly faster and with higher peak concentrations than 'human' insulin but a controlled trial failed to show that B10Asp had any benefit in terms of glycaemic control when compared to 'human' insulin.

From this study it was obvious by 1995 that manipulations of the subcutaneous absorption of rapid-acting [regular] insulin have only very little clinical impact on HbA1c and may explain why analogue insulin produces only less than 5% of the total variations in HbA1c. Much greater percentages of the total variation in HbA1c is accounted for by:

- the size of the insulin dose
- the amount and timing of carbohydrate intake
- the timing of exercise in relation to carbohydrate intake and/or insulin administration
- the effects of stress or intercurrent illness
- psychosocial aspects

- residual beta cell function [the amount of insulin that is still produced by the body's own cells].

All clinical trials with B10Asp were stopped in 1992 when it was shown to promote breast cancer in rats. Nevertheless, in 1996 the first rapidly absorbed insulin analogue, lispro [Humalog], reached the market amidst warnings from Professor Stephanie Amiel [Diabetic Medicine, 1998;15;537-538] that *"there remains a risk of unexpected problems with any new agent and we should remember that the structure of the new insulin is a little closer to IGF than the old insulin".* The closeness to IGF-1 is important because it has broad range of effects including promotion of cell survival, cell proliferation of cells, inhibition of apoptosis, stimulation of metabolism.

In 2000 the first slowly absorbed long-acting insulin analogue, glargine [Lantus] was introduced. It has a flat, apparently peakless activity, and a duration of 24hours. This was followed in 2004 by the introduction of determir [Levemir], a once or twice daily long-acting insulin analogue.

Treatment Goals

Good control is not only the avoidance of high blood glucose levels [hyperglycaemia] but also the avoidance of low blood glucose levels [hypoglycaemia]. Hypoglycaemia is a daily fear of people with diabetes so a reduction in hypoglycaemic events can improve quality of life. It is therefore important to look at the effects of insulin analogues on hyper- and hypoglycaemia.

Short-acting insulin analogues

As could have been expected from the B10Asp study, Humalog and NovoRapid have barely shown any clinical benefits over GM 'human' insulin in terms of blood glucose levels as measured by HbA1c and daily blood glucose tests.
Studies comparing control of hypoglycaemia for Humalog and NovoRapid with GM 'human' insulin showed the following:

Type of hypoglycaemia	**Number of studies analysed**	**Effects of using Humalog or Novolog/NovoRapid**
Frequency of mild hypoglycaemia	22 studies	Reduction in 5 studies
Frequency of severe hypoglycaemia	12 studies	No change in 10 studies
Frequency of nocturnal hypoglycaemia	24 studies	Reductions in 6 studies (19)

A Cochrane Review [2004] of short-acting analogues supported all the above findings and concluded that:

- short-acting insulin analogues have only a minor benefit of short acting insulin analogues in the majority of diabetic patients treated with insulin
- until long term efficacy and safety data are available we suggest a cautious response to the vigorous promotion of insulin analogues
- due to fears of potentially carcinogenic and proliferative effects, most studies to date have excluded patients with advanced diabetic complications
- for safety purposes, a long-term follow-up study of large numbers of patients who use short acting insulin analogues is needed.

Long-acting insulin analogues

The clinical benefits of glargine [Lantus] as measured by HbA1c were small in comparison to GM 'human' insulin and when compared to twice daily long-acting [NPH] 'human' insulin there was no difference in hypoglycaemia.
At the time of writing, detemir [Levemir] has only been on the market a short time but manufacturers information shows little, if any, improvement in HbA1c and it is claimed that there is less weight gain when compared to 'human' insulin.

Studies carried out on selected patient groups
It is important to note that all clinical studies with insulin analogues have been conducted on carefully selected patient groups that have excluded all those with diabetic complications and any other conditions. So the effects of insulin analogues on people with these conditions is not known. Equally unknown are the effects of insulin analogues on mortality and other hard endpoints like blindness, amputation and end stage renal disease.

It is worth noting that the Drugs and Therapeutics Bulletin [Oct 2004 Vol 41;No10] reported on the use of insulin analogues as first line treatment: *"In our view, this approach is not justified given that what still needs to established about the analogues - long-term benefits and safety. Also there is no convincing evidence to justify switching patients from existing conventional therapy to analogues if they have appropriate glycaemic control without troublesome hypoglycaemia."*

Safety issues - the potential for carcinogenic effects

As discussed, the rapid acting analogues B10Asp was shown to induce or promote breast cancer in Sprague-Dawley rats [type of rat used because it is most likely to produce breast cancer if a risk is present]. B10Asp was called the 'super-mitogen' and subsequent analogues reaching the market have been measured against this for their carcinogenic potential.
It was increasingly recognised that changing the physico-chemical properties of the GM 'human' insulin molecule will inevitably change its biological properties although manufacturers tried to play down the potential risks. Long before the European Medicines Evaluation Agency [EMEA] was asked to approve long-acting insulin analogue, glargine, it was found to be highly mitogenic [caused cell proliferation] on in-vitro testing with human osteosarcoma cells [cancerous cells from tissue surrounding bone]. On February 17, 2000 this information, still unpublished, was presented to the EMEA by the manufacturers, Aventis, in an oral explanation. The EMEA accepted the company's claim that this information was irrelevant and subsequently approved the drug. In June 2000, a paper publicly disclosed the mitogenicity of insulin glargine [Lantus] on osteosarcoma cells and in June 2001 Aventis publicly confirmed this information in an abstract presented to the American Diabetes Association.
Recently even more abnormal biological actions of insulin analogues as compared to 'human' insulin have been identified by various researchers:

- Humalog and NovoRapid /NovoLog inhibit thrombocyte function
- Humalog inhibits apoptosis in tumour (insulinoma) cells and protein degradation.
- A new insulin analogue, insulin Glusilin (Aventis) inhibits apoptosis in tumour (insulinoma) cells.
- Lantus, but not Humalog, increases serum IGF-1 concentrations in diabetic patients.
- On the receptor level eg of osteosarcoma cells, rat cardiomyocytes, human skeletal muscle cells, Lantus binds less to the insulin receptor and more to the IGF-1 receptor than does human insulin, and causes abnormal post-receptor signalling compared to human insulin.

[Published data on NovoRapid /Novolog are scarce].

In most instances, the animal toxicology experiments presented to the drug regulatory boards [the EMEA and the FDA] for the approval of insulin analogues were flawed. The experiments were not in accordance with the EMEA 2001 recommendations and are not suitable to rule out clinically relevant carcinogenicity of these insulins.

- Humalog was studied in rats which do not develop breast cancer (Fischer 344 rats
- Lantus was studied in dosages much lower than those of B10 Asp that induced breast cancer in cancer-prone rats
- the exposure time of the rats against Lantus was too short, as many rats died from hypoglycaemia before the end of planned 24-months observation period.
- Standard 2-year carcinogenicity studies in animals have not been performed or published to evaluate the carcinogenic potential of Humalog and NovoRapid/NovoLog.

Toxicology studies

Insulin analogue	**Experimental design**	**Dosage**	**Duration**	**Outcome**
B10 Asp	Sprague-Dawley rats	20-200 U/kg	12 months	breast cancer+++, dose-related
Humalog	344 Fischer rats	20-200 U7kg	12 months	no breast cancer
Lantus	Sprague-Dawley rats	5-12.5 U/kg	<24 months	malignant fibrohisticytoma++ malignant lymphoma (+)
NovoLog NovoRapid	Sprague-Dawley rats	10-200U/kg	12 months	breast cancer with 200 U/kg, significant difference to untreated controls, no significant difference to regular human insulin

Conclusions

Insulin analogues are new biotechnological products with unknown biological effects.

The actions of natural insulin in humans and animals has been brought about by evolution over millions of years and the delicate balance between its metabolic and mitogenic efficacy functions very well in every species in order to maintain survival. This cannot be said of artificial insulin analogues that interfere with this balance in unpredictable and unknown ways.

This lack of information prompted the EMEA [2001] to call for better pre-clinical testing of insulin analogues in order to definitely rule out any relevant carcinogenicity of these compounds. The 'Points to consider document

CPMP/SWP/372/01 on the non-clinical assessment of the carcinogenic potential of insulin analogues states:
"Native human insulin has, in addition to its metabolic actions, a weak mitogenic effect. This effect has become important for the safety of insulin analogues,……since structural modifications of the insulin molecule could increase the mitogenic potency, possibly resulting in growth stimulation of pre-existing neoplasms..."
"Although enhanced insulin-like growth factor 1(IGF-1) receptor activation and/or aberrant signalling through the insulin receptor have been implicated, the mechanism(s) responsible for the mitogenic activity of insulin analogues remain to be clarified..."

According to this same EMEA document, insulin analogues should be investigated on neoplastic [tumour] tissue rather than on non-neoplastic [normal] tissue, including in-vivo studies with tumour tissues transplanted on immunodeficient animals:
"Since there is evidence that receptor in neoplastic [tumour] tissues may react differently from those in normal tissues, it is desirable that the choice of test systems will cover testing of mitogenicity in non-neoplastic as well as neoplastic tissues."
"Due to substantial background data on spontaneous tumour incidence, the rat may be considered a suitable species and in view of the responsiveness to AspB10....at present the Sprague-Dawley rat may be thought of as first-hand choice. ... other species or models, like the promotion of established human tumour cell lines grafted on immunodeficient animals might be considered."

As evidence that IGF-1 promotes colonic-, breast-, prostatic-, and lung cancer growth is accumulating, it is mandatory that insulin analogues should be studied preferably on these neoplastic tissues. However, none of these investigations have so far been carried out or published.

In a public meeting on May 5, 2004 Professor Jürgen Eckel, Germany, announced that he is to carry out a systematic investigation of the mitogenic potency of insulin analogues. However, it will take years for the results of this investigation to be completed and published. Unless cancer growth promotion is properly excluded, the safety of insulin analogues will remain unknown and patients will be unable to assess their risks and benefits in order to make an informed choice of treatment. If patients safety is to be protected and their rights to an informed choice is to be respected, it is essential that patients are provided with the facts as they stand. When the clinical benefits of insulin analogues have proved to be negligible in terms of diabetes control, even a minimal carcinogenic risk could be classed as unacceptable by some patients.

Regulatory requirements for insulin analogues: weighing therapeutic benefits against potential carcinogenicity

Summary

Recent events with Cox-2 inhibitors have demonstrated that there is a need for greater effort into research before a new drug reaches the market and for improved post-marketing surveillance. There is a wide range of drugs available and the pattern of toxicity is likely to change with the introduction of new biotechnology products.

Insulin analogues are just such a product as they are artificial derivatives of the natural hormone insulin, designed to improve the absorption profiles compared to human insulins after subcutaneous injection. However, analogues also differ from human insulin in their biological effects such as, effects on mitogenicity, apoptosis, glucose and lipid metabolism, thrombocyte function and protein degradation, with unknown consequences. While the therapeutic effects have been investigated extensively and found to be negligible, the biological effects of insulin analogues remain to be systematically studied. Of special concern is that the carcinogenic potential of insulin analogues remains to be determined on human carcinoma tissue, according to the recommendations issued by the European Agency for the Evaluation of Medicinal Products (EMEA Points to consider document CPMP/SWP/372/01).

Introduction

Spontaneous adverse drug reactions reporting schemes, such as the Yellow Card Scheme in the United Kingdom, are the cornerstone of post-marketing drug safety surveillance and remain the only way of monitoring the safety of a drug throughout its life on the market. A problem with spontaneous reporting is that less than 10% of all serious and 2-4% of non-serious adverse reactions are reported (1). It must be hoped that the recent introduction in the United Kingdom of patients being able to report adverse drug reactions (2) will improve the number of reports and the post-marketing surveillance system, assuming that patients' reports carry the same weight as those from doctors and healthcare professionals.

Adverse events associated with the effects of the anti-depressant. paroxetine [Seroxat] highlighted in the BBC programme, Panorama, resulted in the Committee on Safety of Medicines [CSM] issuing a safety statement about the recommended dose (3) in March 2003. However, this statement was not based on new evidence but *"on a review of the original dose finding studies carried out for the licensing of paroxetine".* In other words, information the CSM hadthe information before the drug reached the market and once again lives were unnecessarily harmed or lost as a result of this system failure.

Pirmohamed et all point out (1) that there is a wide range of drugs available and the manifestations of drug toxicity may vary, may affect any organ system and that the pattern of toxicity is likely to change with the introduction of new biotechnology products. An early example of this was the introduction of genetically engineered tryptophan in 1988, withdrawn within months because it caused 37 deaths and 1500 people to be permanently disabled (4). Genetic engineering was implicated because the toxin responsible had never been shown to be present in non-genetically engineered tryptophan that had been used for many years without these adverse effects.

Human insulin was the first genetically engineered drug to be marketed in 1982. In common with tryptophan, regulatory authorities considered the method of

production to be immaterial because natural animal insulin had one of the best safety profiles on the market with the only major side-effect being hypoglycaemia, technically caused by overdose (5). In effect regulatory authorities considered the new laboratory produced human insulin to be substantially equivalent to natural animal insulin used for more than 60 years. Patients were changed to the new 'human' insulin not for clinical reasons but on the assumption of superiority and not evidence of its superiority over its animal insulin predecessors.

An estimated 10% of patients reported, and continue to report, adverse effects when using genetically engineered human insulin. Despite awareness that genetic variability leads to differences in drug response between individuals (6) and that the clinical consequences of individual variation in drug response can be great, the adverse reactions with genetically engineered insulins have been largely ignored and they have become first-line treatment for people requiring insulin. However, there have been no large-scale studies to compare human and animal insulins and the vast majority of studies that have been carried out are classed as being of 'poor methodological quality' in a Cochrane Review (7). Not only was human insulin given marketing authorisation without consideration of the method of manufacture but post marketing studies have been of poor quality.
With this background for such a widely prescribed, and therefore highly profitable product as insulin, it is unsurprising that the rofecoxib and paroxetine situations have arisen. If patients are to be protected, regulatory bodies need to reconsider drugs that have already received marketing approval and this is particularly applicable to the more recently developed insulin analogues.

Hundreds of human insulin derivatives, nowadays called analogues have been designed by chemists since the molecular structure of human insulin became known in the 1960s (8,9). While scientists were using these compounds to study structure-function relationships of the insulin molecule, insulin manufacturers were interested in their commercial potential after recombinant DNA biotechnology had opened the way for industrial production. Novo Nordisk announced the production of insulin analogues for therapeutic purposes in 1988 (10). Their prototype analogue, B10Asp, was designed to aggregate less than regular human insulin in pharmaceutical preparations. Pharmaceutical regular human insulin molecules aggregate in the vial to hexamers which, after subcutaneous injection, must disintegrate to insulin monomers before they can enter the circulation. This process of hexamer disintegration takes about 10-15 minutes inside the subcutaneous fat tissue. There is no such time lag after intramuscular injection, perhaps due to the better vascularisation of muscular tissue, and hence faster wash-out of injected insulin. The analogue B10 Asp, by virtue of a slight modification of the native human insulin molecular structure, did not aggregate as much as regular human insulin and was absorbed from the subcutaneous tissue about 15 minutes earlier than human regular insulin.

However, a controlled trial failed to show any benefit in terms of blood glucose regulation of B10Asp versus regular human insulin, although B10Asp was absorbed into the circulation significantly faster and with higher peak concentrations than human insulin (11). From this study it was obvious in 1995 that manipulations of the subcutaneous absorption of rapid acting (regular) insulin have only very little clinical impact on HbA1c, and may explain only less than 5% of total variation in HbA1c. Much greater percentages of the total variation in HbA1c are accounted for by the size of the insulin dose, the amount and timing of carbohydrate intake, the timing of exercise in relation to the carbohydrate intake and/or the insulin application, effects from stress or intercurrent illness on insulin sensitivity, psychosocial aspects and residual ß-cell function (12).

All clinical trials with B10Asp were suspended in 1992, when this compound was shown to promote breast cancer in rats (13). Nevertheless, rapidly absorbed 'monomeric' regular insulin analogue Lispro (Humalog®) was launched in 1996 and reached the UK market in 1998 when Amiel (5) warned that there remains a risk of unexpected problems with any new agent and "we should remember that the structure of the new insulin is a little closer to IGF structure than the old insulin".

Therapeutic potentials

Clinical superiority of Humalog® over human insulin in terms of blood glucose regulation with HbA1c and blood glucose daily profiles was barely detectable (14), as could have been expected from the B10Asp study (11). The same holds true for another insulin analogue, Aspart (Novolog®/ NovoRapid®) as despite its faster subcutaneous absorption, the effects on blood glucose regulation were very similar to those of regular human insulin. In 2000, Aventis launched a slowly absorbed insulin analogue, Glargine (Lantus®); again, the clinical benefits in comparison to human insulin were small (Table 1).

Table 1: Effect of insulin analogues on controlling hyperglycaemia

Aspart (NovoRapid® NovoNordisk) + NPH human insulin versus regular human insulin + NPH human insulin

Number of patients in studies	Changes in HbA1c measurements
1070 Type 1 diabetic patients in Europe:	HbA1c - 0.12% within 6 months
884 Type 1 diabetic patients in USA:	HbA1c - 0.15% within 6 months, -0.14% within 12 months

(NovoNordisk, scientific information on NovoRapid®/NovoLog 1999, 2000 (15))

Glargine (Lantus®,Aventis) + regular human insulin versus NPH human insulin + regular human insulin

Number of patients in study	Changes in HbA1c measurement
333 Type 1 diabetic patients	HbA1c – 0.14% versus NPH human insulin (15,16)

Glargine (Lantus®, Aventis) + insulin Lispro (Humalog®, Lilly) versus NPH human insulin + insulin lispro

Number of patients in study	Changes in HbA1c measurement
619 Type 1 diabetic patients	HbA1c no statistically significant difference (17)

Note: note that a -0.15% change in HbA1c translates into 5mg/dl (0.27mmol/l) change in blood glucose (18)

Table 2: Effect of insulin analogues on controlling hypoglycaemia

Humalog® or Novolog®/NovoRapid®, versus regular human insulin

Type of hypoglycaemia	Number of studies analysed	Effects of using Humalog® or Novolog/NovoRapid®

Frequency of mild hypoglycemia	22 studies	Reduction in 5 studies
Frequency of severe hypoglycaemia	12 studies	No change in 10 studies
Frequency of nocturnal hypoglycaemia	24 studies	Reductions in 6 studies (19)

Glargine (Lantus®) once per day versus NPH human insulin twice per day
No difference in hypoglycaemia (16)

In summary, the beneficial effects of insulin analogues on control of hyper- and hypoglycaemia in diabetic patients were nearly nil. A previous review article (20), and a most recent Cochrane review have come to the same result (21). All clinical studies with insulin analogues had been performed in carefully selected patient groups, excluding those with diabetic complications and co-morbidity. Hence the effects of analogues on these conditions are not known, nor the effects on mortality and other hard endpoints like blindness, amputation, end stage renal disease.

Safety issues: carcinogenic potential

After B10Asp was shown to induce or promote breast cancer (13) in Sprague-Dawley rats, which have a high spontaneous incidence rate of breast cancer and this insulin analogue was called "super-mitogen" (22), it was increasingly recognised that changing the physico-chemical properties of the human insulin molecule will inevitably change its biological properties:
"Mutation of the insulin molecule through recombinant DNA technology has produced 'monomeric' insulin, which does not form hexamers and is therefore more readily absorbed following subcutaneous injection. The pharmacokinetics and biological actions are thus altered... " (23)

However, the manufacturers tried to play down potential risks with the manufacturers claiming that the biological differences of Humalog® to human insulin were not harmful:
"Insulin receptor binding: equipotent to insulin IGF-1 receptor binding: approx.160% as potent as insulin DNA synthesis: marginally more potent than insulin(approx.1-4x); possibly explained by enhanced IGF-1 receptor affinity" (24).

Insulin Glargine (Lantus®) was found to be highly mitogenic on in-vitro testing with human osteosarcoma cells long before the EMEA had been asked for approval of the compound (25). On February 17, 2000 this information, still unpublished, was reported to the EMEA in an oral explanation by Aventis. The EMEA accepted the company's claim that the finding was irrelevant, and subsequently approved the drug (24). A paper (26) in June 2000 publicly disclosed the mitogenicity of insulin Glargine on osteosarcoma cells and in June 2001, Aventis publicly confirmed this information in an abstract presented to the ADA (27):

"Lantus® binds more actively to IGF-1 receptors: In human hepatoma cells (Hep G2), Lantus® affinity for the IGF-1 receptor was 5-7 fold relative to human insulin...In human osteosarcoma cells, IGF-1 receptor affinity of Lantus® was 3.5-7.6 fold relative to human insulin....in a second study on osteosarcoma cells, IGF-1 receptor affinity of Lantus® was 14 fold relative to human insulin....and

thymidine uptake (i.e. incorporation into DNA) in response to Lantus® was 6.1 fold higher compared with human insulin.. "(27)

Recently, even more abnormal biological actions of insulin analogues (as compared to human insulin) have been identified by occasional investigations of various researchers. Humalog® and NovoRapid®/NovoLog® inhibit thrombocyte function (28,29); Humalog® inhibits apoptosis in tumour (insulinoma) cells (30), and protein degradation(31). A new insulin analogue, insulin Glusilin (Aventis) inhibits apoptosis in tumour (insulinoma) cells (30). Lantus®, but not Humalog®, increases serum IGF-1 concentrations in diabetic patients (32,33). On the receptor level e.g. of osteosarcoma cells, rat cardiomyocytes, human skeletal muscle cells, Lantus® binds less to the insulin receptor and more to the IGF-1 receptor than does human insulin, and causes abnormal post-receptor signalling compared to human insulin (21,29,34). Published data on NovoRapid®/Novolog® are scarce (26,35,36).

The animal toxicology experiments, presented to the drug regulation boards, such as the FDA or EMEA, for approval of the insulin analogues (Table 3), in most instances were flawed, and not in accordance with the recommendations issued by the EMEA 2001. These experiments are not suitable to rule out clinically relevant carcinogenicity of these compounds. Humalog® was studied in rats which do not develop breast cancer (Fischer 344 rats (37)), while Lantus® was studied in dosages much lower than those of B10 Asp (13) that had induced breast cancer in cancer-prone rats(38). Furthermore, the exposure time of the rats against Lantus® was too short, as many rats died from hypoglycaemia before the end of planned 24-months observation period.

Table 3 Toxicology studies

Insulin analogue	**Experimental design**	**Dosage**	**Duration**	**Outcome**
B10 Asp (6)	Sprague-Dawley rats	20-200 U/kg	12 months	breast cancer+++, dose-related
Humalog® (29)	344 Fischer rats	20-200 U7kg	12 months	no breast cancer
Lantus® (30)	Sprague-Dawley rats	5-12.5 U/kg	<24 months	malignant fibrohisticytoma++ malignant lymphoma (+)
NovoLog® (31)	Sprague-Dawley rats	10-200U/kg	12 months	breast cancer with 200 U/kg, significant difference to untreated controls, no significant difference to regular human insulin

Standard 2-year carcinogenicity studies in animals have not been performed or published to evaluate the carcinogenic potential of Humalog® and NovoLog ®(39).

Conclusion

Insulin analogues are new biotechnological pharmaceuticals with unknown biological effects. Natural insulin, be it human or animal insulin, has been brought about by evolution over millions of years; its delicate balance between metabolic and mitogenic efficacy is very well functioning in every species to maintain survival. This cannot be said of artificial insulin analogues, which interfere with this balance in an unpredictable way. This lack of information prompted the EMEA in 2001 to call for better pre-clinical testing of insulin analogues in order to definitely rule out relevant carcinogenicity of these compounds (40).
"Native human insulin has, in addition to its metabolic actions, a weak mitogenic effect. This effect has become important for the safety of insulin analogues, i.e. compounds derived from insulin with a molecular composition and/or structure that has been modified as compared to native human insulin, since structural modifications of the insulin molecule could increase the mitogenic potency, possibly resulting in growth stimulation of pre-existing neoplasms..."
"Although enhanced insulin-like growth factor 1(IGF-1) receptor activation and/or aberrant signalling through the insulin receptor have been implicated, the mechanism(s) responsible for the mitogenic activity of insulin analogues remain to be clarified..."(40)

According to the EMEA document (40), insulin analogues should be investigated on neoplastic rather than on non-neoplastic tissues, including in-vivo studies with tumour tissues transplanted on immunodeficient animals.
"Since there is evidence that receptor in neoplastic tissues may react differently from those in normal tissues, it is desirable that the choice of test systems will cover testing of mitogenicity in non-neoplastic as well as neoplastic tissues."
"Due to substantial background data on spontaneous tumour incidence, the rat may be considered a suitable species and in view of the responsiveness to AspB10....at present the Sprague-Dawley rat may be thought of as first-hand choice. ... other species or models, like the promotion of established human tumour cell lines grafted on immunodeficient animals might be considered." (39)

Since evidence is accumulating that IGF-1 promotes colonic-, breast-, prostatic-, and lung cancer growth (41) it is mandatory that insulin analogues should be studied preferably on these neoplastic tissues. However, neither of these investigations have so far been carried out or published. In a public meeting on May 5,2004 Professor Jürgen Eckel, Germany (22,30) announced that he is about to start a systematic investigation of the mitogenic potency of insulin analogues. It will take years for the results of this investigation to be completed and published.

Unless cancer growth promotion is properly excluded, the safety of insulin analogues will remain unknown and patients will be unable to assess their risks and benefits in order to make an informed choice of treatment. If patients safety is to be protected and their rights to an informed choice is to be respected, it is essential that they are provided with the facts as they stand. As the clinical benefits of insulin analogues have proved to be negligible in terms of diabetes control, some patients may consider that even a minimal carcinogenic risk of insulin analogues may be unacceptable.

The long and short-term health of patients must be protected by greater effort being put into researching the safety of new drugs and by greater vigilance on the part of regulators before they reach the market. For physicians prescribing drugs and for patients' exercising their rights to an informed choice of treatment, decisions are made on the basis of weighing up the risks and benefits of the various therapies that are available to them. In order to truly achieve this, there

needs to be greater transparency and more effort put into good quality clinical research before new drugs reach the market accompanied by more effective and more vigilant post-marketing surveillance.

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Contact details:
Insulin Dependent Diabetes Trust
PO Box 294, Northampton
NN1 4XS, United Kingdom
Tel 01604 622837 Fax 01604 622838 e-mail
enquiries@iddtinternational.org
Website www.iddtinternational.org