Introduction

The importance of good glycaemic control is well known fact based on available evidences from landmark studies. Insulin is the most powerful agent in reducing blood sugars and its use is crucial in achieving near normal glycaemic targets for most patients with diabetes. The benefit of insulin is always overshadowed by certain limitations and drawbacks which include hypoglycaemia, weight gain, high variability, less predictability and fear of injection. The available basal insulin in the market has drawbacks like not complete 24 hours coverage for all patients of diabetes, high inter and intra variability and hypoglycaemia especially nocturnal along with fixed time dosing every day. The next generation insulin needs to address these issues which can lower the glycaemic variability or fluctuations of mean glucose every day and also day to day along with nocturnal hypoglycaemia and also have complete 24 hours basal coverage for all patients with freedom from dose timing constraints when required as per patient needs and their schedules. Insulin degludec seems to address these existing issues which can lower the glycaemic variability or fluctuations of mean glucose every day and also day to day along with nocturnal hypoglycaemia and also have complete 24 hours basal coverage for all patients with freedom from dose timing constraints when required as per patient needs and their schedules. Insulin degludec seems to address these existing limitations and understanding the differences with existing basal analogues in terms of pharmacokinetic and pharmacodynamic will aid physician to understand the value addition of this new insulin.

Development of Insulin Degludec

The active form of human insulin consists of a 21 amino acid A chain and a 30 amino acid B chain linked by two disulphide bonds. Naturally occurring insulin in the beta cells self-associates into dimers and three of these will combine with two zinc ions to form a hexameric complex (an adaptation for maximal storage capacity within beta cell). Insulin from these hexamers by the process of exocytosis rapidly dissociate from the beta cells and gets converted to biologically active monomers which enter the blood stream and act on the insulin receptors at the target tissue. When exogenous insulin gets formulated for clinical use, it is injected as hexamers in the subcutaneous use from which monomers are absorbed through capillaries (Figure 1). For designing the rapid acting analogues the main criteria is to ensure rapid dissociation of hexamers to monomers and brisk absorption through capillaries whereas in case of basal insulin analogue the criteria is reversed and a delay in absorption is the key answer- for a slow continuous basal insulin profile which mimics physiological secretion. The basal insulin in market namely NPH, Glargine and Detemir have different methods of protraction- NPH by poorly soluble protamine insulin formulation, Glargine by pH dependent precipitation of insulin and detemir by albumin binding. The within-subject variability of the analogues glargine and detemir were compared with the NPH insulin in a randomised double-blind study in people with type 1 diabetes. The pharmacodynamic and pharmacokinetic (serum concentrations of insulin detemir,
human insulin, and insulin glargine) properties of the basal insulin preparations were recorded for 54 subjects in euglycaemic clamp technique. The coefficient of variation (CV) for the pharmacodynamic end points was 27% for detemir 48% for glargine and 68% for NPH insulin. Thus the existing analogues have lowered the variability in comparison with NPH but still needs improvisation as the peaks and troughs in their PK profiles leads to unwanted hypo and hyperglycaemia. Moreover, the available basal analogues have rising insulin requirements especially day time indicating not a true 24 hour profile for all types of diabetes patients.

The concept of developing a new analogue is in the fact that the absorption of insulin from subcutaneous space is related to the molecular size achieved and hence having higher molecular weight is the key for slow continuous absorption. This can be achieved by forming multi hexamers in the injected subcutaneous space by designing a new insulin analogue which can achieve this formation.

The molecular sizes of insulin complexes correlate with the rate of absorption and thus duration of insulin action of injected insulin. The rapid acting analogues dissociates into monomers quickly resulting in rapid absorption into blood through capillaries. The long acting analogues delay the release of monomers from hexamers and are dependent on molecular size of insulin formed in subcutaneous space. Formation of long chain multihexamers results in heavy molecular weight complex and slow release of insulin monomers to the blood stream (Figure 1).

Insulin degludec is a new basal analogue which has the same amino acid sequence as human insulin, except that the amino acid threonine at position B30 was removed and a 16-carbon fatty diacid chain hexadecandioyl was added at B29 via a glutamic acid spacer (linker) L-γ-Glutamate (Figure 2).

In the formulation insulin degludec is a soluble basal insulin analogue with neutral pH which in the presence of zinc, chloride and phenol tends to self-associate into stable, soluble di-hexamers. Phenol at either ends of di-hexamers prevent further association of individual di hexamers and stabilises the molecule in formulation. Once insulin degludec is injected, phenol disperses and the ends of di-hexamers are free to associate with other di-hexamers with the help of fatty acid and glutamic acid spacer to form long chains of multi-hexamers. This can be described as string of pearls like structure in the S.C. space. Zinc now slowly is absorbed from the multi hexamers at the either end and the gradual decrease in zinc concentration causes the bonds between the zinc ions and the fatty diacid side chains to break, thus allowing the continuous dissociation of insulin degludec monomers from the ends of these multi-hexamer chains. This results in a slow and continuous delivery of insulin monomers from the S.C. injection site into the circulation.

Non-clinical Pharmacology and Toxicology

Insulin degludec receptor binding kinetics is similar to human insulin and is a full agonist to insulin receptor. It binds to both isoforms of human insulin receptor (HIR-A and HIR-B) and maintains the metabolic response similar to human insulin. In experiments involving adipocytes, hepatocytes and skeletal muscles in rodents’ insulin degludec elicited the same metabolic responses and same maximal effect as HI. There have been suggestions that the modified amino acid sequence of insulin analogues may alter their receptor interaction properties like increased IGF-1R activation, thereby raising the possibility of increased incidence or progression of cancer. The affinity for the human insulin-like growth factor–1 receptor (IGF-1R) was low (just 2% relative to human insulin) and the mitogenic potential was measured in various cell lines including MCF-7 (Michigan Cancer Foundation-7 cell line) which concluded that the mitogenic potency was only 4-14% relative to human insulin. This new designer molecule thus proved its metabolic potency similar to HI and also did not compromise on safety.

The change in insulin structure was not found to result in clinically relevant immunogenicity. Across six phase 3a trials, the immunogenic responses (antibody levels) to long-term treatment with insulin degludec were low, with no clinically relevant impact on glycosylated haemoglobin (HbA1c) or total daily insulin dose.

The distribution, metabolism and excretion of insulin degludec have mainly been investigated in non-clinical studies. The fatty diacid side chain of insulin degludec allows it to bind strongly but reversibly to albumin, resulting in a plasma protein binding of > 99%. This adds little to its duration of action, but is likely to buffer any changes due to acute changes in absorption rate if any can occur. The in vitro protein binding studies showed that the
common protein-bound drugs such as ibuprofen, warfarin, acetylsalicylate and salicylate do not affect degludec binding to human serum albumin. Degludec displacement of other albumin-bound drugs is unlikely since the concentration of degludec is very low compared to the albumin concentration (>10,000-fold) and degludec occupies < 0.01% of the albumin molecules. Therefore, the pharmacokinetic (PK) properties of degludec would not be affected in vivo by other albumin-bound drugs or by even very large changes in albumin concentration. As with any other insulin product, elimination of insulin degludec is primarily via insulin receptor-mediated internalisation. The initial peptide cleavage of Insulin degludec occurs within the cell and is the same as seen for human insulin. The fatty acid side chain is extensively metabolised similarly to other naturally occurring fatty acids.  

Pharmacokinetics and Pharmacodynamics

The clinical pharmacology programme comprised a number of trials investigating the pharmacokinetic (PK) and pharmacodynamic (PD) properties of insulin degludec in patients with type 1 and type 2 diabetes. The programme also included trials that studied the properties of insulin degludec in special populations (i.e., patients with renal or hepatic impairment, elderly patients, children and adolescents, and patients of different race/ethnicity).

The PD (glucose-lowering effects) of Insulin degludec was evaluated using the euglycaemic clamp technique, a validated method regarded as the gold standard when assessing the effect of exogenous insulin. In a euglycaemic clamp, the drop in blood glucose with insulin administration is counteracted by variable intravenous (i.v.) glucose infusion. The amount of i.v. glucose needed (glucose infusion rate, GIR) to maintain a stable blood glucose level after insulin injection is a measure of the glucose-lowering effect of the insulin.  

Insulin degludec was evaluated for PK/PD compared with glargine. A randomised, double blind, two-period, crossover trial, with 66 patients with T1D received one of the doses (0.4, 0.6 or 0.8 U/kg) of degludec or glargine for 8 days. A euglycaemic clamp study for 42 hours was conducted after 8 days of treatment along with blood sampling for kinetics over 120 h with target euglycaemic blood glucose at 100mg/dL. Insulin degludec had a flat stable PD glucose-lowering effect beyond 42 h at all the doses administered 0.4, 0.6 and 0.8U/kg. The PK characters showed a flat and stable insulin degludec concentrations with equal distributions in the first and second 12-h post-dosing periods (AUC0-12h /AUC total =0.5) However for insulin glargine the concentration was higher during the first 12 h (AUC0–12h /AUC total =0.6). The detection of insulin degludec and insulin glargine was also evaluated in which degludec was detectable in serum at 120 h following final dose for all subjects at all three dose levels, whereas glargine tended to become undetectable 36–48h post-dosing. The mean terminal half-life was evaluated and for insulin degludec the t 1/2 was 25.4 h and for glargine it was 12.5 h (Table 1).

In another study for type 2 diabetes patients a similar clamp study was conducted with 49 subjects. They were injected with insulin degludec 0.4, 0.6 and/or 0.8 U/kg once daily for two 6-day periods and had an interval of 13–21 days in between. After the 6th day of once daily dosing, the subjects underwent a 26-h euglycaemic glucose clamp with the blood glucose concentration at the target level of 90 mg/dL along with serum insulin concentration measurement up to 120 h post dosing. The results were flat and stable glucose lowering profiles which were determined by GIR profiles were obtained during the entire dosing interval for all three dose levels (0.4, 0.6, 0.8U/kg) of insulin degludec (Figure 3). The glucose-lowering effect of insulin degludec was evaluated using the euglycaemic clamp technique, a validated method regarded as the gold standard when assessing the effect of exogenous insulin.

### Table 1: Mean terminal half-life at steady state-insulin degludec vs IGlar

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<tr>
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<th>Insulin degludec</th>
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<tr>
<td>0.4 U/kg</td>
<td>25.9</td>
<td>11.5</td>
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<tr>
<td>0.6 U/kg</td>
<td>27.0</td>
<td>12.9</td>
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<tr>
<td>0.8 U/kg</td>
<td>23.6</td>
<td>11.9</td>
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<td>Mean half-life</td>
<td>25.4</td>
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### Fig. 3: Glucose lowering effect at three different dose levels 0.4, 0.6 and 0.8U/kg which increases steadily with increasing doses

### Fig. 4: 6 hourly split showing equal 25% distribution of glucose lowering effect of insulin degludec
effect of insulin degludec increased linearly with increasing dose in patients with type 2 diabetes.\textsuperscript{14}

The glucose-lowering effect of insulin degludec was similar in the first 12 h after dosing and following 12 h and was close to 50% for all three dose levels and when divided into four 6h intervals insulin degludec glucose lowering effect was approximately 25% at all three dose levels (Figure 4). PK in this study showed that insulin degludec was detectable for at least 120 hours post dosing and had a mean terminal t1/2 of across the three dose levels was 25.1 h.

Pharmacodynamic variability was evaluated for insulin degludec compared to insulin glargine in 54 type 1 diabetes subjects. They were randomly allocated to either 0.4 U/kg of Insulin degludec or IGlar once daily dosing and on days 6, 9 and 12 underwent 24-h euglycaemic glucose clamps. The variability was analysed using a linear mixed model from the GIR profiles during the euglycaemic clamps.\textsuperscript{15} Insulin degludec had four-times lower day-to-day variability compared to insulin glargine in glucose-lowering effect with Coefficients of variation (CV%) for AUC GIR,0-24  20% for insulin degludec vs. 82% for insulin glargine and for the last 22 h CV 22% vs. 92% respectively (Figure 5).\textsuperscript{15}

**Steady State Insulin Levels without Insulin Stacking**

Insulin when reaches the systemic circulation in excess amount leads to hypoglycaemia referred to as “stacking” and is typically seen with the repeated administration of rapid-acting insulin to correct raised glucose levels. Administration of a second dose of rapid acting insulin which reaches systemic circulation before the residual dose of previous dose of rapid acting insulin leads to greater than desired insulin concentration in blood which results in hypoglycaemia. This inappropriate buildup of insulin is referred to as stacking and usually occurs with rapid acting insulin which gets absorbed into circulation and is usally acute in nature.

Whereas insulin degludec which has a unique mode of protraction of forming multi hexamers in SC space and has a slow release of monomers into circulation will not cause stacking and hypoglycaemia. This basal insulin analogue with a desirable pharmacokinetic (PK) property of flat peakless profile and > 24 hour duration, should allow reliable once-daily dosing for all patients, has raised concern of excessive accumulation of insulin in the circulation. The difference between the two, rapid acting insulin and basal insulin like degludec is the fact that the PK profile is entirely different and blood concentration of insulin is dependent on the PK profiles of these insulin. For rapid acting insulin, they achieve large peak: trough ratio in blood so that insulin concentration rises rapidly to prevent an excessive post-meal hyperglycaemia and drops quickly to avoid late post-prandial hypoglycaemia.\textsuperscript{16} Whereas basal insulin achieves steady state PK profile with low peak: trough ratios and insulin amount in blood is constant and predictable (Figure 6). The unique feature of insulin degludec is it reaches a steady state with once daily dosing in which concentration of insulin remains constant because the 24-hour elimination rate equals the 24-hour absorption rate and hence insulin levels do not increase further with repeated equivalent doses at appropriately spaced intervals.\textsuperscript{16} The evidence lies in the fact that once daily dosing of this molecule with half-life of 25 hours in long term studies of 52 weeks and also extension studies of 105 weeks did not cause stacking but in fact had lower hypoglycaemia episodes compared to glargine. Insulin follows first order kinetics wherein the dose of drug eliminated is dependent on the dose administered. When dosed at half-lives the drug concentration reaches a steady state where the amount of dose given will be equal to amount of drug eliminated and a constant steady
state level is maintained in the blood. This is achieved with insulin degludec and hence results in a smooth flat basal profile as desired for ideal basal insulin. As a rule of thumb it takes about 4–5 half-lives to reach steady state and after 5 half-lives the PK levels reach 99% of the constant concentration.16 Whereas after 3 half-lives the PK levels will already approach 90% of steady state concentration and is seen with insulin degludec in both type 1 and type 2 diabetes patients. This results in insulin degludec to reach a stable steady-state condition with minimal fluctuation in insulin concentration and therefore flatter glucose-lowering activity (Figure 6).16

PK and PD in Special Populations

The other trials evaluated PK/PD parameters in different patient sub-populations. The PK of insulin degludec was estimated in an open-label, parallel-group study in subjects with different grades of hepatic impairment and in subjects with normal hepatic function following single dose administration of 0.4 U/kg Insulin degludec. The groups were divided into mild, moderate, severe hepatic impairment, or normal hepatic function. Blood samples for PK analysis were collected before and up to 120 hrs after dose administration. The mean PK was similar to all the groups and was similar to normal hepatic function subject. The analyses included total exposure, maximum concentration and apparent clearance and were similar with no influence on grade of hepatic impairment.17 Similarly in another study, patients with different grades of renal impairment mild, moderate, severe renal impairment, or ESRD (End stage renal disease) along with normal renal function PK profiles were estimated. Renal impairment had no statistically significant effect on total exposure, maximum concentration or apparent clearance. The ESRD subjects on haemodialysis also had similar PK characteristics unaltered compared to normal renal function group (Figure 7).18

In addition, the ultra-long PK profile of Insulin degludec observed in adults is preserved in patients under different age-groups: children, adolescents and elderly patients.19,20 Insulin degludec provides similar PK responses in Black, White and Hispanic/Latino subjects with type 2 diabetes. No racial and ethnic differences were observed in the PD profile of Insulin degludec in patients with type 2 diabetes.

Summary

There is a need for ideal basal insulin which can overcome the unmet need of a truly once daily insulin, with a flat peakless profile. Useful for all types of patients Insulin degludec is next generation insulin with a unique mode of protraction of forming soluble multi-hexamers and slow continuous absorption giving it a flat profile compared to the existing basal insulin. In patients with type 1 diabetes or with type 2 diabetes, at steady-state, the mean terminal half-life of insulin degludec was 25 hours, i.e., approximately twice as long as for insulin glargine (half-life of 12.1 hours). In once-daily dosing regimen it reaches steady state after approximately 3 days. The duration of action of insulin degludec was estimated to be beyond 42 hours in euglycaemic clamp studies and this gives the unique opportunity of flexible time dosing which is not an available option with the existing basal insulin. The glucose-lowering effect is evenly distributed across a 24-hour dosing interval with insulin degludec having 4 times lower variability than insulin glargine. This is an important attribute given the narrow therapeutic window of insulin and the goal of achieving night time and inter-prandial glycaemic control without increasing the risk for hypoglycaemia, a goal that is challenging given the variability of absorption and lower PK half-lives of current basal insulin products. The combination of the ultra-long, flat and stable profile with an improved hour-to-hour and day-to-day variability could present an improved risk–benefit trade-off with the lower risk of hypoglycaemia, allowing for targeting improved levels of glycaemic control.

References


