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REVIEW ARTICLE

Toxicological Evaluation of Insulin Detemir

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Nanocochleate – A New Drug Delivery System

Summary

Diabetes mellitus is a disease in which a person has a high blood glucose level as a result of either because the body does not produce enough insulin (Type 1), or because the body cells do not properly respond or resistant to the insulin that is produced (Type 2). Insuin therapy is widely used in patients with these two types of diabetes. Modification of the human insulin molecule results in a slower distribution to peripheral target tissues, a longer duration of action with stable concentrations and thus a lower rate of hypoglycemia. Insulin analogs are synthesized to minimize unpredictable side effects of NPH (neutral protamine Hagedorn) insulin. Insulin detemir is an insulin analog that provides effective therapeutic options for patients with type 1 and type 2 diabetes. Several trials showed that insulin detemir did not display any significant differences in glycated hemoglobin (HbA_{1c}) levels when compared to NPH and insulin glargine. It is comparable with insulin glargine in significantly reducing rates of all types of hypoglycemia. Clinical studies have demonstrated that detemir enabled significantly lower within-subject variability and no or less weight gain than NPH insulin and glargine. Recent pharmacodynamic studies have pointed out that detemir could be used once daily in many patients with diabetes. This review will focus on the toxicological profile of insulin detemir: pharmaco/toxico-kinetics and dynamics, safety/efficacy, adverse reactions, drug interactions, and its mutagenecity/carcinogenicity.

***Key Words**: Diabetes mellitus, hyperglycemia, insulin analog, insulin detemir.*

TÜRKÇE BAŞLIK EKSİK

Özet

Diyabet vücudun yeteri kadar insulin üretememesi (Tip 1) veya hücrelerin üretilen insüline yeterli cevap vermemesi/resistan olması (Tip 2) nedeniyle kan şekerinin yüksek olarak görüldüğü bir hastalıktır. İnsan insulin molekülünün modifikasyonu insülinin peripheral dokulara yavaş dağılımını, stabil konsantrasyonlarda daha uzun sure etkili olmasını ve hipogliseminin daha az sıklıkla görülmesini sağlar. İnsülin analogları NPH (doğal protamin Hagedorn) insülinin öngörülemez yan etkilerini azaltmak için sentezlenmişlerdir. İnsülin detemir tip 1 ve tip 2 diyabeti olan hastalar için etkin terapötik bir seçenek olan bir insulin analogudur. Birçok araştırma insulin detemirin NPH veya insulin glarjine oranla, glikolize hemoglobin (HbA_{1c}) düzeylerinde bir fark oluşturmadığını göstermiştir. Hipogliseminin tüm tiplerinin ortaya çıkma oranını azaltması bakımından insulin glarjin ile benzer etkinliktedir. Kilinik çalışmalar detemir kullanımı ile bireyler arası farklı etkinlik görülme olasılığının azaldığını ve NPH insulin ve glarjine göre daha az sıklıkla kilo alımını sağladığını göstermiştir. Son farmakodinamik çalışmalar detemirin diyabet hastalarında günde bir kez kullanımının uygun olduğunu göstermiştir. Bu derlemede insulin detemirin farmako/toksiko-kinetiği ve dinamiği, güvenirliği/etkinliği, yan etkileri, ilaç etkileşmeleri ve mutajenisite/karsinojenisitesi değerlendirilerek toksikolojik profilinden söz edilecektir.

***Anahtar Kelimeler:** Diyabet, hiperglisemi, insulin analoğu, insulin detemir.*

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Introduction

Basal insulin therapy is an integral part of the intensive management of type 1 diabetes and also often used in type 2 diabetes (1). Type 1 diabetes mellitus is caused by absolute insulin deficiency resulting from autoimmune destruction of pancreatic β-cells. The autoimmune insulitis is characterized by a presence of auto-reactive T-lymphocytes and auto-antibodies against the antigen structures of β-cell. An approximate population incidence of type 1 diabetes is 0.0025% with the highest incidence at the age of 13 to 15 (2). However, significant ethnic differences have been observed, with the lowest incidence in China and Japan (0.004% and 0.016%, respectively) and the highest in Finland (0.040%) (3,4). For the etiopathology of type 1 diabetes, the substitution of missing insulin represents the only therapeutic option. Since the publication of the "Diabetes Control and Complications Trial (DCCT trial)", most of the patients with type 1 diabetes are treated using an intensified regimen of basal-bolus therapy (5). On the other hand, type 2 diabetes mellitus is a heterogenous metabolic disorder characterized by a relative insulin deficiency resulting from a reduced sensitivity of tissues to insulin and impairment of insulin secretion from pancreatic β-cells. Some patients are characterized by predominant insulin resistance and relative insulin insufficiency, while the others have a major defect in insulin secretion combined with insulin resistance. Type 2 diabetes represents more than 80% of the diabetes cases. The prevalence of type 2 diabetes is growing much more than has been previously estimated, in particular due to the increasing prevalence of obesity. It has been documented that before the development of severe comorbidities, earlier initiation of insulin therapy in type 2 diabetes, is required for improvement of metabolic control and prevention of micro- and macro-vascular complications (6). A therapeutic approach of earlier insulin initiation may have a role in the protection of β-cells from functional impairment due to long-term influence of hyperglycemia (7). The recent evidence-based shift towards an algorithm of early initiation and aggressive titration of insulin therapy in the management of type 2 diabetes requires the use of an effective insulin formulation that is both safe and acceptable to both patients and physicians (8).

Addition of insulin to oral anti-diabetic therapy has been traditionally started with basal NPH (Neutral Protamine Hagedorn) insulin. Until the recent introduction of basal insulin analogs, NPH insulin has been the most frequently used basal insulin, usually administrated in the evening. It is characterized by peaks in plasma insulin concentrations 5 to 10 h after administration, increased risk of hypoglycemia during the night, and duration of action of approximately 12 to 18 h that may contribute to hyperglycemia in the morning. With NPH insulin, differences in crystal size and inadequate resuspension cause changes in absorption kinetics and dosing precision and result in unpredictable glucose levels (9). However, insulins with long effectiveness and low side effects were needed. Therefore, it was important to develop new basal insulins to minimize these concerns. The introduction of basal insulin analogs has resulted in a series of clinical trials that provided information on the most effective way of using these insulins in the treatment of type 1 and 2 diabetes (10).

An insulin analog is an altered form of insulin, different from any occurring in nature, but still available to the human body for performing the same action as human insulin in terms of glycemic control. Through genetic engineering of the underlying DNA, the amino acid sequence of insulin can be changed to alter its pharmacokinetic (absorption, distribution, metabolism, and excretion) characteristics. Officially, the U.S. Food and Drug Administration (FDA) refers to these as "insulin receptor ligands", although they are more commonly referred to as insulin analogs (11). Normal unmodified insulin is soluble at physiological pH. Analogs have been created that have a shifted isoelectric point so that they exist in the solubility equilibrium in which most precipitates out but slowly dissolves in the bloodstream and is eventually excreted by the kidneys. These insulin analogs are used to replace the basal level of insulin, and may be effective over a period of about 24 h (12).

An ideal insulin regimen in patients with diabetes would mirror the 24-h insulin profile of a non-diabetic person, thereby preventing hyperglycaemia

without inducing hypoglycaemia. Until recently, available insulins had pharmacokinetic disadvantages, compared to physiological insulin secretion. The advent of the long-acting insulin analogs, insulin detemir and glargine, in the last decade has revolutionized insulin therapy in type 2 diabetes. Their unique pharmacokinetic and pharmacodynamic properties have offered tangible advantage over the conventional intermediate and long-acting insulin preparations in terms of improving glucose control as well as reducing risk of hypoglycemia and weight gain (12). Insulin detemir is a new basal insulin analog recently available for commercial use. Clinical trials have demonstrated lower fasting plasma glucose levels, lower variability in plasma glucose, predictable action profile and a reduced risk of nocturnal hypoglycaemia and weight gain, compared to conventional basal insulins (1). This study reviews the properties and potential use of insulin detemir and its toxicological outcomes.

INSULIN DETEMIR

Insulin detemir is a novel, biologically engineered analog of human insulin that has been successfully developed for clinical use in diabetes as basal insulin (13). The structure of insulin detemir is given in Figure 1. In detemir, the threonine in position B30 of human insulin has been omitted, and a C14 fatty acid chain (myristic acid) has been attached to the lysine at position B29. These changes increase its tendency to self-associate, leading to slow systemic absorption from the injection site. Reversible binding of detemir molecules to albumin at the injection site slows absorption further, while albumin binding in the circulation may also buffer changes in absorption rate, potentially limiting pharmacodynamic variability (14,15). As a result, detemir has a long and relatively flat time–action profile which is consistent from one injection to another (16).

The primary activity of insulin detemir is the regulation of glucose metabolism. Insulins, including insulin detemir, exert their specific action through binding to insulin receptors (13). The basal level of insulin may be maintained up to 20 h, but the time is clearly affected by the size of the injected dose. Insulin detemir has a high affinity for serum albumin, increasing its duration of action.

Mechanism of extended action of insulin detemir

The details of the mechanism of prolongation of action of insulin detemir have been elucidated in a series of physicochemical and pharmacological studies, comparing the properties of various acylated and non-acylated analogs differing in terms of self-association and albumin affinity (14). The data suggest that insulin detemir is hexameric following injection; but that hexamer–dihexamer equilibrium is established as pharmaceutical preservatives (phenol and cresol) are depleted from the injection site and exchanged for physiological electrolytes. Dihexamers are thought to form via contact between the myristic acid groups, which are situated at the poles of the hexameric complex (17). Self-associated insulin is more slowly absorbed into the circulation than monomeric insulin, and dihexamerisation is likely to delay dissociation of the complex into monomers. Data from studies of the disappearance rates of radiolabelled insulin analogs in porcine models confirm that the self-association state of insulin has a major influence on the subcutaneous depot residence time (14). However, these experiments also showed that this process is influenced by the extent to which these analogs bind to albumin in interstitial fluid at the injection site. It is likely that insulin detemir is bound to albumin in both self-associated and monomeric states, and that self-associated insulin detemir may bind more than one albumin molecule. The data show that the long disappearance time of labelled insulin detemir from the injection depot is due to both self-association and albumin binding (14).

Pharmaco/Toxicokinetics of Insulin Detemir

After subcutaneous injection of insulin detemir in healthy subjects and in patients with diabetes, insulin detemir serum concentrations indicated a slower, more prolonged absorption over 24 h in comparison to NPH human insulin. This insulin remains liquid in the subcutaneous tissue, providing greater surface area with reduced variability in absorption. Maximum serum concentration (C_{max}) is reached between 6 and 8 h after administration. The mean duration of action of insulin detemir ranged from

5.7 h at the lowest dose to 23.2 h at the highest dose (sampling period 24 h). The absolute bioavailability of insulin detemir is approximately 60% (14). In the interstitial fluid, albumin reduces insulin receptor affinity, and therefore results in the relatively low biological efficacy of the insulin analog compared with human insulin and the side chain fatty acid interacts with the neighbouring insulin molecules and further prolongs the rate of absorption (18, 19).

There is strong binding to albumin at the injection site, in the plasma (98 % remains bound to albumin) and in the target tissue (96 % remains bound to albumin). This protects insulin detemir from liver clearance which is only about 1/500 or 8% of the single pass extraction of human insulin. Detemir has a small apparent volume of distribution of approximately 0.1 L/kg and after subcutaneous administration, has a terminal half-life of 5 to 7 h depending on dose (20). Insulin, in general, is primarily metabolized by the liver (approximately 50%), the kidney (30%), and other (mainly muscle) tissues, with less than 1% excreted unchanged in the urine. Hepatic extraction of insulin may be significantly decreased in obese patients (150% ideal body weight or more). Insulin enters insulin-sensitive cells after binding to specific receptors, exerts its effect by a protein "second messenger", and is enzymatically degraded within cells by insulin protease or glutathione insulin transhydrogenase (GIT) enzymes. The exact metabolic process is not completely understood. Renal metabolism of insulin increases in patients with liver disease. After glomerular filtration, insulin is reabsorbed by proximal tubular cells, and is then enzymatically degraded to oligopeptides and amino acids. Insulin also diffuses from peritubular capillaries, and becomes bound to the contraluminal membranes of tubular cells. This binding stimulates reabsorption of sodium, phosphate, and glucose (21). There are no data on the plasma clearance of insulin detemir. The average plasma clearance of human regular insulin ranges from 7 to 33 mL/min/kg in nonobese, nondiabetic subjects with normal renal and hepatic function. Plasma clearance appears to be indirectly related to the plasma concentration of insulin, averaging 13.4 mL/min/kg at infusion rates of 20 mU/kg/min and 9.3 mL/min/kg at infusion rates of 200 mU/kg/min (assuming 70 kg bw) in young nondiabetic patients with normal renal and hepatic function (22).

Several studies were performed which compared the pharmacodynamic profiles of insulin detemir and other insulin analogs. The repeat-clamp study that compared the pharmacodynamic profile of the two basal analogs and NPH insulin in type 1 diabetes had, as its primary objective, the examination of within-subject variability (16). This study has shown significantly lower within-subject variability of insulin detemir than that of the comparators. The continued duration of action at 24 h of studied insulins was 14% for NPH insulin, 24% for insulin detemir and 39% for glargine. Porcellati et al. compared the pharmacodynamics of basal insulin analogs in 24 patients with type 1 diabetes after a 2-week treatment with glargine or detemir once daily in a randomized, double-blind, crossover study. Glucose infusion rate was similar with detemir and glargine for the first 12 h. However, plasma glucose increased progressively after 16 h with detemir demonstrating a lower effect than insulin glargine during the period of 12 to 24 h (17). In another randomized, double-blind trial with 3 euglycemic glucose clamp experiments, 27 insulin-treated patients were examined. They received 0.4, 0.8 and 1.4 IU/kg of either insulin detemir or glargine. The duration of action was comparable between the two analogs and it increased with a rise in the doses. Within-subject variability was lower for detemir, and between-subject variability did not differ between treatments (24). Recently, a double-blind, randomized, crossover study in patients with type 2 diabetes examined the glucose-lowering effect of detemir and glargine, using an assessment of continuous glucose monitoring. Over a 24-h period, once-daily dosing with insulin detemir provided a similar glycemic control to that of insulin glargine after both had been titrated to the same glucose target (25).

Despite a lower rate of hypoglycemia in patients treated with basal insulin analogs, it is important to study symptom awareness of either insulin treatment. Hormone and symptom response in healthy subjects was tested during a hypoglycemic clamp with insulin

detemir and human insulin in random order. Insulin detemir increased symptom awareness during hypoglycemia (sweating, especially, was earlier and faster) compared to human insulin in healthy individuals, whereas counter-regulatory hormone response and cognitive function were unaltered (26).

Dosage and Dosage Forms of of Insulin Detemir

Insulin detemir is available in easy-to-use pen injection devices, which are discrete, convenient, and can increase a patient's confidence levels with regard to performing self-injections (27). Insulin detemir is marketed in Turkey and other countries in the forms of FlexPen (disposible pen, 100 U/ml) and penfill (cartridge, 100 U/ml). Dosing for patients with type 1 or type 2 diabetes should be individualized. The starting dose of 0.2–0.5 U/kg/day should be given in the evening at dinner or bedtime. This dose should be increased until the desired fasting plasma glucose concentration has been obtained. Meal-time insulin should be considered according to the patient's insulin requirements. The total daily dose of detemir should be split into 2 injections, morning and evening, if pre-dinner target levels cannot be reached (28).

Insulin Detemir in Special Populations

1. Pregnancy: The effect of pregnancy on the pharmacokinetics and pharmacodynamics of detemir has not been studied in depth**.** Until now, there are no clinical studies or reports on the use of insulin detemir in pregnant women with diabetes. However, clinical studies are ongoing. Detemir is classified as Category C by FDA. In a fertility and embryonic development study, insulin detemir was administered to female rats before mating, during mating, and throughout pregnancy at doses up to 300 nmol/kg/d [3 times the recommended human dose, based on plasma area under curve (AUC) ratio]. Doses of 150 and 300 nmol/kg/d produced numbers of litters with visceral anomalies. Doses up to 900 nmol/kg/d (approximately 135 times the recommended human dose based on AUC ratio) were given to rabbits during organogenesis. Drug-dose related increases in the incidence of fetuses with gall bladder abnormalities such as small, bilobed, bifurcated and missing gall bladders were observed at a dose of 900 nmol/kg/d. The rat and rabbit embryofetal development studies that included concurrent human insulin control groups indicated that insulin detemir and human insulin had similar effects regarding embryotoxicity and teratogenicity (29,30).

2. Children and Adolescents: The pharmacokinetic properties of detemir were investigated in children (6 to 12 years) and adolescents (13 to 17 years) and adults with type 1 diabetes. Similar to NPH human insulin, slightly higher plasma AUC and C_{max} were observed in children by 10 % and 24 %, respectively, compared to adolescents and adults. There was no difference in pharmacokinetics between adolescents and adults (31). On the other hand, the adeloscents may benefit from the reduced risk of excessive weight while using determir (32).

3. Geriatrics: In a clinical trial investigating differences in pharmacokinetics of a single subcutaneous dose of detemir in young (25 to 35 years) versus elderly (≥68 years) healthy subjects, higher insulin AUC levels (up to 35 %) were found in elderly subjects due to a reduced clearance. As with other insulin preparations, detemir should always be titrated according to individual requirements (31).

4. Patients with Renal and Hepatic Impairment: Individuals with renal impairment showed no difference in pharmacokinetic parameters as compared to healthy volunteers. However, literature reports have shown that clearance of human insulin is decreased in renally impaired patients (31). On the other hand, individuals with severe hepatic dysfunction, without diabetes, were observed to have lower AUCs as compared to healthy volunteers. Careful glucose monitoring and dose adjustments of insulin, including detemir, may be necessary in patients with hepatic and/or renal dysfunction (31).

Adverse Reactions of Insulin Detemir

1. Hypoglycemia and Hyperglycemia: The risk of hypoglycemia is one of the unwanted consequences of the treatment algorithm for early initiation and aggressive titration of insulin therapy in type 2 diabetes. As with all insulins, the timing of hypoglycemia may differ among various insulin

formulations. Glucose monitoring is recommended for all patients with diabetes. Any change of insulin dose should be made cautiously and only under medical supervision. Changes in insulin strength, timing of dosing, manufacturer, type (e.g., regular, NPH, or insulin analogs), species (animal, human), or method of manufacture (rDNA versus animal-source insulin) may result in the need for a change in dosage. The risk is particularly pronounced with conventional intermediate and long-acting insulin preparations such as NPH and ultralente due to peak activity and unpredictable profile. Concomitant oral antidiabetic treatment may need to be adjusted. In trials of up to 6 months duration in patients with type 1 and type 2 diabetes, the incidence of severe hypoglycemia with detemir was comparable to the incidence with NPH, and, as expected, greater overall in patients with type 1 diabetes (33).

The smooth pharmacokinetic profile of the long-acting insulin analogs detemir and glargine delivers a relatively predictable and peak-less insulin level which is less prone to inflicting hypoglycemia. Detemir is not to be used in insulin infusion pumps (31).

In the two studies by Raslova et al. and Haak et al. which compared once or twice daily detemir with once or twice daily NPH in a basal-bolus regimen, there was no significant difference in the risk of both overall and nocturnal hypoglycemia between the two groups (30,34). However, in a multicenter trial, 476 patients with high levels of glycated hemoglobin (HbA_{1c}) were randomized to the addition of twice-daily insulin detemir or NPH insulin. For over 24 weeks, insulin doses were titrated toward pre-breakfast and pre-dinner plasma glucose targets of ≤6.0 mmol/L. This resulted in comparable reductions of HbA_{1c} for detemir and NPH (from 8.6 to 6.8 and from 8.5 to 6.6%, respectively). Compared with NPH insulin, in patients treated with insulin detemir the risk for all and for nocturnal hypoglycemia was reduced by 47% and 55%, respectively ($p < 0.001$) (35). Similarly, another trial examined the effect of an evening detemir, a pre-breakfast detemir, or evening NPH insulin administered at initial doses of 10 IU in 498 patients treated with ≥1 OAD. Similar reductions of HbA_{1c} of all three regimens were found found after the titration of the administered insulins. All-day and nocturnal hypoglycemias were reduced significantly with morning and evening detemir. Nocturnal hypoglycemia was reduced further, by 87%, with morning detemir compared with evening NPH ($P < 0.001$) (36). The risk of hypoglycemia was also not significantly different when insulin detemir was compared with glargine as add-on to oral antidiabetics (37). On the other hand, in a 26-week multinational, multicenter, randomized treat-to-target trial, oral antidiabetics were discontinued and subjects were randomized to analog basal-bolus therapy (insulin detemir once daily and insulin aspart at Meal-time) or biphasic insulin aspart 30, twice daily. Both insulin analog regimens enabled a majority of people with type 2 diabetes to reach $HbA_{1c} \leq 7.0\%$ after the failure of oral antidabetics and oral antidabetic-basal insulin therapy. Insulin-treated patients had more benefit from the transfer to analog basal-bolus therapy, while insulin-naive individuals had more benefit from the biphasic analog regimen (38).

The signs of mild to moderate hypoglcemia include sweating, dizziness, palipitation, tremor, hunger, restlessness, lightheadedness, trouble in concentrating, headache, drowsiness, sleep problems, anxiety, blurred vision, slurred speech, depressed mood, irritability, abnormal behaviour, unsteady movement and personality changes. If there was severe hypoglycemia, the symptoms might include disorientation, unconsciousness, seizures, and death (31, 39).

Inadequate dosing or discontinuation of treatment may lead to hyperglycemia and, in patients with type 1 diabetes, diabetic ketoacidosis. The first symptoms of hyperglycemia usually occur gradually over a period of hours or days. They include nausea, vomiting, drowsiness, flushed dry skin, dry mouth, increased urination, thirst and loss of appetite as well as acetone breath. Untreated hyperglycemic events are potentially fatal. Detemir is not intended for intravenous or intramuscular administration. The prolonged duration of activity of insulin detemir is dependent on injection into subcutaneous tissue.

Intravenous administration of the usual subcutaneous dose could result in severe hypoglycemia. Absorption after intramuscular administration is both faster and more extensive than absorption after subcutaneous administration (28).

2. Skin and Appendages: Mild injection site reactions occurred more frequently with detemir than with NPH human insulin and usually resolved in a few days to a few weeks. As with any insulin therapy, lipodistrophy may occur at the injection site and delay insulin absorption. Other injection site reactions with insulin therapy may include redness, pain, itching, hives, swelling, pruritus and inflammation. Continuous rotation of the injection site within a given area may help to reduce or prevent these reactions. On rare occasions, injection site reactions may require discontinuation of detemir. In some instances, these reactions may be related to factors other than insulin, such as irritants in a skin cleansing agent or poor injection technique (40-42).

3. Systemic allergy: Generalized allergy to insulin, which is less common but potentially more serious, may cause rash (including pruritus) over the whole body, shortness of breath, wheezing, reduction in blood pressure, rapid pulse, or sweating. Severe cases of generalized allergy, including anaphylactic reaction, may be life-threatening (43). Type I, type III and type IV allergies were also reported with insulin detemir usage (44-46).

4. **Others:** Insulin requirements may be altered during intercurrent conditions such as illness, emotional disturbances, or other stresses.

Drug Interactions of Insulin Detemir

Drug interactions of insulin detemir are summarized in Table 1. The results of *in vitro* and *in vivo* protein binding studies demonstrated that there was no clinically relevant interaction between insulin detemir and fatty acids or other protein bound drugs (28,31).

Efficacy and Safety of Insulin Detemir

Several studies conducted to assess the efficacy and safety of insulin detemir in type 1 diabetes have demonstrated that detemir offers comparable levels of HbA_{1c} reduction to both NPH and glargine as well as achieving better fasting plasma glucose levels and less intrapatient variability compared to NPH. Moreover, these favorable outcomes of detemir are accompanied by a lower risk of hypoglycemia in overall and nocturnal hypoglycemia. The relative efficacy and safety of insulin detemir in type 2 diabetes has also been investigated in multinational, open-label, randomized trials. These studies compare insulin detemir to NPH and insulin glargine either as part of basal-bolus regimen or as an add-on to oral antidabetics (28,31). These studies performed on both type 1 and type 2 diabetes are summarized below:

1. Type 1 Diabetes and Insulin Detemir

Insulin glargine and insulin detemir, each compared with NPH insulin, were examined by Pieber et al. to study glycemic control and risk of hypoglycemia of twice-daily insulin detemir with once-daily insulin glargine in 320 subjects with type 1 diabetes. The treatment did not show any significant differences in body weight gain between insulin detemir and insulin glargine, or a difference in HbA_{1c}. But the risk of severe hypoglycemia (RR 0.25, 95% CI 0.07 to 0.86) and the rate ratios for severe and nocturnal hypoglycemia were significantly lower in favor of insulin detemir (47).

A trial comparing insulin detemir versus NPH insulin in children and adolescents with type 1 diabetes did not show any differences in HbA_{1c} or frequency of severe hypoglycemia. However, benefits in favor of insulin detemir were found in a lower risk of nocturnal hypoglycemia. In this study, lower and more predictable fasting plasma glucose and lower body mass index were also the advantages of the treatment with insulin detemir (48).

The long-term efficacy and safety of insulin detemir compared to NPH insulin was examined in a 2-year, randomized, controlled trial in patients with type 1 diabetes using a treat-to-target basal-bolus regimen with insulin aspart. In this study, 22% of patients treated with insulin detemir reached a $HbA_{1c} \leq 7.0$ % in the absence of confirmed hypoglycemia during the

last month of treatment vs 13% on NPH insulin (p= 0.02). Detemir was associated with a 69% lower risk of major hypoglycemic episodes compared to NPH (p<0.001). The risk of nocturnal hypoglycemia was 46% lower with detemir than with NPH (p<0.001). Moreover, patients treated with detemir gained less weight (detemir 1.7 kg, NPH 2.7 kg; p= 0.02) (49).

A large, multi-national, prospective, observational study PREDICTIVE evaluated the safety and efficacy of insulin detemir in patients with diabetes in daily practice. The European cohort included 20,531 patients. A subgroup of 4782 type 1 diabetes patients was transferred from a basal-bolus regimen with NPH insulin (n=3117) or insulin glargine (n=1665) to insulin detemir basal-bolus therapy; or from a human insulin basal-bolus regimen (n=570) to insulin detemir/insulin aspart. After mean follow-up of 14.4 weeks HbA_{1c} and hypoglycemia were reduced by 0.6% and 54% (p<0.0001, respectively) with weight neutrality. Mean fasting glucose and within-patient fasting glucose variability significantly decreased in all patients (p<0.0001) (50).

2. Type 2 Diabetes and Insulin Detemir

The first two studies with insulin detemir in type 2 diabetes were of a similar design. They were multinational, open-label, randomized, parallel group trials comparing efficacy and safety of basal-bolus therapy using either insulin detemir in combination with meal-time insulin aspart versus NPH insulin in combination with meal-time regular human insulin or basal-bolus insulin regimen comprising either insulin detemir or NPH insulin both in combination with Meal-time insulin aspart (51,52). Patients received basal insulin either once or twice daily according to their pretrial insulin treatment and insulin aspart or regular insulin at mealtimes. In the first study, a 22-week long therapy of 395 people with type 2 diabetes resulted in comparable HbA_{1c} between treatments, with decreases from their baselines of 0.65% and 0.58% in the detemir and the NPH group, respectively. Treatment with insulin detemir plus aspart was associated with a significantly lower within-person variation in self-measured fasting plasma glucose. The relative risk of nocturnal hypoglycemia was significantly lower in the detemir group (RR 0.54, 95% CI 0.30 to 0.97; p<0.04) (53). In a second study, a 26-week long therapy of 505 patients resulted in comparable glycemic control but significantly lower within-subject variability and less weight gain in the detemir group compared to patients treated with NPH insulin (1.0 and 1.8 kg, respectively, p = 0.017). Insulin detemir was well tolerated and had a similar safety profile to NPH insulin (52).

Efficacy and tolerability of insulin detemir or NPH insulin added to oral therapy for type 2 diabetes was examined in a treat-to-target titration protocol. In a parallel-group, multicenter trial, 476 patients with high levels of HbA_{1c} was randomized to addition of twice-daily insulin detemir or NPH insulin. Over 24 weeks, insulin doses were titrated toward pre-breakfast and pre-dinner plasma glucose targets of ≤6.0 mmol/L. This resulted in comparable reductions of HbA_{1c} for detemir and NPH (from 8.6 to 6.8 and from 8.5 to 6.6%, respectively). Compared with NPH insulin, in patients treated with insulin detemir the risk for all and for nocturnal hypoglycemia was reduced by 47% and 55%, respectively (p<0.001) (54).

Another trial examined the effect of an evening detemir, a pre-breakfast detemir, or evening NPH insulin administered at initial doses of 10 IU in 498 patients treated with ≥1 oral antidiabetics. Similar reductions of HbA_{1c} of all three regimens were found after titration of administered insulins. All-day and nocturnal hypoglycemias were reduced significantly with morning and evening detemir. Nocturnal hypoglycemia was reduced further, by 87%, with morning detemir compared with evening NPH (p<0.001) (55).

In a 26-week multinational, multicenter, randomized treat-to-target trial, oral antidabetics were discontinued and subjects were randomized to analog basal-bolus therapy (insulin detemir once daily and insulin aspart at meal-time) or biphasic insulin aspart 30, twice daily. Both insulin analog regimens enabled a majority of people with type 2 diabetes to reach HbA_{1c} ≤ 7.0 % after the failure of oral antidiabetics and oral antidiabetic-basal insulin

therapy. Insulin-treated patients had more benefit from the transfer to analog basal-bolus therapy, while insulin-naive individuals had more benefit from the biphasic analog regimen (56).

3. Insulin Detemir and Body Weight

Insulin therapy in type 2 diabetes is commonly associated with weight gain and often results in nonadherence to insulin therapies. Insulin-related weight gain can be detrimental to the patient with diabetes for a number of reasons like increased blood pressure, cholesterol and triglyceride levels, decreased high-density lipoprotein cholesterol and increased waist-to-hip ratio (57,58). As these changes are known to be associated with increased cardiovascular risk, it is possible that they will to some extent limit the prognostic benefits gained from improved glycemic control. Weight gain is also associated with deterioration in glycemic control, leading to an increase in insulin resistance (59). There is therefore a need to minimize weight gain with insulin therapy. Insulin detemir has favorable pharmacodynamic properties that are thought to provide a theoretical weight neutral advantage over the conventional basal insulin preparations. The mechanisms behind the weight-sparing effect of insulin detemir are still being clarified. Reduced risk of hypoglycemia with insulin detemir, coupled with a more consistent and reliable delivery of desired dose than is available with traditional basal insulin, has been proposed to minimize defensive snacking by patients, and help to limit weight gain. However, reduced risk of hypoglycemia, which decreases defensive snacking by patients, is unlikely to fully explain the weight-sparing effect of insulin detemir (60). It has been suggested that due to prolonged action via acylation and albumin binding, insulin detemir may differentially influence hepatocytes more than peripheral tissues, thus effectively suppressing hepatic glucose output without promoting lipogenesis in the periphery (61). The second theory suggests that insulin detemir may be more effective than human insulin in communicating satiety signals within the central nervous system. This hypothesis of a direct effect of insulin detemir on the brain to reduce food intake was tested in healthy volunteers using the method of magnetoencephalography to examine the activity of the cerebral cortex in lean and overweight non-diabetes humans during a two-step hyperinsulinemic euglycemic clamp with human insulin, saline infusion and insulin detemir. Despite cerebrocortical resistance to human insulin, insulin detemir increased beta activity in overweight human subjects, similar to human insulin in lean subjects. It was suggested that the decreased cerebral beta-activity response in overweight subjects could be restored by insulin detemir (62).

In patients with type 1 and type 2 diabetes, detemir was associated with somewhat less weight gain than NPH. In a study with patients with type 1 diabetes, treatment with insulin detemir led to a slight (0.2–0.3 kg) decrease in weight, whereas NPH insulin resulted in weight gains ranging from 0.4 to 1.4 kg over 6–12 months (63-65). Home et al. postulated that the smaller number of hypoglycemic events associated with insulin detemir correlates with a reduced need for caloric intake for treating or preventing hypoglycemic events, thus leading to a lower chance of weight gain than treatment with NPH insulin (66). Insulin detemir has likewise caused less weight gain compared to glargine when added to oral agents at comparable level of glucose control (p=0.012) (37).

In a more recent multinational, 52-week, open-label, parallel-group, noninferiority, treat-to-target trial Hollander et al. determined that at the 52th week, mean weight gain was significantly lower with detemir than with glargine (2.8 vs 3.8 kg; mean difference, –1.04; 95% CI –2.08 to –0.01; $p<0.05$). There was no significant difference between detemir and glargine in terms of change in fasting sugar (7.19% and 7.03%, respectively) or risk of hypoglycemia. Mean HbA_{1c} and mean decrease in HbA_{1c} from baseline were more in insulin glargine group but none of these differences reached statistical significance (67). In another study by Hallschmid et al., the reserarchers compared acute effects of human insulin and detemir on EEG measures and food intake in 15 healthy men during 2 hyperinsulinemic euglycemic clamps that included an insulin bolus injection (human insulin, detemir) followed by a steady 90-minute infusion. Twenty minutes after infusion, subjects were allowed to eat ad libitum from a test buffet. While inducing

comparable peripheral effects, detemir exerted stronger acute effects on brain functions than human insulin and triggered a relative decrease in food consumption by 303 kcal ($p<0.04$), suggesting an enhanced anorexigenic impact of detemir compared to human insulin on central nervous networks that control nutrient uptake (68).

4. Mitogenic Properties of Insulin Detemir: Besides influencing glucose metabolism, insulin exerts other biological functions such as proliferation, differentiation, and cell apoptosis. Insulin activity is driven through specific insulin receptors. At low concentrations its intercellular activity leads to a very quick appearance of metabolic effects. At higher concentrations insulin affects processes such as growth promotion by binding and stimulating insulin-like growth factor type I (IGF-I) **(69)**. The B26-B30 region of the insulin molecule has been critical for insulin receptor recognition, but the C-terminal end of the insulin B chain seems to be important in insulin binding to the IGF-I receptor. Multiple factors such as residence time on the receptor, dissociation rate, rate of receptor internalization and the degree of phosphorylation of signaling proteins can affect the mitogenic potencies of insulin analogs **(70)**. Insulin analogs are developed to modify the structure of the human insulin molecule in order to more accurately approximate the endogenous secretion of insulin. Changing the structure of the insulin molecule, however, may significantly alter both its metabolic and mitogenic activity and have raised concerns about the safety of the insulin analogs **(69)**.

In vitro experiments showed that insulin detemir's affinity for the insulin receptor was 50% lower and it dissociated from the receptor two times faster than native insulin, which resulted in a 50-fold lower metabolic potency (27%) if adjusted for albumin binding only. The affinity for the IGF-1 receptor was 15 times lower than that of human insulin, and growth-promoting effects using human osteosarcoma cells (Saos/B10) were >250-fold lower than that of human insulin (11% if corrected for albumin binding) (71).

Wada et al. studied intracellular signaling properties of NPH, glargine and detemir insulins in various cultured cells and receptors. For the metabolic signaling, glargine and NPH insulin induced comparable dose-dependent phosphorylation of the studied receptors, whereas detemir-induced kinetics were markedly lower in adipocytes and myocytes. The authors concluded that their results indicate that glargine has comparable properties to human insulin in metabolic and mitogenic signaling and action. In contrast, detemir-induced metabolic signaling was less potent in all cell types studied, and it was reduced further by increasing concentrations of albumin (72).

5. Insulin Detemir and Mutagenicity/ Carcinogenicity: There is epidemiological evidence that diabetes is an independent predictor of cancer of the breast, colon, bladder, liver, pancreas and endometrium. However, clinical data on the relationship between risk of cancer and insulin treatment are sparse. IGF-I has been implicated as playing an important role in the progression and, potentially, in the development of human cancers. Epidemiological data have revealed that patients with high levels of IGF-I have an increased risk of developing cancer compared with those with IGF-I levels in the low and normal ranges. Last but not least, based on the expected role of IGF-I in tumor growth, anti-IGF-I receptor antibodies have been explored for the treatment of certain cancers (73).

Recent epidemiological studies suggested that treatment with insulin glargine might promote cancer growth. Concerning the relationship of insulin analogs with cancer promotion, a meta-analysis was performed to assess the risk of cancer during treatment with insulin detemir. The analysis showed that insulin detemir had a lower or similar occurrence of a cancer diagnosis compared with patients treated with NPH insulin or insulin glargine, respectively (73). Standard two-year carcinogenicity studies in animals have not been performed. Insulin detemir tested negative for genotoxic potential in the *in-vitro* reverse mutation study in bacteria, human peripheral blood lymphocyte chromosome aberration test, and the *in-vivo* mouse micronucleus test (28,31).

However, the results of cell culture studies give

conflicting results. In a study conducted by Rensing et al., five types of human adenocarcinoma (breast, colon, pancreas, lung and kidney) were evaluated for the presence of insulin receptors on angiogenic structures. In an *in vitro* angiogenesis assay, human insulin, insulin lispro, insulin glargine and insulin detemir were evaluated for their potential to increase capillary-like tube formation of human microvascular endothelial cells (hMVEC). Insulin receptors were found to be strongly expressed on the endothelium of microvessels in all evaluated adenocarcinomas, in addition to variable expression on tumour cells. Low or no detectable expression of insulin receptors was seen on microvessels in extratumoral stroma. Incubation with commercially available insulin compounds increased capillary-like tube formation of hMVEC *in vitro*. The researchers suggested that all tested insulin compounds might stimulate tumour growth by enhancing local angiogenesis (74). In another study performed on MCF-7 breast cancer cells, the mitogenic potential of the serum of diabetic patients treated with different insulin analogs were investigated. The results showed that serum containing insulin glargine was 1.11 (95% CI 1.05–1.18) fold more mitogenic than human insulin-containing serum ($p<0.005$); mitogenicity of serum containing detemir was 0.99 (95% CI 0.98–1.02) fold that of human insulin-containing serum (75).

In another cell culture study performed on HCT-116 (colorectal cancer), PC-3 (prostate cancer) and MCF-7 (breast adenocarcinoma) cell lines, the cells were treated with insulin, IGF-I or insulin analogs, and proliferation and protection from apoptosis were measured by cell counting and fluorescent-activated cell sorter (FACS) analysis, respectively. Western blots were used to identify signalling molecules activated by the analogs. Glargine, detemir and lispro had proliferative effects that resemble IGF-I action. Insulin, however, did not stimulate cellular proliferation. In addition, glargine and detemir displayed an IGF-I-like anti-apoptotic activity. Glargine, like insulin and IGF-I, induced phosphorylation of both ERK and AKT and this suggested that the analog was able to stimulate both the Ras-Raf-mitogen-activated protein kinase (MAPK) and PI3K-AKT pathways. Furthermore, glargine induced both insulin receptor and IGF- insulin receptor phosphorylation. It was concluded that glargine, detemir and lispro, unlike regular insulin, exhibit *in vitro* proliferative and anti-apoptotic activities in a number of cancer cell lines. These actions resemble some of the effects of IGF-I, a growth factor involved in cancer initiation and progression. Insulin had no increased IGF-I activity. The specific receptor/s involved in mediating analogs' actions remains to be identified (76).

Based on published clinical data from different studies which did not have sufficiently long-term duration and size, it is not possible to draw definite conclusions on the risk of cancer promotion by insulin analogs.

CONCLUSION

Patients with type 2 diabetes should start an intensive therapy of hyperglycemia at an early stage and should prefer those therapeutic options which provide the possibility to reach HbA_{1c} goals individually with a low risk of hypoglycemia or other adverse effects of treatment. Insulin detemir is a basal insulin analog that provides an effective therapeutic option for patients with type 1 and type 2 diabetes. For glycemic control, no significant differences were found in HbA_{1c} levels when insulin detemir is compared with NPH insulin (77).

Detemir is comparable with insulin glargine in significantly reducing the rate of all types of hypoglycemia. Besides, it provides more favorable clinical benefit of no or less weight gain than NPH insulin and glargine. It has been demonstrated that insulin detemir is responsible for significantly lower within-subject variability than NPH insulin and insulin glargine. Recent pharmacodynamic studies have shown that detemir can be used once daily in many patients with diabetes. Patients can be safely skip from one basal insulin to another, but close monitoring during this switch-offs is necessary as there may be dose differences with requirements for a higher dose of insulin detemir (78). However, insulin detemir, bind to albumin rather than fat like earlier insulin varieties, and results including long-term adverse effects from long-term usage (e.g. more than 10 years) have never been released. In

conclusion, detemir should be used with care under the supervision of a physician and adverse reactions should be treated as soon as possible.

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