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Comparison of subcutaneous absorption of insulin glargine (Lantus®) and NPH insulin in patients with type 2 diabetes
### OVERVIEW

The ability to produce insulin analogs with modified pharmacokinetic properties by recombinant DNA technology has provided the opportunity to create insulin preparations that more closely mimic physiological insulin secretion patterns. Insulin glargine was designed specifically to provide the basal insulin requirement and was developed on the novel premise that the introduction of amino acid modifications that increase the isoelectric point of the native insulin molecule towards neutrality would result in precipitation of the insulin in the subcutaneous compartment and result in delayed absorption. Insulin glargine fulfilled this expectation and is characterized by delayed subcutaneous absorption and consequently prolonged action, the duration of which is almost comparable with endogenous insulin.

The successful production of novel human insulin analogs offers enormous opportunity to improve the care of persons with diabetes and correct utilization offers the best opportunity to date to mirror normal physiologic insulin secretion. However, such structural changes to the insulin protein clearly have the potential to alter kinetic and dynamic actions of the molecule and hence safety profile in a dramatic and detrimental way. Of particular interest is the insulin receptor binding properties of an analog, the binding affinity at other analogous receptors (especially IGF-1) and the associated biologic responses that such binding elicits. The studies that characterize the actions of insulin glargine with respect to these actions have been investigated in detail.

In this chapter, we describe the molecular features of insulin glargine and examine how the molecular structure directly influences its biologic effects. We present pre-clinical summaries presenting findings on receptor kinetics, signaling and mitogenicity, and on toxicology. Clinical studies describing the pharmacokinetic and pharmacodynamic characteristics of insulin glargine are reviewed in detail, including studies which have examined the influence of insulin glargine on vascular function and diabetic retinopathy. A short overview of the study and a brief description of the study design are provided in shaded boxes. In the summary, an interpretation of the relevance of these studies as a prelude to the clinical trial program for insulin glargine is presented.
MOLECULAR CHEMISTRY OF INSULIN GLARGINE

Insulin glargine (previously HOE 901) is an analog of human insulin produced by recombinant DNA technology utilizing non-pathogenic laboratory strains of *Escherichia coli* (K 12) as the production organism (McKeage and Goa, 2001). Two modifications have been made to the human insulin structure to produce the analog, insulin glargine (Figure 14) (Owens et al., 2001). Firstly, two positively charged arginine molecules have been added, which elongate the C-terminus of the β-chain. This alters the isoelectric point from pH 5·4 to pH 6·7, making the molecule, which is soluble at slightly acidic pH, less soluble at the physiologic pH of subcutaneous tissue. As insulin glargine is formulated at an acidic pH of 4·0, a second modification is needed to stabilize the insulin to prevent deamidation and dimerization via the acid-sensitive asparagine residue at position 21 in the α-chain. Neutrally charged glycine has therefore been used to replace the asparagine at position A21, to ensure good stability. When insulin glargine (pH 4) is injected into the subcutaneous space (pH 7·4), the acidic solution is neutralized, leading to precipitation. This amorphous precipitation in the subcutaneous tissue delays absorption, resulting in an extended duration of action (Figure 15).

INSULIN GLARGINE FORMULATION

Chemically, insulin glargine is: A21(Gly)-B31,32 (Arg)2 – human insulin. The empirical formula of insulin glargine is C267H404N72O78 S6. Insulin glargine has a molecular weight of 6063 daltons. Insulin glargine injection is a sterile clear solution of aqueous liquid at pH 4. Each ml of insulin glargine injection contains 100 units (3.6378 mg) of insulin glargine, 30 µg of zinc, 2.7 mg m-cresol, 20 mg of glycerol 85% and water (Lantus® Package Insert, 2002).

Insulin glargine hexamerization

The formation of an insulin hexamer is dependent on the presence of zinc ions, and, as noted, the ionic strength directly influences the equilibrium of hexamer association-dissociation (Berchotl and Hilgenfeld, 1999). The classical hexamer coordinates with six insulin molecules. The insulin molecules present in a hexamer adopt one of two conformations, the T or R state. These states reflect the spatial structure of the first eight amino acids of the β-chain N-terminal, which form an extended “finger” in the T state and are α helical in the R state (Figure 17).

In the presence of small amounts of phenol or cresol molecules, which are found in commercial formulations of insulin and act as a preservative, the equilibrium is moved towards the R state. The phenolic molecules interact with the dimer-dimer interface and bind at two largely hydrophobic pockets at each dimer-dimer interface and stabilize the R state α helices. This stabilization of the R state plays a key role in stabilizing the insulin crystals because of a reduced tendency for the hexamer to dissociate. This is because the α helical shape of the B1–B8 sequence restricts zinc ion diffusion from the hexamer. Once the hexamer complexes dissociate, it is the formation of dimers and monomers, which can be absorbed through the capillary membrane, that allow biological activity (Figure 15).

Although native insulin binds six phenol molecules per hexamer (two per dimer),...
conformational studies with insulin glargine have shown that the glargine hexamer differs in its ability to bind phenol, and possesses the capacity to incorporate an additional (seventh) phenol molecule as a result of their novel structure (Figure 18). The extra phenol binds at the mouth of a channel created by the helical shape of the N-terminal B1–B8 sequences, and acts as a plug, reducing solvent access to the zinc ion and its ligands.

It is thought that the capacity to bind an extra phenol molecule relates to inter-hexameric association. This seems to result from distinct features of the glycine residue at position 21 in the A-chain. The 21A-Gly residue forms part of the hexamer-hexamer interface close to the seventh phenol molecule. Thus, it seems that 21A-Gly enhances the stabilization of the hexamic complex and further contributes to the protracted activity characteristic of insulin glargine. The delayed absorption is associated with the relatively constant insulin supply, much like that of endogenous basal insulin secretion in non-diabetic subjects in the post-absorptive state.

**Insulin glargine exists as a solution at pH 4**

Insulin glargine injection is an acidic solution (pH 4) and does not exist as a suspension in acidic conditions. The solution state of insulin glargine is distinct from other intermediate- and long-acting insulins. The intermediate-acting neutral protamine Hagedorn (NPH) insulin and lente insulin exist as stable protamine suspensions formed in the presence of low concentrations of zinc. The long-acting ultralente insulin also exists as a suspension in its treatment formulation (reviewed by Barnett, 2003). The requirement to resuspend these insulin preparations before administration is a major limitation, especially for their intended use as a basal insulin required to provide peakless and stable action. There is marked inter- and intra-patient variability in the response of patients to the NPH and lente insulins, and this is particularly noted with ultralente insulin. Failure to mix adequately is common (Jehle et al., 1999), and even on vigorous mixing, reproducibility of dosing is difficult to achieve (Rosskamp and Park, 1999).

**PRE-CLINICAL PHARMACOLOGY STUDIES**

**Insulin and IGF-1 receptor kinetics; insulin signaling; mitogenicity**

**Growth promoting and metabolic activity of the human insulin analogue [GlyA21, ArgB31, ArgB32] insulin (HOE 901) in muscle cells.**


Insulin glargine shows similar IGF-1 receptor-mediated metabolic and growth promoting activity compared to human insulin in rat muscle cell line models.

**STUDY RATIONALE**

Modification of the insulin molecule can lead to altered interaction with the insulin receptor and the homologous IGF-1 receptor (IGF-1R). A previous analog, Asp (B10) insulin, showed increased affinity to the insulin receptor and enhanced IGF-1R binding, which was associated with carcinogenic properties in vivo. Therefore, insulin glargine (HOE 901) was investigated in this context.

**OBJECTIVES**

To compare the growth-promoting and metabolic activities of insulin glargine and native human insulin in muscle tissue using cardiac myoblasts and rat ventricular cardiomyocytes.

**STUDY DESIGN**

An in vitro IGF-1R binding and intra-cellular signaling study undertaken in cardiac myoblasts obtained from the rat heart muscle cell line H9c2 and in adult rat ventricular cardiomyocytes.

**KEY FINDINGS**

- The IGF-1R binding and growth-promoting activity of HOE901, human insulin and Asp (B10) were investigated using the rat myoblast cell line H9c2, which expresses high levels of IGF-1R.
- IGF-1R binding was determined by incubating various concentrations of [125I]-labeled insulins with cultures of H9c2 cells in the absence or presence of the insulins for a period of 90 minutes at 37°C. Bound radioactivity was determined by gamma counting.
- Growth-promoting activity was assessed by measuring the incorporation of [3H]-thymidine in H9c2 cells following incubation with each of the three insulins for 16 hours.
- The relative metabolic potency of the three insulin preparations was determined by measuring the uptake of [14C]-labeled 3-O-methylglucose in rat cardiomyocytes, which express high levels of the insulin-sensitive glucose transporter, GLUT4.

**References**


**Figure 18.** The binding site for the seventh phenol molecule in insulin glargine. The electron density for the phenol is displayed as heavy shading. This phenol provides an inter-hexamer link between the parent hexamer (lower half of figure) and the neighboring hexamer (upper portion of figure). Residues of the neighboring hexamer are indicated by #. The hydrogen bond between the phenolic hydroxyl group and GlyA17.2 of the neighboring hexamer is indicated by a broken line. (Modified from *Biopolymers* 1999; 51:165–172. © 1999 Wiley Periodicals, by permission of John Wiley & Sons, Inc.)
slightly higher affinity than that of human insulin (half-inhibitory concentrations of 70 and 101 nM, respectively).

- The growth-promoting activity of insulin and insulin glargine, as measured by thymidine uptake, were essentially identical with approximately a 2-fold increase in DNA synthesis. In comparison, Asp (B10) caused a greater increase in thymidine uptake (~4-fold) (Figure 19b).

- At lower concentrations, closer to physiological levels, there was no significant difference in the growth-promoting activities of the three insulins (19-41% over basal).

**EDITORS COMMENTARY**

The growth-promoting activity and maximal metabolic activity of human insulin and insulin glargine, mediated by IGF-1R in rat muscle cells, are essentially identical. No increased mitogenic effect was evident with insulin glargine in relation to the interaction with the IGF-1R. Other studies examining the interaction of insulin glargine with the insulin receptor have shown normal association and even enhanced disassociation kinetics and unaltered patterns of insulin receptor signaling. Taken together, these findings indicate that the mitogenic potential of insulin glargine is essentially similar to that of human insulin at physiological concentrations.

**Additional references**


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**Figure 19.** (a) Competition of insulin and insulin analogs for [125I]-labeled IGF-1 binding in comparison to unlabeled IGF-1. (b) Effects of insulin, insulin analogs and IGF-1 on [3H]-thymidine incorporation in H9c2 myoblasts. Data are mean values ± S.E.M. (Reprinted from *European Journal of Pharmacology* 1997; 320: 259–265. Reprinted by permission of Elsevier Science B.V.)
In vitro pharmacology studies with insulin glargine and human insulin: IGF-1 receptor binding and thymidine incorporation.


Insulin glargine shows low affinity for the IGF-1 receptor and similar levels of mitogenic activity compared to human insulin in the majority of cell lines studied.

**STUDY RATIONALE**

Alterations in insulin molecular structure can change its interaction not only with the insulin receptor, but also with the structurally homologous insulin-like growth factor-1 (IGF-1R) receptor.

**OBJECTIVES**

To compare the IGF-1R binding affinity and mitogenic potential of insulin glargine and human insulin with endogenous IGF-1.

**STUDY DESIGN**

- In a second study involving osteosarcoma cells, the affinity of insulin glargine for the IGF-1 receptor was 14 fold higher than human insulin but approximately 2000 fold lower than IGF-1.
- In rat cardiomyoblasts, the IGF-1 receptor affinity was only slightly higher for insulin glargine than human insulin.
- Insulin glargine and human insulin stimulated thymidine uptake in rat cardiomycoblasts to a similar extent. In contrast, the mitogenic activity of the insulin analog Asp (B10) was significantly higher (p<0.005) and comparable to IGF-1.
- In human osteosarcoma cells, thymidine uptake in response to insulin glargine was 6.1 fold higher than human insulin. Compared to insulin glargine, IGF-1 caused a higher level of thymidine incorporation.

**KEY FINDINGS**

- In human hepatoma HepG2 cells, the affinity for the IGF-1R affinity was 5-7 fold higher for insulin glargine than human insulin, but 300-500 fold lower than that of endogenous IGF-1.
- In human osteosarcoma cells, the IGF-1R affinity of insulin glargine was 3.5-7.6 fold higher than human insulin but 200 fold lower than IGF-1.

**EDITORS COMMENTARY**

This series of experiments indicates that in vitro, insulin glargine and human insulin have low affinities for the IGF-1R compared to endogenous IGF-1 and that insulin glargine and human insulin stimulate thymidine uptake to a similar extent in the majority of cell lines examined. Although differences between insulin glargine and human insulin-stimulated thymidine uptake were apparent in the osteosarcoma cell line investigated, the increase was only detected at high concentrations that are not expected in the clinical environment.
**Effects of the long-acting insulin analog insulin glargine on cultured human skeletal muscle cells: comparisons to insulin and IGF-1.**


Using a culture system of human skeletal muscle cells derived from healthy subjects and persons with T2DM, insulin glargine behaved with similar activity to human insulin with regard to insulin and IGF-1 receptor binding, and both metabolic and mitogenic responses, including signaling events downstream from the response.

**STUDY RATIONALE**

The C-terminus of the insulin β chain, which is modified in insulin glargine, is known to significantly influence the interaction with the IGF-1 receptor (IGF-1R). Given that the IGF-1R can mediate growth-promoting effects of ligands other than its cognate hormone, the potentially altered receptor binding characteristics and metabolic and mitogenic responses, including signaling events proximal to these responses, of insulin glargine required detailed investigation.

**OBJECTIVES**

To compare the metabolic and mitogenic responses to insulin glargine, human insulin and IGF-1 in cultures of differentiated human skeletal muscle cells (HSMC) isolated from both healthy subjects and persons with T2DM.

**STUDY DESIGN**

A series of in vitro insulin and IGF-1R binding, metabolic and mitogenic potency and intracellular signaling studies, comparing human insulin with insulin glargine conducted in HSMC.

**Patient cells and culture**

HSMC were obtained via muscle biopsies from healthy subjects (n=17) and persons with T2DM (n=16) and were grown in culture and differentiation was induced. Cells were incubated for 4 hours at 12˚C with [125I]-labeled insulin (final concentration of 67 pM) or IGF-1 (final concentration of 39 pM) in the absence or presence of varying concentrations of unlabeled regular human insulin, insulin glargine or IGF-1.

**Assays**

Displacement of labeled insulin or IGF-1 was used to represent binding affinities to HSMC.

Metabolic and mitogenic effects were assessed by measuring glucose uptake (0-methyl glucose) and [3H]-thymidine incorporation respectively.

Intracellular signal transduction was determined by examining the level of intracellular phosphorylation of key proteins in signaling pathways (the serine/threonine kinase, Akt, and MAPK) following stimulation of cells for 15 minutes at 37˚C with each of the ligands by Western blotting of cell lysates with antibodies to phosphotyrosine.

**KEY FINDINGS**

**Receptor binding**

- Human insulin and insulin glargine were equally potent in their ability to displace bound [125I]-labeled insulin from the insulin receptor, in cells from both healthy persons and those with T2DM.
- Human insulin and insulin glargine had similar affinities for the IGF-1 receptor, except at supra-physiological concentrations. At concentrations of >100 nM, insulin glargine minimally displaced bound [125I]-labeled IGF-1 from its receptor (≈0.5% of the potency of unlabeled IGF-1) (Figure 22).

**Metabolic activity – glucose uptake**

- All three ligands stimulated glucose uptake similarly in a dose dependent manner in healthy HSMC. The sensitivity of glucose uptake was greatest in response to IGF-1 and lower but similar for human insulin and insulin glargine.
- Maximum human insulin and insulin glargine stimulated rates of glucose uptake were similar in T2DM cells, but both rates were significantly lower than those in healthy subjects. The maximal response to IGF-1 was greater than that of either human insulin or insulin glargine (Figure 23a and 23b).

**Mitogenic activity – thymidine uptake**

- Thymidine incorporation into DNA was dose dependent for all three insulin molecules, but the dose-response curves were shifted far to the right for human insulin and insulin glargine, with no significant difference between human insulin and insulin glargine (Figure 24).
- Human insulin and insulin glargine showed equivalent but greatly reduced sensitivities (<1%) compared to IGF-1. Insulin glargine had a lesser effect on thymidine incorporation than human insulin.

**Intracellular signaling**

- Phosphorylation of Akt was slightly greater in response to IGF-1 compared to human insulin and insulin glargine.
- In cells from healthy subjects, the greatest MAPK phosphorylation was attained after IGF-1 stimulation (p<0.05 vs. insulin glargine and human insulin). Insulin glargine and human insulin were equipotent (Figure 25a and 25b).

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**Figure 22.** Comparison of affinities for IGF-1 receptor presented as displacement of specific IGF-1 binding, normalized to cell protein in (a) cells from normal subjects; (b) cells from patients with T2DM. (Reprinted from Journal of Clinical Endocrinology & Metabolism 2001; 86:5838–5847. Reprinted with permission from The Endocrine Society).

**Figure 23.** Dose response curves of deoxyglucose uptake calculated as a function of the basal (no added insulin) activity in (a) cells from healthy subjects; (b) cells from subjects with T2DM. Results are the average ± S.E.M. (Reprinted from Journal of Clinical Endocrinology & Metabolism 2001; 86: 5838–5847. Reprinted with permission from The Endocrine Society).
Overall, these findings indicate that human insulin and insulin glargine are comparable with respect to insulin receptor binding, IGF-1 receptor binding, stimulation of glucose uptake and mitogenic potential. Insulin glargine displaced human insulin from the insulin receptor with an almost identical efficacy to the native hormone. Although differences between insulin glargine and human insulin were seen with respect to IGF-1 receptor binding, the increase was small and only detected at high concentrations that are not expected in the clinical environment.

In HSMC employed in this study, the mitogenic potential of insulin glargine was not different from human insulin, with both showing elevated mitogenic potential at very high non-physiologic concentration levels. The use of HSMC has advantages over the system of Saos/810 human osteosarcoma cells previously described (Kurtzhals et al., 2000; page 39). The relative expression levels of insulin and IGF-1 receptors are similar to skeletal muscle cells in vivo, which is the major target for insulin and therefore suggests that this represents a valid physiological model to assess the actions of insulin glargine.

**Additional references**

**STUDY DESIGN**
A series of *in vitro* insulin receptor binding, metabolic and mitogenic potency studies using hamster, mouse and human cell lines.

**OBJECTIVES**
To investigate the relationships between insulin structure, insulin receptor and IGF-1 receptor binding characteristics, and the metabolic and mitogenic potency of various insulin analogs (insulin aspart, insulin lispro, insulin glargine and insulin detemir) and other reference insulin analogs.

**STUDY RATIONALE**
Previously, an experimental rapid-acting insulin analog, Asp (B10), was shown to cause mammary tumors in female Sprague-Dawley rats. Therefore, as new insulin analogs are introduced into patient care, it is important to define their insulin receptor binding, metabolic and mitogenic characteristics.

**Insulin receptors dissociation studies**
- Chinese hamster ovary (CHO) cells over expressing HRI were incubated with [*125*I]-labeled insulin.
- The dissociation of radioactivity was measured following the addition of unlabeled human insulin.
- The dissociation of radioactivity was measured following the addition of [125I]-labeled insulin.

**Metabolic potency**
- Uptake of [*3H*]-labeled glucose by mouse adipocytes was measured in a dose-response study and EC50 determined.
- Relative metabolic potencies were calculated as the ratio of EC50 values for human insulin and individual insulin analogs.

**Mitogenic potency**
- Uptake of [*3H*]-thymidine in a human osteosarcoma cell line Saos/B10 predominately expressing IGF-1 receptors was measured.
- Relative mitogenic potency was calculated as the ratio of EC50 values for human insulin and individual insulin analogs.

**Correlations of receptor binding and metabolic and mitogenic potencies of insulin analogs designed for clinical use.**

Insulin analogs (including insulin lispro, aspart, glargine and detemir) differ with respect to insulin and IGF-1 receptor binding characteristics and metabolic and mitogenic capacity in *in vitro* cell line models.

**EDITORS COMMENTARY**
Overall, these findings indicate that human insulin and insulin glargine are comparable with respect to insulin receptor binding, IGF-1 receptor binding, stimulation of glucose uptake and mitogenic potential. Insulin glargine displaced human insulin from the insulin receptor with an almost identical efficacy to the native hormone. Although differences between insulin glargine and human insulin were seen with respect to IGF-1 receptor binding, the increase was small and only detected at high concentrations that are not expected in the clinical environment.
### Key Findings

The insulin and IGF-1 receptor binding properties and metabolic and mitogenic potencies of the series of insulin analogs investigated are detailed below.

<table>
<thead>
<tr>
<th>Analog</th>
<th>Insulin receptor affinity (%)</th>
<th>Insulin receptor off-rate (%)</th>
<th>Metabolic potency (lipogenesis) (%)</th>
<th>IGF-1 receptor affinity (%)</th>
<th>Mitogenic potency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human insulin</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Asp (B10)</td>
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<td>14 ± 1</td>
<td>207 ± 14</td>
<td>587 ± 50</td>
<td>975 ± 173</td>
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<td>Aspart</td>
<td>92 ± 6</td>
<td>81 ± 8</td>
<td>101 ± 2</td>
<td>81 ± 9</td>
<td>58 ± 22</td>
</tr>
<tr>
<td>Lispro</td>
<td>84 ± 6</td>
<td>100 ± 11</td>
<td>82 ± 3</td>
<td>156 ± 16</td>
<td>66 ± 10</td>
</tr>
<tr>
<td>Glargine</td>
<td>86 ± 3</td>
<td>152 ± 13</td>
<td>60 ± 3</td>
<td>641 ± 51</td>
<td>783 ± 132</td>
</tr>
<tr>
<td>A21Gly</td>
<td>78 ± 10</td>
<td>162 ± 11</td>
<td>88 ± 3</td>
<td>42 ± 11</td>
<td>34 ± 12</td>
</tr>
<tr>
<td>B31B32diArg</td>
<td>120 ± 4</td>
<td>75 ± 8</td>
<td>75 ± 5</td>
<td>2,049 ± 202</td>
<td>2,180 ± 390</td>
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<tr>
<td>Detemir</td>
<td>46 ± 5 and 18 ± 2</td>
<td>ca 27</td>
<td>ca 27</td>
<td>16 ± 1</td>
<td>ca 11</td>
</tr>
</tbody>
</table>

**Editors Commentary**

This study was conducted using a range of in vitro cell line models measuring parameters potentially related to efficacy and safety. In the cell lines studied, the amino acid modifications in the rapid-acting insulin analogs, insulin aspart and insulin lispro, had no significant influence on metabolic and mitogenic potency of human insulin.

Insulin glargine, in these in vitro models, exhibited differences compared to human insulin. Relative to human insulin, the affinity of insulin glargine for solubilized insulin receptor was 80-90%, with an off-rate of about 150%. Insulin glargine had a six-fold greater affinity for IGF-1 receptors and an eight-fold greater ability to promote DNA synthesis compared with human insulin in the Saos/B10 osteosarcoma cell line.

This is the only study to demonstrate an increased mitogenic potency of insulin glargine in comparison to human insulin. These results have been assessed and reviewed at length (Kellerer and Häring, 2001). The authors emphasized the very high expression levels (>30,000 per cell) of IGF-1 receptors and the low expression of insulin receptors (<1000) which makes it likely that all insulin receptors exist as insulin/IGF-1R hybrid proteins, thus excluding the effect of any binding to endogenous insulin receptors in experiments with Saos-B10 cells.

### STUDY RATIONALE

Numerous studies had analyzed the mitogenic potential of insulin glargine. Two experts reviewed the in vitro models that had been employed, in the light of the findings reported by Kurtzhals et al. who had suggested that mitogenic responses to insulin glargine may be a cause for concern.

**OBJECTIVES**

To compare the features and characteristics of the in vitro models used previously to assess mitogenic potential in studies of insulin glargine.

**STUDIES AND CELL LINES REVIEWED**

The studies by Bähr et al. (1997) of rat cardiac myoblasts and ventricular cardiomyocytes (page 31), Berti et al. (1998) of transfected rat-1 fibroblasts (page 33) and Kurtzhals et al. (2000) of human osteosarcoma cell lines (page 39).

**KEY FINDINGS**

- Bähr et al. found no increased mitogenic potential in rat cardiac myoblasts and ventricular cardiomyocytes expressing low levels of insulin and IGF-1R.

**Editors Commentary**

This review indicates that the cell lines used to assess the mitogenic potential of insulin glargine have different features which impact significantly on how the in vitro findings can be interpreted and potentially translated to assessments of the in vivo situation. The expert commentary concludes that it would be inappropriate to assign any message regarding mitogenic potential to insulin glargine therapy from the published findings.
Insulin-like growth factor 1 receptors are more abundant than insulin receptors in human micro- and macrovascular endothelial cells


Human endothelial cells express insulin receptor and functioning IGF-I receptors; insulin glargine had a higher affinity for the IGF-I receptor in human coronary artery endothelial cells than human insulin, but this had no effect on DNA synthesis, even at high concentrations.

STUDY RATIONALE

Endothelial cell dysfunction is thought to play an important role in the process of diabetes-related micro- and macroangiopathy. There is, however, little information on insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF-1R) expression in human endothelial cells or on the biological effect of human insulin and insulin analogs binding at these receptors.

OBJECTIVES

To characterize IR and IGF-1R receptor gene expression, ligand binding and receptor activation characteristics, and to examine the biological effect of IGF-1, human insulin and insulin glargine in vitro on human dermal microvascular endothelial cells (HMVEC) and human aortic (macrovascular) endothelial cells (HAEC).

STUDY DESIGN

IR and IGF-1R gene expression, receptor binding and phosphorylation, metabolic and mitogenic potency studies were conducted, comparing the effects of IGF-1, human insulin and insulin glargine in human endothelial cells in vitro.

Commercial cultures of HMVEC and HAEC were grown under standard conditions. The mRNA expression of the IR and IGF-1R genes was determined by quantitative real-time RT-PCR. Ligand binding by receptors was determined using [125I]-labelled insulin and [125I]-IGF-1. Receptor phosphorylation was determined by immunoprecipitation and Western blotting. DNA synthesis and glucose incorporation was quantified by measuring [3H]-thymidine and [3H]-glucose incorporation, respectively, into HMVEC cells only. Findings are presented as means ± SE.

KEY FINDINGS

- Gene expression of IR and IGF-1R was demonstrated in HMVEC and HAEC. IGF-1R was expressed at significantly higher levels (5-8 fold) compared to IR (p<0.001).
- Specific binding of [125I]-IGF-1 to HMVEC was 1.6 ± 0.2% of the total [125I]-IGF-1 added, which fits a one-site binding model. The concentration of unlabeled ligand required for half-maximal displacement (EC50) was 5.7 x 10–10 M for IGF-1, 2.2 x 10–7 M for insulin and 2.5 x 10–9 M for insulin glargine.
- The specific binding of [125I]-IGF-1 in HAEC was 1.9 ± 0.1% of the total [125I]-IGF-1 added. EC50 was 4.3 x 10–10 M for unlabelled IGF-1, 5.8 x 10–8 M for insulin and for insulin glargine, 9.9 x 10–8 M.
- The specific binding of [125I]-insulin was lower than [125I]-IGF-1 in both cell types (0.5 ± 0.2% in HMVEC and 0.2 ± 0.04% in HAEC).
- The β subunit of the IGF-1R was phosphorylated by IGF-1 at a concentration of 10–8 M in HMVEC and HAEC. A much higher insulin concentration (10–6 M) was required for phosphorylation in HAEC. In HMVEC, IGF-1R was phosphorylated by insulin glargine at concentrations of 10–6 M and weakly at 10–8 M.
- The incorporation of [3H]-thymidine into DNA of HMVEC was highly significant at 10–7 M concentration of IGF-1 (P < 0.002), with no effect with either insulin or insulin glargine.
- IGF-1 significantly stimulated glucose incorporation at 10–7 M (P = 0.008), 10–8 M (P = 0.02), and 10–9 M (P = 0.009); there was no effect evident with either insulin or insulin glargine.

EDITORS COMMENTARY

These in vitro studies show that micro- and macrovascular cell lines express IGF-1R and IR that bind ligand, with a higher population of IGF-1R present. The binding studies showed that insulin was approximately a thousand-fold less potent, and with insulin glargine a hundred-fold less potent, than IGF-1 at displacing [125I]-IGF-1 from its receptor. This represents a 10-fold difference between human insulin and insulin glargine with regard to affinity for the IGF-1 receptor in HMVEC. Phosphorylation of the IGF-1R receptor was achieved at a higher concentration (10–6 M) of both insulin and insulin glargine compared to IGF-1 (10–8 M) in HMVEC and HAEC. There was no mitogenic or metabolic effect of both insulin and insulin glargine, whereas IGF-1 increased both significantly at higher concentrations of 10–7 M.

Additional references

1. Chisalita SI, Arnqvist HJ. IGF-1 receptors are more expressed than insulin receptors in human coronary artery endothelial cells. Diabetes Metabolism 2003; 29(Spec No 2):Abstract 1191.
Toxicology

Evaluation of the reproductive toxicity and embryotoxicity of insulin glargine (LANTUS) in rats and rabbits.


In rats, insulin glargine has no adverse effects on reproduction, embryofetal and post-natal development. In rabbits, maternal and embryofetal toxicity was related to the hypoglycemic effect of the insulin.

STUDY RATIONALE

Before treating women with DM who are, or are planning to become pregnant, with novel insulin analogs, such as insulin glargine, careful safety evaluation is required. Animal studies were therefore undertaken to explore the effect of insulin glargine on reproduction, the embryo and the fetus.

OBJECTIVES

To determine the effect of daily subcutaneous administration of insulin glargine and NPH insulin on reproduction and/or embryofetal development in rats and rabbits.

STUDY DESIGN

Reproductive toxicity

Rats (25 male, 25 female) received daily subcutaneous injections of insulin glargine (1, 3 or 10 U/kg), NPH insulin (3 U/kg) or a control solution during the pre-mating and mating periods (males and females), throughout pregnancy (females) and 21-day lactation period (females). The lowest dose of insulin glargine was approximately twice the maximal therapeutic dose used in humans (0.5-1.0 U/kg). Effects on reproduction were assessed by measuring sperm motility in male rats and by monitoring the normal development of pups grown to maturity.

Embryotoxicity

Female rats (20 per group) were given daily subcutaneous injections of insulin glargine (2, 6.3 or 20 U/kg), NPH insulin (6.3 U/kg) or a control solution between days 7-18 of pregnancy. The lowest dose of insulin glargine was approximately twice the anticipated maximal therapeutic dose used in humans (0.5-1.0 U/kg).

Female rabbits (n=20) were treated with insulin glargine (0.5, 1.0 or 2.0 U/kg) or NPH insulin (1 U/kg) between days 6-18 of pregnancy. The range of insulin glargine doses used was based on the range at which no signs of maternal or embryofetal toxicity were expected and where signs of intolerance in the parental animals and conceptuses were expected. Embryotoxicity was assessed by sacrificing pregnant females on the 21st and 29th day after mating and examining fetuses for abnormalities.

KEY FINDINGS

Reproductive toxicity

• Treatment with insulin glargine or NPH insulin led to a dose-dependent hypoglycemic response with respect to degree and duration.
• Hypoglycemia in the groups of rats receiving 1 or 3 U/kg insulin glargine did not result in clinical signs and no impairment in behavior or general condition was observed.
• In the group receiving 10 U/kg insulin glargine, 5 female rats died or were killed. These events were thought to be caused by hypoglycemic shock. In the remaining rats, no adverse effects on physical condition, fertility, course of pregnancy, parturition, or the post-natal development of off-spring were observed.
• The physical development, sensory functions, sexual maturation, behavior and fertility of off-spring were unaffected by insulin administration.
• Sperm motility was unaffected by administration of insulin glargine or NPH insulin.
• Autopsy of parent animals and their offspring showed no detrimental effects of insulin glargine or NPH insulin on internal organs.

Embryotoxicity

• In rats, insulin glargine and NPH insulin had no effect on pregnancy, intrauterine death rate or intrauterine development.
• Morphological, cross-section and skeletal examinations of rat fetuses revealed no malformations, abnormalities or developmental retardation that was considered to be compound-induced.
• In rats, administration of insulin glargine or NPH insulin resulted in dose-dependent hypoglycemia with respect to severity and duration.

• No impairment of behavior or general condition was observed in the groups of rabbits receiving 0.5 or 1.0 U/kg insulin glargine. However, two animals in the 2.0 U/kg group and one in the NPH insulin group developed hypoglycemic shock and died or were killed.
• In the group of rabbits receiving 0.5 U/kg insulin glargine, pregnancy and intrauterine development were unaffected by treatment.
• In the groups of rabbits receiving 1.0 or 2.0 U/kg insulin glargine there was a dose-dependent increase in abortion, or dead fetuses with an increase in early intrauterine death in the 2.0 U/kg group. In the NPH insulin group, there was an increase in the rates of abortion and early intrauterine death.
• At the highest insulin glargine dose, compound-dependent changes in the fetuses included a slight increase in ventricular dilation of the brain and of blood in the thoracic cavity.

EDITORS COMMENTARY

Apart from toxicologic effects induced by hypoglycemia, insulin glargine had no independent effect on the reproduction, embryonic development and post-natal development in rats. Rabbits, however, proved to be more sensitive to hypoglycemia with increased rates of abortion, dead fetuses and early intrauterine death related to the insulin dose. The results from this study show that any apparent effect of insulin glargine on reproduction and embryology in rats and rabbits is due to hypoglycemia and not any toxic effects of insulin glargine itself. Of note, insulin glargine is not approved for use in human pregnancy.
Evaluation of the carcinogenic potential of insulin glargine (LANTUS) in rats and mice.


Insulin glargine did not exhibit a systemic carcinogenic effect in mice or rats after exposure over a two year period.

**STUDY RATIONALE**

Careful assessments are needed of the mitogenic and carcinogenic potential of modified insulins before clinical administration. Several cell lines have been used to assess the mitogenicity of insulin glargine, but there was a need to assess the carcinogenic potential of insulin glargine using *in vivo* models.

**OBJECTIVES**

To determine if insulin glargine has any carcinogenic potential by conducting studies in rodents (NMRI mice and Sprague-Dowley rats) over a two year duration.

**STUDY DESIGN**

General toxicity studies, followed by carcinogenic studies in mice and rats using a range of doses of insulin glargine including NPH insulin for comparison with saline and vehicle as control.

Mice (10 male and 10 female), (NMRI species) received daily subcutaneous injections of 5, 10 and 20 U/kg of insulin glargine and general toxicity was assessed at 3 and 6 months. Based on these results, 2 year carcinogenicity studies were performed using mice (50 of each sex) and rats (Sprague-Dowley, 50 of each sex).

The animals received over the 2 year period once daily subcutaneous injections of either vehicle solution (control), saline (control), insulin glargine (2.5 or 12.5 U/kg) or NPH insulin (12.5 U/kg in mice and 5 U/kg in rats) for comparative purposes.

The lowest dose used in the carcinogenicity studies was approximately two-times higher than the maximal therapeutic dose used in humans (0.5 - 1.0 U/kg). Health and survival checks were conducted frequently. Hematology parameters were measured and necropsy examinations conducted.

**KEY FINDINGS**

- In mice, the mortality rate over the 2 year study was comparable between the treatment groups.
- Mortality rates in male rats showed a significant increase in animals treated with vehicle solution, insulin glargine and NPH insulin, compared to saline treated control animals (p<0.05). In female rats a significant increase in mortality was only observed in animals receiving the highest dose of insulin glargine and NPH insulin, compared to control animals.
- There was no increased incidence of mammary tumors in both mice (table 2) and rats (table 3) in the insulin glargine-treated groups compared with saline control and NPH insulin-treated groups.
- Malignant fibrous histiocytoma (MFH) at the injection site were found in significantly greater numbers in the insulin glargine-treated (2 U/kg) male mice and in the vehicle and all insulin glargine-treated male rats. This effect was not dose dependent.

**TABLE 2.** Incidence of mammary tumors in female mice in a 2-year carcinogenicity study.

<table>
<thead>
<tr>
<th>Test</th>
<th>Dose (U/Kg/d)</th>
<th>Duration (months)</th>
<th>Mammary tumors (n)</th>
<th>Benign</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td>0</td>
<td>24</td>
<td></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>24</td>
<td></td>
<td>0</td>
<td>0</td>
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<tr>
<td>Insulin glargine</td>
<td>2</td>
<td>24</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>24</td>
<td></td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>24</td>
<td></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>NPH insulin</td>
<td>12.5</td>
<td>24</td>
<td></td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

**TABLE 3.** Incidence of mammary tumors in female rats in the 2-year carcinogenicity study.

<table>
<thead>
<tr>
<th>Test</th>
<th>Dose (U/Kg/d)</th>
<th>Duration (months)</th>
<th>Adenoma Carcinoma</th>
<th>Adenoma Carcinoma</th>
<th>Carcinoma* Mixed malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td>0</td>
<td>24</td>
<td>0</td>
<td>9</td>
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<tr>
<td>Vehicle</td>
<td>0</td>
<td>24</td>
<td>1</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>Insulin glargine</td>
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<td>24</td>
<td>3</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td></td>
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<td>24</td>
<td>1</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>24</td>
<td>0</td>
<td>7</td>
<td>15**</td>
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<tr>
<td>NPH insulin</td>
<td>5</td>
<td>24</td>
<td>1</td>
<td>7</td>
<td>28</td>
</tr>
</tbody>
</table>

*Adenocarcinoma arising in fibroadenoma **p<0.05 compared with the NaCl control

**EDITORS COMMENTARY**

Unlike the findings previously with the insulin analog, Asp (B10), no increased systemic carcinogenicity was observed with insulin glargine over a 2-year period of exposure. Although hepatocellular adenomas and carcinomas were found in mice, these are tumors which are commonly found. The MFH described at the injection site are a recognized phenomenon in laboratory rodents, and are considered to be a result of the local inflammatory reaction.

**ADDITIONAL REFERENCES**

Mixture of regular human insulin and insulin glargine injected subcutaneously in healthy dogs does not increase risk of hypoglycaemia.


The subcutaneous administration of a mixture of insulin glargine and regular human insulin did not result in any safety hazard in dogs.

STUDY RATIONALE
The modifications introduced to the human insulin amino acid sequence to produce insulin glargine alter the isoelectric point, resulting in insolubility at physiologic pH. Mixing insulin glargine with regular human insulin is not recommended. This study investigated if inadvertent mixing of the two insulins would present a safety hazard.

OBJECTIVES
To compare the glucose-lowering effect of a mixture of insulin glargine and regular insulin after subcutaneous injection in dogs.

STUDY DESIGN
Insulin glargine and regular insulin were administered to healthy dogs as a mixture, as separate injections or individually. Blood glucose levels were monitored over 24 hours.

Healthy, male, fasted, beagle dogs (n=56) were randomized to one of four groups and received subcutaneous injections of insulin or insulin mixtures. The groups were:
1. A mixture of pre-mixed regular insulin (0.1 U/kg) and insulin glargine (0.1 U/kg)
2. Single injections of regular human insulin (0.1 U/kg) and insulin glargine (0.1 U/kg)
3. Regular human insulin (0.2 U/kg)
4. Insulin glargine (0.2 U/kg)

Blood glucose levels were determined before injection and at a series of time points over 24 hours. Hypoglycemia was defined as a blood glucose level of < 40 mg/dL (2.22 mmol/L).

KEY FINDINGS
- No episodes of severe hypoglycemia occurred in any animal in each of the four treatment groups.
- The incidence of hypoglycemia was highest with regular human insulin only (group 3, 9/14 dogs).
- Groups 1, 2, and 3 were each characterized by a rapid onset of insulin action that had similar glucose lowering effects, reflecting the activity of the regular human insulin.
- In contrast, group 4 had a different profile with delayed, but sustained, effectiveness and a significantly lower nadir.

EDITORS COMMENTARY
This study provides useful safety data on the effect of inadvertent mixing and subsequent administration of insulin glargine and regular human insulin. Mixing insulin glargine with other insulins is not recommended due to the sensitivity of insulin glargine to changes in pH. The study, conducted in dogs, suggests that inadvertent mixing would not constitute a safety hazard. In reality, mixing of the two insulins resulted in similar glucose-lowering effects, but the formation of a milky precipitate should alert users to realize that mixing was inappropriate.

CLINICAL PHARMACOLOGY STUDIES
Pharmacokinetic, pharmacodynamic and metabolic studies

Comparison of the pharmacokinetics/dynamics of GLY(A21)-ARG(B31,B32)-human-insulin (HOE71GT) with NPH-insulin following subcutaneous injection by using euglycaemic clamp technique.


Early formulations of insulin glargine (HOE71GT) showed prolonged action and less pronounced peaks of insulin action compared to NPH insulin in healthy subjects.

STUDY RATIONALE
When this study was presented in 1994, research was ongoing to find a new insulin that would satisfy the requirement for a long acting basal insulin. This study set out to investigate the time-action profile of a new, potentially long-acting insulin analog (HOE71GT), later to be developed and known as HOE901 and then insulin glargine.

OBJECTIVES
To compare the time-action characteristics (pharmacodynamics) of a subcutaneous injection of two formulations of HOE71GT differing in their zinc content with NPH insulin in healthy subjects over a 24 hour period, post administration.

STUDY DESIGN
Randomized, double-blind, crossover design, HOE901 with either 15 or 80 µg/ml zinc was injected in 12 healthy male subjects. On each study day, subjects received a single subcutaneous injection of either HOE71GT or NPH insulin at a dose of 0.2 U/kg body weight. Somatostatin was given intravenously to suppress exogenous insulin secretion and C-peptide secretion was suppressed during the entire clamp period of 24 hours.

KEY FINDINGS
- The mean time point of the maximum insulin action, as determined by the glucose infusion rate, was significantly delayed with HOE71GT compared to NPH insulin (12.13 ± 3.75 hours [HOE71GT-15] and 12.98 ± 4.75 hours [HOE71GT-80] vs. 6.54 ± 2.92 hours, respectively; p<0.01).
- The maximal action of insulin (mg kg min) was significantly lower for both analogs (2.14 ± 0.75 [HOE71GT-15] and 1.90 ± 0.62 [HOE71GT-80] vs. 4.02 ± 2.22, respectively; p<0.01).
- The duration of insulin action was longer than 24 hours for both formulations of HOE 71GT compared to NPH insulin (16.22 ± 1.45 hours) (Figure 26).
Clinical Pharmacology Studies

Chapter 2 • Insulin Glargine Chemistry and Pharmacology

Pharmacokinetics of $^{125}$I-labelled insulin glargine (HOE 901) in healthy men.


Insulin glargine exhibits prolonged absorption from the subcutaneous site of injection in healthy subjects, with the same rate of absorption irrespective of injection site.

**STUDY RATIONALE**

In early studies insulin glargine was formulated with different zinc concentrations. There was a need to assess the subcutaneous absorption of formulations containing 15 or 80 µg/ml of zinc and to examine if site of injection altered the absorption characteristics.

**OBJECTIVES**

**Study one**

To compare the absorption rates from subcutaneous tissue and the appearance in plasma of two formulations of insulin glargine (containing 15 or 80 µg/ml zinc) to NPH insulin.

**Study two**

To examine the influence of the site of subcutaneous injection on the absorption of insulin glargine (containing 30 µg/ml zinc).

**STUDY DESIGN**

Randomized, single center, crossover studies, single-blinded (study one) or unblinded (study two) in healthy male subjects using $^{125}$I-radio-labeled insulins and external gamma radiation counting to define the rate of absorption from the subcutaneous site of injection.

The rate of absorption from subcutaneous tissue was determined from levels of residual radioactivity following injection of $^{125}$I-labeled insulin (at residue A14). Residual radioactivity was determined over time by placing a gamma counter 50mm from the subcutaneous injection site and the radioactivity counted for periods of 5 minutes, over the 24 hour post-injection period.

The primary pharmacokinetic endpoint was the time in hours for 25% of the administered radioactivity to disappear ($T_{75}$%). The appearance of (exogenous) insulin in plasma was estimated from measurements of immunoreactive insulin and C-peptide concentrations in plasma.

**Study one**

Insulins (0.15 U/kg) and placebo were administered by subcutaneous injection into the abdominal wall in 12 healthy male volunteers on four study days, separated by washout periods of >7 days.

**Study two**

Absorption of insulins were determined following subcutaneous injection into the anterior abdominal wall, arm or thigh in 12 healthy male subjects on three study days, separated by washout periods of 7-14 days.

**KEY FINDINGS**

**Study one**

- Mean $T_{75}$% for NPH insulin was significantly shorter compared to insulin glargine [15] or insulin glargine [80] (3.21 vs. 8.75 vs. 11.01 hours, respectively, p<0.0001).
- Mean residual radioactivity after 24 hours was lower for NPH insulin compared to insulin glargine [15] or insulin glargine [80] (21.90 ± 9.83% vs. 43.84 ± 15.04% vs. 52.17 ±

**Editors Commentary**

This "euglycemic" clamp study was one of the earliest research studies published in the development program of insulin glargine (reviewed by Rosskamp and Park, 1999). The study was performed to characterize the action profile over 24 hours after subcutaneous injection at the abdominal site of two early formulations of insulin glargine (HOE71GT), which differed in their zinc content. Previous X-ray crystallography studies had shown that the changes to the amino acid sequence altered the association properties, making the hexamer structure more stable (Hilgenfeld et al., 1992). In animal models, the addition of small amounts of zinc had further prolonged the duration of action and therefore early human studies had used different zinc content. The median glucose infusion rate is shown in Figure 15. NPH insulin showed a characteristic profile, achieving its maximum effect after 5 hours and decreasing in activity from 10 hours. In contrast, HOE71GT was characterized by a longer duration of action over the 24-hour clamp period with a constant peakless profile of insulin action compared to NPH insulin, irrespective of the zinc content of the formulation. These features suggested that insulin glargine may be suitable for providing basal insulin requirements in persons with DM and that further research was warranted.

References

15.84%, respectively, p<0.0001) (Figure 27a).

- Plasma exogenous insulin levels between 0-6 hours were significantly higher with NPH insulin compared to insulin glargine [15] and insulin glargine [80] (6.69 ± 4.34 vs. 3.14 ± 1.91 vs. 2.95 ± 2.59 mU/L, respectively, p=0.0017) (Figure 27b).
- The blood glucose levels over 0-6 hours after insulin administration were significantly lower for NPH insulin compared with glargine [15] and [80] (4.84 ± 0.61 vs. 5.17 ± 0.42 vs. 5.28 ± 0.34 respectively; p<0.001).

**Study two**

- No significant differences in the absorption of insulin glargine [30] at the three injection sites were found, as shown by T50% (hours): arm (11.9 ± 6.2), thigh (15.3 ± 6.2) and abdomen (13.2 ± 4.6) (Figure 28a) and residual radioactivity (after 24 hours): arm (47.7 ± 17.9 %), thigh (56.3 ± 14.8 %) and abdomen (57.2 ± 16.0 %).
- Comparing the three injection sites, no significant differences in exogenous plasma insulin concentrations (Figure 28b) or plasma glucose levels were found.

**Editors Commentary**

The subcutaneous absorption of insulin glargine was clearly prolonged compared to NPH insulin. The exogenous plasma insulin profiles demonstrated a peak with NPH insulin at 3-5 hours, compared with a slower absorption and relatively peakless plasma insulin profile for both formulations of insulin glargine. The approximate time to disappearance of 50% radioactivity from the abdominal wall injection site (T50%) for insulin glargine from the two studies was around 24 hours compared to 11 hours with NPH insulin.

There was little or no difference in the rate of absorption of insulin glargine between the main subcutaneous injection sites – an important fact to establish before widespread clinical usage. These studies provide clear evidence that insulin glargine is absorbed from the subcutaneous tissue at approximately half the rate of the ‘intermediate – acting’ NPH insulin suggesting insulin glargine is better suited as a basal insulin.

**Additional References**

Clinical Pharmacology Studies

Chapter 2 • Insulin Glargine Chemistry and Pharmacology

Time-action profile of the long-acting insulin analog insulin glargine (HOE901) in comparison with those of NPH insulin and placebo.


Insulin glargine has a peakless time-action profile that lasts 24 hours, whereas NPH insulin has a distinct peak with a shorter duration of action.

STUDY RATIONALE

The time-action profile of insulin glargine (zinc concentration of 30µg/ml) had not been compared to NPH insulin in healthy volunteers using a euglycemic glucose clamp technique. Therefore, a clinical study was conducted to fully characterize the insulin glargine-associated glucose lowering effect.

OBJECTIVES

To compare the time-action pharmacodynamic profile of a subcutaneous injection of insulin glargine and NPH insulin in healthy, subjects over a 30 hour period, post administration.

STUDY DESIGN

Randomized, doubleblind, placebo-controlled, 3-way cross-over, ‘euglycemic’ glucose clamp study.

Subjects, timing and medication

Healthy male volunteers (age 27 ± 4 years with BMI 22.2 ± 1.8 kg/m² and insulin antibody negative) received a single subcutaneous injection of insulin glargine, NPH insulin or placebo on one of three study days in random order, with a washout period of 7 days between the study days.

‘Euglycemic’ glucose clamp

Each study day required the subjects to have fasted overnight. They were connected to a Biostator and a ‘euglycemic’ glucose clamp was established using a constant intravenous infusion of insulin (0.15 mU/kg/min) and a variable glucose infusion rate. After a baseline period of 2 hours, subjects received a subcutaneous injection of insulin glargine, NPH insulin (0.4 U/kg) or placebo. Glucose infusion rates (GIRs) were established to maintain blood glucose levels at 90 mg/dL (5 mmol/L) over the 30 hour post-administration period. Blood samples were collected every 30 minutes to determine blood glucose levels and every 60 minutes to determine serum insulin and serum C-peptide levels.

KEY FINDINGS

- The hypoglycemic activity (reflected in the GIR) of insulin glargine increased slowly to a plateau level after 4 hours and remained relatively constant for the remainder of the observation period (30 hours) (Figure 29).
- NPH insulin required a pronounced peak in glucose infusion approximately 4-6 hours after injection, followed by a steady decline over the remainder of the study period.
- The maximal hypoglycemic activity (GIRmax) observed with insulin glargine was lower and occurred later (tmax) compared to NPH insulin (GIR: 5.3 ± 1.1 vs. 7.7 ± 1.3 mg/kg/min, p<0.05 and (tmax) 8.6 ± 4.4 vs. 5.4 ± 1.0 hours, p<0.01).
- Similarly, total metabolic activity (AUC 0-30 hours) was lower with insulin glargine compared to NPH insulin (7.92 ± 1.28 vs. 9.24 ± 1.29 g/kg, p<0.05).

EDITORS COMMENTARY

This well designed study was conducted as a randomized, placebo controlled trial utilizing the euglycemic glucose clamp, to determine the time-action characteristics of insulin glargine and NPH insulin. The placebo arm ensured that the metabolic effect i.e. the glucose requirements elicited by constant basal insulin infusion used, was known under these conditions. It is reasonable to assume that the time action profiles measured are predictive of those in persons with DM.

This study shows that the activities of insulin glargine increased to a plateau within 4 hours. Insulin glargine showed a smooth, peakless time-action profile that lasted more than 24 hours, whereas NPH insulin had a distinct peak 4-7 hours after injection. These characteristics suggest insulin glargine may be suitable for supplying basal insulin as a once daily, subcutaneous injection.

Additional references

Comparison of subcutaneous absorption of insulin glargine (Lantus®) and NPH insulin in patients with type 2 diabetes.


Insulin glargine has a longer presence in subcutaneous tissue with much slower absorption profile compared to NPH insulin, resulting in improved insulin supply.

**STUDY RATIONALE**

Previous studies in healthy subjects had shown that insulin glargine exhibits delayed absorption characteristics compared to NPH insulin, but there was a need to examine these findings in persons with T2DM.

**OBJECTIVES**

To compare the subcutaneous absorption characteristics of insulin glargine to NPH insulin in persons with T2DM.

**STUDY DESIGN**

An insulin glargine pharmacokinetic study with a randomized two-way cross-over design in insulin-naïve persons with T2DM comparing [125]I-radiolabeled NPH insulin and insulin glargine using external gamma radiation measurement.

Insulin-naïve persons (n=14) with T2DM (age 40-70 years, BMI <30 kg/m², HbA1c <10%, and 10 treated with OHA) were administered, by subcutaneous injection into the anterior abdominal wall, NPH insulin glargine or insulin (0.3 U/kg), [125]I-radiolabeled at residue A14, on two study days, separated by washout periods of >7 days. After insulin administration, participants were studied for 48 hours.

Residual radioactivity was determined by placing a gamma counter 50 mm from the injection site and the radioactivity counted for 5 minute intervals at 1, 2, 4, 6, 8, 12, 16, 20, 24, 36 and 48 hours after injection. During the first 24 hour period, patients remained fasted and samples were taken for blood glucose and C-peptide measurement.

The rate of absorption from subcutaneous tissue was calculated from levels of residual radioactivity following injection of [125]I-labeled insulin. The primary pharmacokinetic endpoint was the time in hours for 25% of the administered radioactivity to disappear (T75%). Secondary variables were the times taken for 50% and 75% of the administered radioactivity to disappear (T50% and T25%) and the mean residual radioactivity at the injection site 24, 36 and 48 hours after injection. The primary pharmacodynamic variables were plasma glucose and C-peptide concentrations.

**KEY FINDINGS**

- Median values for disappearance of radioactivity were significantly longer for insulin glargine compared with NPH insulin (Table 4).
- The median residual radioactivity at the injection site was significantly higher for insulin glargine compared to NPH insulin (Figure 30 and Table 5).
- Mean plasma glucose levels reached a minimum after 14.6 ± 1.3 hours and 9.0 ± 2.0 hours in response to insulin glargine and NPH insulin, respectively.

**Additional references**

Comparison of biphasic insulin aspart (BIAsp 30) and insulin glargine (IGlarg) during isoglycaemic clamp studies in persons with type 2 diabetes.
Abstract 573-P

Following injection of BIAsp 30 insulin, the plasma insulin concentrations increased rapidly, reaching distinct peaks 2 - 3 hours after injection, in contrast to insulin glargine which had a peakless profile with a plateau between 6 - 16 hours.

STUDY RATIONALE
In current clinical practice for persons with T2DM the next treatment step after treatment failure with OHAs is supplementation with insulin. This may be achieved by either the introduction of a basal insulin preparation with continued OHAs or a premixed insulin preparation which provides both basal and prandial insulin requirements. This study was undertaken to evaluate these two very different approaches to insulin therapy by comparing the pharmacokinetic and pharmacodynamic profiles of BIAsp 30 administered twice-daily and insulin glargine administered once-daily at the same total daily dose.

OBJECTIVES
To compare the pharmacokinetic and pharmacodynamic properties of BIAsp 30 and insulin glargine in persons with T2DM.

SUBJECTS
Twelve persons with T2DM, insulin naïve and treated with diet and OHAs were enrolled (mean ± SD: age 58.8 ± 8.9 years, BMI 31.0 ± 3.0 kg/m² and HbA₁c 7.1 ± 0.6 %).

STUDY DESIGN
In brief: A single-center, randomised, 24 hour isoglycaemic clamp study comparing the effect of either 0.5 U/kg of BIAsp 30, given as two doses 12 hours apart (0.25 U/kg at 08:30 and 0.25 U/kg at 20:30) or the same total daily dose (0.5 U/kg) of insulin glargine at 08:30, both subcutaneously into the anterior abdominal wall.

This was a randomised, open, single-centre, two-way crossover study. Patients were studied on two separate study days, 7(±3) days apart. On study day one, subjects were randomised to receive either 0.5 U/kg of BIAsp 30 given as two doses 12 hours apart (0.25 U/kg at 08:30 and 0.25 U/kg at 20:30) or the same total dose (0.5 U/kg) of insulin glargine at 08:30 both subcutaneously into the anterior abdominal wall. An isoglycaemic clamp was carried out over the following 24 hours. After a washout period of approximately 7 days, all 12 persons returned to the clinic for a further study day on which they received the comparator preparation.

During each of the study days, patients remained fasting and on bed rest during a 24 hour hypoglycemic “clamp” study. Fasting samples were taken at -30, -20, -10 and 0 minutes, following which a bolus dose of either 0.25 U/kg BIAsp 30 or 0.5 U/kg insulin glargine, according to a randomization schedule, was administered subcutaneously into a skin fold of the anterior abdominal wall.

Patients randomised to BIAsp 30 received a second dose (0.25 U/kg) 12 hours later. Samples for determination of plasma glucose concentrations were taken at 10-minute intervals. The glucose infusion rate was adjusted to maintain blood glucose concentrations at fasting levels. Frequent samples for plasma insulin and C-peptide concentrations were also taken over the 24-hour study period.

KEY FINDINGS
- The mean doses of BIAsp 30 (46.5 ± 6.6 U (range 36 - 50 U)) and insulin glargine (46.5 ± 4.0 U (range 37 - 50 U)) were comparable between treatment groups.
- Plasma glucose remained constant throughout the 24-hour clamp period. CV of plasma glucose during clamps (derived by calculating the CV of deviation (%) of all glucose during the clamp from the target value) was 6.3% for BIAsp 30 and 4.3% for insulin glargine, respectively.
- Following each injection of BIAsp 30, GIR increased rapidly reaching a distinct peak (Figure 31). A flatter post-injection GIR profile was observed following injection of Insulin glargine.
- GIR AUC0-24h was approximately 34% higher following BIAsp 30 than after insulin glargine (2.51 ± 0.36 vs. 1.87 ± 0.12 g/kg/minute, p=0.037).
- Plasma insulin rose more rapidly and had a higher peak following injection of BIAsp 30 than insulin glargine. Insulin AUC0-24h was 28.2% higher after BIAsp 30 than insulin glargine (4514 ± 404 vs. 3521 ± 368 pmol/L/hour, p=0.001).
- Plasma C-peptide levels fell below baseline levels following both injections (A and B) of BIAsp 30 but were unchanged following injection of Insulin glargine.
- Over the 24-hour study period the C-peptide AUC0-24h following BIAsp 30 was significantly lower than for insulin glargine (19.7 ± 2.4 vs. 22.4 ± 3.1 nmol/L/hour, p=0.029).

Figure 31. (a) Glucose infusion rates after subcutaneous injection. (b) Serum insulin concentrations after subcutaneous injection.

EDITOR’S COMMENTARY
This study shows that following each injection of BIAsp 30 insulin, plasma insulin concentrations increased rapidly reaching distinct peaks 2 - 3 hours after injection, in contrast to insulin glargine which had a peakless profile with a plateau between 6 - 16 hours. The glucose infusion rate reflect ed the insulin profiles. The AUC0-24h for plasma insulin and GIR were approximately 30% higher following the two injections of BIAsp 30 than insulin glargine despite the same total daily dose.
An assessment of the variability in the pharmacodynamics (glucose lowering effect) of HOE901 compared to NPH and ultralente human insulins using the euglycemic clamp technique.


And

Reproducibility of serum insulin and glucose infusion rate profiles of insulin glargine compared with NPH insulin and insulin Ultralente.


The intra-subject coefficient of variability in glucose lowering effect with insulin glargine was comparable to NPH insulin and superior to ultralente insulin following subcutaneous administration. A re-analysis using non-parametric methods to account for poorly produced profiles in two subjects showed that insulin glargine is associated with 30-50% less day-to-day variability in GIR profile reproducibility compared to NPH insulin and Ultralente.

**STUDY RATIONALE**

NPH insulin and Ultralente insulin are characterized by poor reproducibility of serum insulin levels and in glucose lowering effect. Insulin glargine has a smooth, peakless time action profile, but the reproducibility of the glucose lowering response of insulin glargine was not defined.

**OBJECTIVES**

To compare the pharmacodynamic variability (glucose lowering effect) of subcutaneous insulin glargine, NPH insulin and Ultralente insulin in healthy volunteers using the “euglycemic” clamp technique.

**STUDY DESIGN**

A single dose, double-blind, randomized, parallel replicate “euglycemic” clamp study undertaken in healthy male subjects assessing between day and within-subject variations in serum insulin concentration and the corresponding glucose lowering effect.

In this double-blind, randomized, parallel replicate design study, 36 healthy male volunteers aged 18 – 45 years were enrolled in three treatment groups (n=12/group). Subjects received two consecutive subcutaneous injections of insulin glargine, NPH insulin or ultralente insulin (0.4 units/kg), with a wash-out period of 7 days between treatments. “Euglycemic” clamps were performed, for up to 24 hours, after subcutaneous administration of the insulins ‘clamped’ at the subjects’ individual fasting blood glucose concentration.

The area under the curve (AUC) of the glucose infusion rate (GIR) (mg/kg) was the main pharmacodynamic variable and was recorded every 10 minutes for 24 hours. Insulin concentrations, corrected for endogenous insulin, were determined every hour.

In the first study, the mean values and coefficients of variation were determined for each of the patient groups. In the second analysis, the profile reproducibility and the standard deviation of these differences were calculated.

- The profile reproducibility was defined as the cumulative absolute differences in insulin concentration and GIR (clamp 1 - clamp 2) (ΔINS–CUMabsolute).
- The standard deviation of these differences was calculated to display a measure of the spread of these differences over time (SD–ΔINSraw).

The cumulative differences are similar to the area between the curves as the values were taken every 10 minutes for GIR and hourly for insulin. Non-parametric tests (Kruskal-Wallace) were employed.

**KEY FINDINGS**

- In the first analysis, intra-subject coefficient of variation during the 24 hour ‘clamp’ (AUC 12-24 hours) period (AUC 0-24 hours) was lowest for NPH insulin (19%) followed by insulin glargine (32%) and ultralente insulin (38%).
- Between 12 and 24 hours during the ‘clamp’ period, intra-subject variability was lowest for insulin glargine (23%) followed by NPH insulin (29%) and ultralente insulin (55%).
- In the second analysis, total insulin exposure (INS–AUC0–24h) was 40% greater for NPH insulin compared to insulin glargine, but suppression of endogenous insulin release (C-peptide) and total glucose disposal (GIR–AUC0–24h) were similar for all groups (Table 6).
- The profile reproducibility was the same for insulin glargine and NPH insulin, but significantly larger for Ultralente (Table 6).
- The standard deviation of between day differences in insulin and GIR showed less variation for insulin glargine profiles as compared with NPH and ultralente. However, these did not reach statistical significance because two subjects treated with insulin glargine were identified with markedly different profiles in exposure and hence GIR (Table 6).

**Additional references**

Determination of intra-subject variability with different insulins is a challenging task. The extrapolation of the findings to the clinical arena is confounded by the magnitude of the variability and multiple factors that determine blood glucose levels. This type of study conducted under rigorous experimental conditions allows correction for certain variables like proper resuspension of NPH insulin and Ultralente and does provide useful, but not definitive, evidence on reproducibility.

The findings were initially presented in 1999 and 2000 and confirmed that insulin glargine had a peakless onset of action after 2-3 hours, which was sustained for more than 24 hours and was consistent between visits. In contrast, the GIR profile of ultralente showed marked differences between the 2 visits, with about a four hour difference in onset of action (about 3 hours and 7 hours after administration). The intra-subject variation was lowest for NPH insulin (19%) followed by insulin glargine (32%) and ultralente insulin (38%), but was lowest for insulin glargine in the 12-24 hour period after injection. It is important to note that with this sort of analysis, the presence of a consistent peak for NPH insulin favors its reproducibility assessment.

In the data, it was apparent that the insulin glargine profiles were particularly poorly reproduced in 2 subjects, for unknown reasons. The re-analysis of the study findings, undertaken using non-parametric methods to give the extreme findings less weight, shows that insulin glargine is associated with 30-50% less day-to-day variability in GIR profile reproducibility compared to NPH insulin and Ultralente.

In healthy subjects, pairwise comparisons of $F_{24}$ showed significantly less fluctuation in mean serum insulin levels after a single dose of insulin glargine, in comparison to NPH insulin and Ultralente (Figure 32).

A complex fluctuation analysis tool, which was used to calculate fluctuation values based on the geometric mean values over a 24 hour period, expressed the findings as $F_{24}$ alone and $F_{24}$ as a percentage of average concentration ($C_{ave}$), expressed as $PF_{24}$. Three studies were analyzed that described findings in subjects with T1DM (2 studies, not described here) or healthy subjects (Scholtz et al., 1999). Healthy subjects ($n=36$) had received a single dose of insulin glargine, NPH insulin or ultralente (0.4 U/kg) on separate days (detailed on page 60).

Key findings

- Insulin glargine exhibits less fluctuation in serum insulin levels compared to NPH insulin or ultralente.

Study rationale

Of clinical concern are the large fluctuations of serum insulin concentrations in persons with DM treated with NPH insulin and ultralente. The within-day fluctuation of insulin glargine levels were undefined.

Objectives

To establish the level of fluctuation from mean serum levels of insulin glargine in comparison to other insulins using an analytical method, applied to previously described study findings.

Study design

Data from three previously described studies were assessed for fluctuations in mean serum insulin levels after single dose administration using analytical methods.

Reviewing healthy subjects only, the analysis of within-day fluctuations of serum insulin levels shows that insulin glargine offers a more consistent serum level compared to NPH insulin and ultralente. This may potentially reduce episodes of hyperglycemia or hypoglycemia.
Physiological responses during hypoglycemia induced by regular human insulin or a novel human analog, insulin glargine.


Insulin glargine is not associated with significant alterations in physiological or biochemical responses to hypoglycemia when compared to regular human insulin.

STUDY RATIONALE

Shortly after human insulin was introduced as a replacement for animal insulins, there were reports from patients of an altered perception of hypoglycemic symptoms. This study examined if the difference between insulin glargine and regular insulin translates into alterations in physiological response to hypoglycemia.

OBJECTIVES

To compare the physiological symptoms, and counter-regulatory hormones during hypoglycemia induced by regular human insulin or insulin glargine in healthy subjects and in persons with T1DM.

STUDY DESIGN

A single-dose, double-blind, randomized, two-way crossover trial in healthy subjects and persons with T1DM, assessing the nocturnal counter-regulatory response and a range of symptoms during a stepped hypoglycemic clamp using regular human insulin or insulin glargine.

KEY FINDINGS

- Controlled hypoglycemia induced by intravenous infusions of human insulin and insulin glargine each produced a similar and significant increase in the total symptom score in normal individuals.
- The peak total symptom scores at the lowest blood glucose level (2.5 mmol/L) elicited by human insulin and insulin glargine were comparable in both normal subjects (human insulin: 18.83 ± 2.68; insulin glargine: 18.5 ± 3.20) and in persons with T1DM (human insulin: 17.46 ± 3.62; insulin glargine: 19.08 ± 3.83).
- The peak epinephrine levels in normal blood glucose levels were comparable in both normal subjects (human insulin: 18.83 ± 2.68; insulin glargine: 18.5 ± 3.20) and in persons with T1DM (human insulin: 17.46 ± 3.62; insulin glargine: 19.08 ± 3.83).
- The peak epinephrine levels in normal subjects during hypoglycemia induced by human insulin and insulin glargine were comparable (767 ± 140.4 pg/ml and 608.8 ± 129.9 pg/ml respectively).
- Human insulin and insulin glargine elicited similar rates of glucose disposal.

Additional references

EDITORS COMMENTARY
Symptoms were experienced in equivalent degrees during hypoglycemia induced by both insulins. The counter-regulatory hormone response did not differ significantly between the two insulins. Comparing human insulin and insulin glargine, the study found similar glucose infusion rates were required during the clamp procedure indicating that the insulins are equipotent with regard to a key biologic property, namely insulin-stimulated glucose disposal. These findings indicate that the structural and physicochemical properties of insulin glargine are not associated with significant alterations in physiological or biochemical responses to controlled hypoglycemia compared to regular human insulin in normal subjects and persons with T1DM. Thus, it is unlikely that insulin glargine has any intrinsic role in the genesis of hypoglycemia unawareness. Nevertheless, further studies to assess the responses to insulin glargine in subjects with previous evidence of hypoglycemia unawareness are certainly warranted.

STUDY RATIONALE
The glucose lowering effect of insulin glargine in comparison with regular human insulin was investigated.

OBJECTIVES
To compare the glucose-lowering effect of equimolar concentrations of insulin glargine and regular semi-synthetic human soluble insulin given intravenously in healthy subjects using the ‘euglycemic’ clamp technique.

STUDY DESIGN
Twenty healthy subjects received insulin glargine and regular insulin as a constant intravenous infusion followed by a ‘euglycemic’ glucose clamp. This study was a single dose, double-blind, randomized two-way crossover study in which healthy subjects (n=20) received insulin glargine and regular insulin (0.1 U/kg) as a 30 minute constant intravenous infusion after which they were clamped for six hours at their individual fasting blood glucose concentration. The area under the curve (AUC) of the glucose infusion rate (GIR) was calculated.

KEY FINDINGS
• The AUC_{0-6} of the GIR for insulin glargine and regular insulin was 663.9 and 734.9 mg/kg, respectively.
• The 90% confidence interval for the mean ratio of insulin glargine to regular insulin was 84.6 – 96.7% (point estimate 90.3%), which was within the pre-defined equivalence range.

Additional References
Intravenous glargine and regular insulin have similar effects on endogenous glucose output and peripheral activation/deactivation kinetic profiles.


Although intravenous glargine has an amino acid sequence distinct from human insulin, when given intravenously it has similar biological activity, as measured by hepatic glucose output and peripheral glucose uptake.

STUDY RATIONALE

Insulin glargine has an amino acid sequence distinct from that of human insulin, which results in kinetic differences. Absorption is delayed, but it was unclear if the amino acid changes alter biological activity.

OBJECTIVES

To compare the effects of intravenously administered insulin glargine and regular human insulin on the activation and deactivation of suppression of endogenous glucose output (EGO) and stimulation of peripheral glucose disposal in healthy subjects.

STUDY DESIGN

Healthy subjects (n=12, age 34.8 ± 2.7 years, BMI 24.2 ± 0.7 kg/m², mean fasting plasma glucose 89 ± 2.2 mg/dL (4.9 ± 0.1 mmol/l) mean fasting insulin 8.1 ± 2.4 µIU/ml) took part in the glucose "clamp" study, which consisted of a basal study period and a glucose clamp study period.

After an eight hour overnight fast, subjects were infused with [3H]-labeled glucose to maintain euglycemia at 90 mg/dL (5 mmol/L).

After basal measurements were complete, a 10 minute priming dose was followed by a continuous intravenous infusion of 40 mU/m²/min for 4 hours of either insulin glargine or regular human insulin, followed by a 3-hour deactivation period. During the 7-hour period, subjects received a continuous, variable infusion of [3H]-labeled glucose to maintain euglycemia at 90 mg/dL (5 mmol/l).

The glucose turnover rate was assessed during both basal and glucose clamp phases using the modified Steele equations for non-steady-state conditions.

In the basal state, EGO is equal to the rate of glucose appearance (Ra).

A50EGO is the time in minutes required for 50% of the active suppression of EGO after insulin infusion.

D50EGO is the time required to achieve 50% deactivation from maximum insulin induced suppression of EGO after cessation of insulin infusion.

 Incremental glucose disposal rate (IGDR) was defined as the difference between the basal glucose disposal rate (GDR) and GDR values during and after cessation of insulin infusion.

A50IGDR was defined as the time from basal glucose disappearance (Rb) to reach half maximum insulin-stimulated glucose disposal rate (Rc).

D50IGDR is the time required for 50% deactivation from maximum insulin-stimulated GDR to half maximum GDR after cessation of insulin infusion.

STUDY RATIONALE

No significant differences were observed in the activation and deactivation kinetics of intravenously administered insulin glargine and regular human insulin with respect to EGO or peripheral glucose disposal.

• Serum insulin concentrations of both were similar during the 4-hour insulin infusion (activation period) and during the 3-hour deactivation period (Figure 33a).

• The mean time required for 50% suppression of EGO (A50EGO) after insulin infusion was similar for regular insulin (73 ± 23 minutes) and insulin glargine (57 ± 20 minutes) (Figure 33b).

• The mean maximum rate of glucose disposal was 10.10 ± 0.77 mg/kg/min for regular insulin and 9.90 ± 0.85 mg/kg/min for insulin glargine (Figure 33c).

• The mean time required for 50% suppression of the incremental GDR (A50IGDR) was 32 ± 5 and 42 ± 10 minutes for regular insulin and insulin glargine respectively.

• The time required for deactivation from maximum insulin-stimulated GDR to half maximum GDR after cessation of insulin infusion (D50IGDR) was 63 ± 5 and 57 ± 4 for regular insulin and insulin glargine respectively.

EDITORS COMMENTARY

These elegant findings show that insulin glargine does not differ from regular human insulin when administered intravenously in normal subjects. This provides good evidence that the different pharmacodynamic effects of regular insulin and insulin glargine observed following subcutaneous administration are due to differences in their absorption, with insulin glargine showing delayed absorption as a consequence of its distinct molecular structure.

Additional References

Clinical Pharmacology Studies

Chapter 2  •  Insulin Glargine Chemistry and Pharmacology

Biotransformation of insulin glargine after subcutaneous injection in healthy subjects.


Insulin glargine undergoes metabolic breakdown both at the injection site and within the circulatory system to yield two products which are structurally similar to human insulin and which possess equivalent metabolic activity to the parent compound.

STUDY RATIONALE

In vivo animal studies have shown that two main metabolites of insulin glargine (designated M1 and M2) are produced. Metabolite one (M1) results from cleavage of the two arginine amino acids (B31 and B32) from the C-terminus of the β chain (A21-Gly-insulin) and metabolite two (M2) from the additional loss of the next amino acid on the β chain (A21-Gly-des-30B-Thr-insulin). Both M1 and M2 have been shown to possess equivalent metabolic activity to that of the parent compound in vitro and in vivo.

OBJECTIVES

To determine the metabolic degradation pattern of insulin glargine in humans at the site of bolus subcutaneous injection and in the systemic circulation over 24 hours following administration.

STUDY DESIGN

Open label, metabolite-profiling study in five healthy subjects obtaining injection site tissue and plasma samples and using high performance liquid chromatography (HPLC) and radioimmunoassay (RIA) techniques to identify insulin glargine metabolites.

Five healthy, male subjects aged 18-50 years with a BMI of 18-26 kg/m² were enrolled. Four subjects received a single subcutaneous injection of insulin glargine (0.6 U/kg) into the anterior abdominal wall; placebo was administered to one subject. Subjects underwent a euglycemic glucose “clamp” procedure over the following 24 hour.

Blood samples were taken at one hour intervals and RIA was used to determine immunoreactive insulin (in plasma and serum) and C-peptide (in serum) concentrations. A tissue sample was obtained by liposuction from the point of subcutaneous injection. One tissue sample was taken at either 2, 6 12 or 24 hours after injection. Fractions containing insulin and insulin metabolites were identified by HPLC based on their retention times using reference compounds. The identity of insulin and insulin metabolites was confirmed by mass spectrometry.

KEY FINDINGS

- Analysis of tissue samples by HPLC and RIA revealed two immunoreactive peaks in varying ratios; the peak eluted first was identified as insulin glargine; the second peak was a mixture of metabolites, M1 and M2. Insulin glargine and the M1 and M2 metabolites were present in an average ratio of 50:50.
- Analysis of plasma samples revealed the presence of insulin glargine and two of the degradation products (M1 and M2) in the circulation in addition to endogenous insulin.

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- Analysis of plasma samples revealed the presence of insulin glargine and two of the degradation products (M1 and M2) in the circulation in addition to endogenous insulin.

EDITORS COMMENTARY

This study examined the 24 hour degradation profile of insulin glargine. Following subcutaneous administration, insulin glargine undergoes metabolic degradation resulting in two metabolites (M1 and M2). Degradation appears to be initiated at the injection site and continued within the circulatory system. The early degradation of insulin glargine in the subcutaneous tissue does not impair its biological activity.

Additional references

Clinical Pharmacology Studies

Chapter 2 • Insulin Glargine Chemistry and Pharmacology

**Metabolic action and cellular processing and degradation of insulin glargine.**


Certain differences in cellular processing and metabolic action occur between regular insulin and insulin glargine.

**STUDY RATIONALE**

The effects of insulin glargine on glucose metabolism and mitogenesis have been extensively investigated as part of its safety evaluation, but other aspects of insulin glargine cellular processing and action have been studied in less detail.

**OBJECTIVES**

To compare insulin glargine to human insulin using HepG2 and 3T3-L1 cell line models to characterize cellular binding, degradation, intracellular processing, and stimulation of DNA synthesis. To study the relative impact of human insulin and insulin glargine on insulin-mediated lipolysis and glucose incorporation into lipids.

**STUDY DESIGN**

Radioiodinated regular human insulin and insulin glargine were used to assess their respective cellular binding, degradation, intracellular processing, and inhibition of epinephrine-stimulated lipolysis in HepG2 cells and 3T3-L1 cell lines.

In HepG2 cells, the cellular binding, degradation and processing of [125I]-labeled human insulin and [125I]-labeled insulin glargine were quantified and effects on DNA synthesis (assessed by thymidine uptake) and protein degradation were measured. The effects of human insulin and insulin glargine on epinephrine-stimulated lipolysis and glucose incorporation into lipids were studied in 3T3-L1 adipocytes.

**KEY FINDINGS**

- The cellular binding of human insulin and insulin glargine were similar in HepG2 cells.
- Degradation of human insulin was significantly higher than insulin glargine (21.6 ± 1.4% vs. 16.3 ± 0.3% degraded/hour; p<0.01) and consequently more degraded human insulin was released, by cells previously loaded with radiolabeled material, compared to insulin glargine (58.3 ± 1.4% vs. 50.1 ± 2.4%; p<0.02). The amount of intact human insulin was concomitantly reduced compared with insulin glargine (35.8 ± 1.4% vs. 44.8 ± 2.6%; p<0.02).
- Protein degradation was significantly higher (~20%) with human insulin compared to insulin glargine (~15%; p<0.05).
- In 3T3-L1 adipocytes, human insulin inhibited epinephrine-stimulated lipolysis to a greater degree compared to insulin glargine (EC<sub>50</sub> = 0.35 vs. 1.40 nM; p<0.001).
- Human insulin and insulin glargine had similar effects on lipogenesis and glucose oxidation to CO<sub>2</sub>.

**EDITORS COMMENTARY**

The assessment of insulin analogs and how their metabolic processing, binding and actions compares to human insulin have not been extensively investigated. Previous studies have however suggested that the mechanism of insulin action is not exclusively related to the extent of receptor-binding and receptor-mediated signal transduction. This study demonstrates in vitro differences in metabolic action and cellular processing between regular human insulin and intact insulin glargine in the cell lines studied. Intracellular processing and degradation of insulin glargine was less than human insulin and more intact insulin glargine was released by cells compared with regular insulin. Insulin glargine was also slightly less effective in reducing lipolysis and inhibiting protein degradation. However, these differences are considered unlikely to have adverse clinical effects and may even partially enhance the effectiveness of insulin glargine as a basal insulin.
Effects of basal insulin treatment on IGF-1: glargine vs. NPH-insulin.

Slawik M, Petersen KG. Diabetes 2002; 51(Suppl 1):A296 Abstract 1202–P.

Insulin glargine has no intrinsic in vivo IGF-1 affinity.

STUDY RATIONALE

Given the role of the IGF-1 receptor in mediating aberrant insulin growth signals and the 6-8 fold higher affinity of insulin glargine for the IGF-1 receptor shown in a specific in vitro model, in vivo assessments of the effect of insulin glargine on IGF-1 levels were warranted.

OBJECTIVES

To determine if insulin glargine has intrinsic IGF-1 activity in vivo, determined indirectly by assessing serum IGF-1 levels in comparison to NPH insulin in persons with T1DM and T2DM.

STUDY DESIGN

Single center, open label study measuring IGF-1 concentrations in serum, one and three weeks after commencement of treatment with either insulin glargine or NPH insulin.

Subjects with T1DM (n=15; mean age: 27 years; mean BMI: 25 kg/m²) or T2DM (n=20; mean age: 66 years; mean BMI: 30 kg/m²) being treated with either NPH insulin or insulin glargine, were switched to the alternative insulin for 3 weeks. Serum IGF-1 and HbA1c were measured at one and three weeks.

KEY FINDINGS

- No reduction in serum IGF-1 values was detected in response to insulin glargine compared to NPH insulin (Figure 35) in persons with T1DM or T2DM.
- The serum IGF-1 levels increased moderately with insulin glargine and this was most marked in males with T1DM.

Figure 35. Serum IGF-1 concentrations in combined group of patients with T1DM or T2DM.

EDIATORS COMMENTARY

The measurement of IGF-1 levels was an indirect means of examining the interaction of NPH insulin and insulin glargine with the IGF-1 receptor. Since IGF-1 levels did not decrease when patients were switched from NPH to insulin glargine, no intrinsic binding activity of insulin glargine at the IGF-1 receptor in vivo is suspected.

Vascular function; retinopathy

Progression of retinopathy with insulin glargine or NPH insulin: A multi-trial analysis.


This comprehensive analysis of data from four phase III clinical trials in persons with T1DM and T2DM was conducted by an expert panel and provides strong evidence and opinion that insulin glargine is not associated with an increased risk of, or progression of, diabetic retinopathy.

STUDY RATIONALE

Given the awareness of the role of the IGF-1 receptor in mediating aberrant insulin growth signals and the implication of IGF-1 as a mediator of progression of retinopathy, the expert panel was convened and a detailed review conducted of four phase III registration studies. The panel sought evidence that there was no association between insulin glargine therapy and diabetic retinopathy progression.

OBJECTIVES

To examine in detail the adverse ophthalmologic outcomes in persons with T1DM or T2DM who had been treated with insulin glargine or NPH insulin within a defined clinical trial environment.

STUDY DESIGN

Retrospective assessment of parameters related to ophthalmologic safety in persons with T1DM or T2DM enrolled in four randomized clinical trials.

The findings of the comprehensive ophthalmologic-related safety parameters included in the four phase III randomized clinical trials in 2207 persons with T1DM or T2DM were reviewed by an expert panel, consisting of four ophthalmologists and one physician, considered experts in the field.

KEY FINDINGS

- The rates of progression of retinopathy were shown to be similar between NPH insulin and insulin glargine, with the largest difference in a T2DM study (2.7% vs. 7.5%, respectively).
- Proliferative retinopathy rates appeared similar between NPH insulin and insulin glargine.
- The rates of CSME were not significantly different between NPH insulin and insulin glargine.
- The rates of CSME were not significantly different between NPH insulin and insulin glargine, based on ophthalmologic examination and fundus photography.
- A similar number of RAES (around 10%) were recorded with each treatment.
- Optic disc swelling was not observed in any patients.
EDITORS COMMENTARY

An extensive clinical evidence base (>2000 patients) from the registration trials was reviewed by the “Retinopathy Expert Working Group”, an independent panel of four ophthalmologists and one endocrinologist, all with a special interest in diabetes. Their final conclusion, that “insulin glargine is not associated with increased risk of development or progression of diabetic retinopathy”, appears to be valid from the detailed review that was undertaken.

There is an ongoing follow-up process, including data from clinical trial experience and post-marketing surveillance, to rule out any potential retinopathy-related long term effects of insulin glargine therapy.

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**STUDY RATIONALE**

Endothelial dysfunction is an important finding in T2DM. Previous studies have shown that six months of insulin therapy with NPH insulin enhances both endothelium independent and dependent vasodilatation in T2DM. The effects of prolonged insulin therapy with the insulin analog, insulin glargine, on vascular function are unknown.

**OBJECTIVES**

To determine whether long term insulin glargine therapy (3-5 years) maintains or further improves endothelial function in insulin-naive patients with T2DM.

**STUDY DESIGN**

Comparison of endothelial function before and after the introduction of insulin glargine in persons with T2DM matched to healthy subjects. The endothelial function was estimated from blood flow response to in vivo intrabrachial artery infusions of endothelial dependent and independent vasodilators.

**KEY FINDINGS**

- Before insulin glargine treatment, as expected the blood flow response to ACh was significantly lower in persons with T2DM compared with healthy controls (p<0.021), but not in response to SNP.
- After 6 months of insulin glargine treatment, blood flow responses to both ACh and SNP increased significantly and were no longer different from control (Figure 36a).
- After 3.5 years of insulin glargine treatment, blood flow during infusion of high dose ACh increased significantly from 8.8 ± 0.9 ml/dL per minute at baseline to 13.0 ± 1.9 ml/dL per minute at 3.5 years (p<0.01 vs. baseline) (Figure 36b).

**Subjects**

Eleven insulin-naive persons with T2DM receiving OHA (age 59 ± 2 years; BMI 29.7 ± 0.9 kg/m²; known duration of diabetes 8 ± 1 years) and 16 healthy, age, gender and weight-matched control subjects were enrolled.

**Treatment groups**

Participants with T2DM received a single subcutaneous injection of insulin glargine once daily at bedtime for 3.5 years with OHA continued unchanged. Control subjects received no treatment.

**Methodology**

In vivo endothelial function was evaluated from blood flow responses to intra-brachial artery infusions of endothelial-dependent (acetylcholine (ACh): doses 7.5 and 15 μg/min) and endothelial-independent (sodium nitroprusside (SNP); doses 3 and 10 μg/min) vasodilators before and after 0.5 and 3.5 years of insulin glargine treatment. BMI, insulin dose and glycemic control were measured at baseline, and after 6 months and 3.5 years.

**3.5 years of insulin therapy with insulin glargine markedly improves in vivo endothelial function in type 2 diabetes.**

• After 3.5 years of insulin glargine treatment, blood flow during infusion of SNP increased significantly from 10.7 ± 0.9 ml/dL per minute at baseline to 13.4 ± 1.0 ml/dL per minute at 6 months (p<0.01) and 16.6 ± 1.5 ml/dL per minute at 3.5 years (p<0.01 vs. 0 years) (Figure 36b).

• During insulin glargine therapy, mean HbA1c levels decreased from 9.1 ± 0.4% to 7.5 ± 0.2% at 6 months (p<0.001) and remained at this level (7.5 ± 0.2%) at 3.5 years (p<0.001).

• The bedtime insulin glargine dose averaged 40 ± 5 IU (0.43 U/kg) at 6 months increasing to 60 ± 10 U (0.63 U/kg) at 3.5 years, there was no significant change in body weight during the study (87.5 ± 3.4, 88.7 ± 3.8 and 89.9 ± 3.9 kg at baseline, 6 months and 3.5 years, respectively).

**Figure 36.** Forearm blood flow response to intra-arterial SNP and Ach infusions in (a) patients with T2DM at 6 months and (b) 3.5 years. (Reprinted from Arteriosclerosis, Thrombosis and Vascular Biology 2004; 24:1–7. Reprinted with permission from LWW Publishing Group).

**EDITORS COMMENTARY**

The effects of hyperinsulinemia on vascular function are continually debated. However, studies show that insulin therapy acutely enhances vasodilation induced by endothelium-dependent vasodilators, such as Ach and activates endothelial nitric oxide synthase increasing the production of nitric oxide in *vivo*. A marked improvement in both endothelium-dependent and endothelium-independent vasodilation was seen at 6 months and a further improvement at 3.5 years from study initiation with insulin glargine treatment. These data support the view that insulin glargine therapy can improve and even reverse endothelial dysfunction and has reaffirmed the fact that insulin has beneficial rather than harmful effects on vascular function.

**Additional references**

Insulin glargine and regular human insulin similarly acutely enhance endothelium-dependent vasodilation in normal subjects


STUDY RATIONALE

The structural changes to insulin glargine may change its binding properties to the insulin receptor and homologous receptors, such as the insulin-like growth factor-1 receptor. Therefore, this study was carried out to examine the impact of insulin glargine on vascular function in normal subjects given that its in vitro effects on endothelium-dependent and endothelium-independent vascular function were previously unknown.

OBJECTIVES

To compare the in vitro effects in healthy subjects of regular human insulin and insulin glargine on endothelium-dependent and endothelium-independent vasodilatation induced by acetylcholine (ACh) and sodium nitroprusside (SNP).

STUDY DESIGN

Normal subjects received infusions of ACh or SNP and blood flow responses were recorded by venous occlusion plethysmography under both normal and hyperinsulinemic conditions, using infusions of saline, regular human insulin or insulin glargine.

Normal, apparently healthy males (n=10; mean ± SD: age: 33 ± 3 years, mean BMI: 23.2 ± 2.4 kg/m²) were studied on two days in a double-blind, cross-over fashion. Subjects were studied under normoglycemic or normo-glycemic hyperinsulinemic “clamp” conditions maintained by intravenous infusion of saline, regular human insulin or insulin glargine (120 min, 1 mU/kg/min). In each study, blood flow responses to intrabrachial artery infusions of ACh and SNP were recorded.

KEY FINDINGS

• Comparing saline to insulin, endothelium-independent blood flow responses to low (3 µg/min) and high (10 µg/min) doses of SNP were unaltered by regular human insulin (11.2 ± 1.1 vs. 12.0 ± 1.7 and 16.8 ± 1.9 vs. 18.4 ± 2.6 ml/dL/min, respectively) or by insulin glargine (12.2 ± 2.6 vs.13.4 ± 4.6 and 19.1 ± 4.2 vs.19.6 ± 5.1 ml/dL/min, respectively), but with no difference evident between the insulins.

• Comparing saline to insulin, endothelium-dependent blood flow responses to both low (7.5 µg/min) and high doses (15 µg/min) of ACh were enhanced significantly by regular human insulin (at low dose 11.5 ± 6.0 vs. 15.8 ± 8.0 ml/dL/min, respectively (p<0.05) and at high dose 14.0 ± 7.5 vs. 21.1 ± 10.4 ml/dL/min, respectively (p<0.01)) and insulin glargine (at low dose 13.9 ± 4.8 vs. 19.3 ± 6.5 ml/dL/min, respectively (p<0.02) and at high dose 17.3 ± 2.1 vs. 23.2 ± 3.1 ml/dL/min, respectively (p<0.02)) but with no difference evident between the insulins.

Glargine and regular human insulin similarly acutely enhance endothelium-dependent vasodilation in normal subjects


STUDY RATIONALE

The structural changes to insulin glargine may change its binding properties to the insulin receptor and homologous receptors, such as the insulin-like growth factor-1 receptor. Therefore, this study was carried out to examine the impact of insulin glargine on vascular function in normal subjects given that its in vitro effects on endothelium-dependent and endothelium-independent vascular function were previously unknown.

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EDITORS COMMENTARY

This study shows a similar acute stimulatory effect on endothelium-dependent vasodilation in normal individuals mediated by regular human insulin and insulin glargine. These results suggest that the difference in structure between insulin glargine and human insulin has no effect on the acute vasodilatory effect of Ach or SNP in a dose response study. These findings are important to consider in the context of an increased binding affinity of insulin glargine in vitro to the IGF-1 receptor and suggest that in vivo, the enhanced binding affinity has no differential effect on vasodilatory function with human insulin and insulin glargine behaving similarly in normal subjects.

Additional references

Clinical Pharmacology Studies

SUMMARY

Developed by Aventis, insulin glargine is the first clinically available recombinant, long-acting insulin analog. The pharmacokinetic imperfections of conventional “intermediate” and “long-acting” insulin preparations used over the last 50 years limit their ability to simulate basal insulin secretion, a prerequisite to normalize glycemia. The occurrence of peak insulin concentrations resulting from their subcutaneous administration causes both an increased risk of hypoglycemia and, due to the limited and variable duration of action, hyperglycemia. The power of recombinant DNA technology to create the designer insulin, insulin glargine, with protracted action, which is administered as a solution without the need for resuspension represents a landmark in modern molecular medicine. A large pre-clinical and clinical investigation program has characterized the binding and signaling characteristics, mitogenic capacity, absorption, metabolism and time-action profile of insulin glargine, validating the molecular design of this molecule, and indicating that this novel insulin molecule provides a long-acting and peakless basal insulin supply.

MOLECULAR CHEMISTRY

Insulin glargine was developed on the novel premise that the introduction of amino acid modifications that increase the isoelectric point of the native insulin molecule towards neutrality would result in precipitation of the insulin in the subcutaneous compartment and result in delayed absorption. The first extended-action insulin analog made by this method was NovoSol Basal (GlyA21, ArgB27, ThrB30-NH₂-insulin), which had an extremely protracted absorption. However, the development was discontinued due to reduced bioavailability (Jørgensen et al., 1989). On the basis of the same concept, a new and novel di-arginyln insulin analog was developed, but this was shown to have a shorter glucose-lowering action than NPH insulin (Zeuzem et al., 1990). It was after further research that an additional modification, substituting a glycine residue at position A21, was shown to alter the kinetics, resulting in an extended duration of action.

The molecular consequences of these alterations made to native human insulin to create insulin glargine result in key advantages for the analog over conventional insulin preparations. The alteration of the isoelectric point renders the insulin glargine molecule insoluble at physiologic pH, which results in amorphous precipitation in the subcutaneous tissue and a slow, peakless release with extended action up to 24 hours. In addition, the solubility of insulin glargine at an acidic pH ensures that the molecule is in solution at the time of injection, removing all concerns over variability in dosing because of a lack of resuspension.

RECEPTOR BINDING, SIGNALING AND MITOGENICITY

The findings from the studies conducted on the rapid-acting insulin analog, Asp [B10], emphasize why close assessment of receptor binding, receptor signaling and mitogenic potential of insulin analogs must be a central goal. Insulin receptor affinity and metabolic potency of insulin molecules are known to correlate with mitogenic potential, and that this is also related to insulin receptor occupancy time and to IGF-1 receptor affinity, as shown with Asp [B10] (Berti et al., 1998). Amino acid substitutions can modify the tertiary structure of the insulin protein in a way that alters interaction with the insulin and IGF-1 receptors and potentially lead to modifications in downstream, intracellular insulin signaling patterns.

In the studies using rat-1 fibroblasts over-expressing the human insulin receptor, insulin glargine behaved in a similar manner to normal human insulin with respect to insulin receptor association/dissociation and phosphorylation/dephosphorylation of the receptor and known substrates in the intracellular signaling cascade (Berti et al., 1998). Both insulins produced similar growth promoting activity as shown by comparable levels of thymidine incorporation. These findings suggest that insulin glargine does not differ significantly from native human insulin with respect to its effects on early intracellular signaling and mitogenic potency.

The growth-promoting activities of insulin glargine and native human insulin mediated by the IGF-1 receptor were compared in muscle tissue using cardiac myoblasts (H9c2 cardiac myoblasts) as a cell model, which express high levels of IGF-1 receptors with no detectable insulin receptors (Bähr et al., 1997). The growth-promoting activities of insulin glargine and human insulin were essentially identical, despite a slightly higher affinity of insulin glargine for the IGF-1 receptor. This finding relates only to the interaction of insulin glargine with the IGF-1 receptor. In contrast with the findings described above, a further study did find that insulin glargine had a 6.5-fold increased affinity for the IGF-1 receptor protein purified from transfected Baby Hamster Kidney cells and an 8-fold increased mitogenic potency, assessed by [³H]-thymidine incorporation, compared with native insulin in human malignant osteosarcoma cell line Saos/B10 that predominantly expresses IGF-1 receptors (Kurzhals, 2000). These findings were reviewed in depth and expert opinion indicated that differences in the findings reported related to the unique characteristics of the cell models used, in particular differences in the relative expression of insulin and IGF-1 receptors (Kellerer and Häring, 2001).

Recently, more studies conducted using human micro- and macrovascular endothelial cells that abundantly express IGF-1 receptors have been published. Again, although a difference in binding between human insulin and insulin glargine was apparent, this was only at very high concentrations (Chisalita and Arnqvist, 2004). A recent study has examined the interaction of insulin glargine with the IGF-1 receptor in vitro and in vivo. Using a sophisticated surface plasmon resonance Biacore analysis, which monitors the progress of biomolecular interactions in real time, insulin glargine had about an 8-fold higher affinity for soluble IGF-1 receptor compared to human insulin, but a 10-fold lower affinity than IGF-1. In addition, insulin glargine had a 14-fold faster association-rate and an almost 2-fold faster dissociation-rate as compared with insulin (Ekström et al., 2004). An in vitro study was then undertaken in 12 adolescents with T1DM who were about to receive a 12-week intensified treatment program with insulin glargine. IGF-1 concentrations increased markedly after 2 weeks of insulin glargine treatment to a level sustained to study end. The authors suggest that this study confirms that insulin glargine has increased affinity for soluble IGF-1 receptor in vitro but that the increase in serum IGF-1 concentrations argues against insulin glargine interactions with IGF-1 receptor in subjects with T1DM.

The findings that insulin glargine dose have a greater capacity to promote DNA synthesis in certain situations compared to human insulin raised concerns, especially given that IGF-1 signaling is known to promote vascular endothelial growth factor-dependent retinal neovascularization, shown in Figure 37. Given this, an independent panel of experts retrospectively reviewed clinical data from 2207 patients enrolled in the registration trials for insulin glargine in persons with T1DM and T2DM (Forjanic–Klapproth and Home, 2001). There was no overall independent increased risk of development or progression of retinopathy and no cases of optic disk swelling were reported with either insulin glargine or NPH insulin, which makes it highly unlikely to be a real concern.

Further carcinogenicity studies carried out on mice and rats show no neoplastic changes after treatment with human insulin or insulin glargine up to 12.5 U/kg/day in rats and mice insulin glargine in persons with T1DM and T2DM and there is no suggestion in Wistar rats and Himalayan rabbits that insulin glargine has reproductive toxicity or embryotoxicity.
remained constant thereafter, with no peak effect and almost constant glucose-lowering activity. The effect lasted up to an average of 24 hours, although in some patients the duration of action was estimated to be in excess of 30 hours (Heinemann et al., 2000).

The results obtained in healthy subjects have been confirmed in intensively treated persons with T1DM. In a study undertaken in 20 subjects with T1DM to indirectly compare the pharmacokinetics and dynamics of insulin glargine with NPH insulin, ultralente and continuous subcutaneous infusion of insulin lispro (CSII), patients were studied on four occasions during a 24-hour isoglycemic clamp (Lepore et al., 2000). In a 2-way, crossover design patients were given 0.3 U/kg of insulin, either NPH insulin or insulin glargine subcutaneously. On two subsequent days, patients received either ultralente or CSII. There was a pronounced peak in action of human NPH insulin after about 4.5 hours and at about 10 hours after ultralente. Insulin glargine had a flat, prolonged action profile, with an onset of action was later than other insulins (p<0.05) (Figures 39a and 39b). Additionally, intersubject variability was lower with insulin glargine than with human NPH insulin and ultralente (p<0.05).

In a comparison study (Porcellati et al., 2002), once daily insulin glargine given in the evening was compared to multiple daily injections of NPH insulin and ultralente. Insulin glargine had a prolonged duration of effect, with a relatively constant, flat time-action profile, compared to other insulins.

The early findings reported by Dreyer showed that insulin glargine was characterized by a longer duration of action with a less pronounced peak of insulin action compared to NPH insulin, irrespective of the zinc content of the formulation (Dreyer et al., 1994). The duration of insulin action was longer than 24 hours for both formulations of insulin glargine compared to NPH insulin, which had duration of action of about 16 hours.

The time-action profile of insulin glargine was described in comparison to NPH insulin over an extended period of 30 hours in healthy subjects using the “clamp” technique (Heinemann et al., 2000). The use of healthy subjects required endogenous insulin secretion to be suppressed, which was achieved by a continuous intravenous infusion of insulin. To correct for the metabolic effect of the infused insulin, a control experiment was conducted in which subjects received a placebo injection. Following subcutaneous injection, insulin glargine levels increased to a plateau within 4 hours of administration and remained constant thereafter, with no peak effect and almost constant glucose-lowering activity. The effect lasted up to an average of 24 hours, although in some patients the duration of action was estimated to be in excess of 30 hours (Heinemann et al., 2000).

The extended duration of action of insulin glargine does not lead to an accumulation of circulating insulin or an increased metabolic effect. Investigations in persons with T1DM...
who received a single daily injection of insulin glargine at bedtime for 11 consecutive days show no accumulation following multiple injections over a 12 day dosing period and that the use of a loading dose or dose reduction after several injections of insulin glargine is unnecessary.

CONCLUSION

The insulin analog, insulin glargine, has a unique molecular structure, the biological consequences of which render the molecule long-acting with a predictable time-action profile and little intra-individual variability in bioavailability, and with no abnormal behavior with respect to mitogenicity.

References

1. Chisalita SI, Arnqvist HJ. Insulin-like growth factor 1 receptors are more abundant than insulin receptors in human micro- and macrovascular endothelial cells. Am J Physiol Endocrinol Metab 2004;286:E896-901.