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Mitogenic potency of insulin glargine

Mitogenne działanie insuliny glargine

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Abstract

The goal of diabetes mellitus treatment is to maintain long-term near-normoglycaemia to prevent the onset or progression of long-term complications. In order to achieve tight glycaemic control and improve the quality of life for diabetic patients, a number of novel insulin preparations, insulin analogues, have been constructed thanks to recombinant DNA technologies and advanced protein chemistry. Because structurally modified insulins may differ from human insulin not only in metabolic but also in mitogenic potencies there were concerns raised about the possibility of increased insulin analogue proliferative action or tumourigenesis. *In vitro* and *in vivo* studies on insulin analogues in comparison to endogenous insulin have been performed to closely monitor the insulin analogue action profiles. Insulin glargine was the only one presenting a significant increase in affinity to insulin-like growth factor type 1 (IGF-1) receptor. However, there was controversy regarding the safety of insulin glargine use because of its potential risk of mitogenicity but it proved to be true only for human osteosarcoma cells Saos/B10. Outcomes of the studies performed on lines other than cancer cells and on animals did not present any increased mitogenic activity nor mitogenic potency of insulin glargine in comparison to human insulin. **(Pol J Endocrinol 2009; 60 (1): 34–39)**

Key words: *insulin analogue, glargine, mitogenicity*

Streszczenie

Celem leczenia cukrzycy jest uzyskanie i stałe utrzymywanie stanu zbliżonego do normoglikemii w celu zapobiegania powstawaniu i progresji późnych powikłań naczyniowych. Aby uzyskać pełną kontrolę gikemii i poprawić jakość życia pacjentów chorujących na cukrzycę, opracowano, dzięki wykorzystaniu technik rekombinacji DNA i zaawansowanych metod modyfikacji białek, wiele nowych preparatów insulinowych — analogów insuliny. Ponieważ strukturalnie zmodyfikowana cząsteczka insuliny może różnić się od cząsteczki ludzkiej insuliny nie tylko wpływem na metabolizm, ale również właściwościami mitogennymi, dlatego zwrócono uwagę na istnienie ryzyka podwyższonej aktywności proliferacyjnej analogów insuliny. W celu dokładnej oceny profilu aktywności insulin analogowych porównano je, zarówno w badaniach *in vitro,* jak i *in vivo*, z insuliną ludzką. Glargina okazała się być jedyną insuliną, która charakteryzuje się znamiennie zwiększonym powinowactwem do receptora insulinopodobnego czynnika wzrostu 1 (IGF-1, *insulin-like growth factor type 1*). Mimo że istniały kontrowersje dotyczące bezpieczeństwa stosowania glarginy z uwagi na ryzyko działania mitogennego, to okazało się ono istotne jedynie w badaniach *in vitro* w odniesieniu do ludzkiej linii komórek kostniakomięsaka Saos/B10. Wyniki pozostałych badań nie wykazały zwiększonej aktywności mitogennej lub potencjału mitogennego w porównaniu z insuliną ludzką.
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Słowa kluczowe: *insulina analogowa, glargine, mitogenność*

Introduction

The purpose of this review is to summarize the outcomes of performed up to date studies regarding the mitogenic potency of insulin analogues. In response to human insulin molecule structure modification, not only metabolic but also supposedly mitogenic activity of insulin analogues may be significantly altered, that is why the potentially dangerous properties of these preparations have been widely examined in both *in vitro* and *in vivo* studies.

In the year 1921, Canadian surgeon Frederic Banting, Charles Best a medical student, physiology professor John J.R. Macleod, and James B. Collip a biochemist discovered insulin by isolating the dog's pancreatic internal extract. A year later insulin was successfully injected a 14-year-old diabetic boy giving rise to the era of insulin therapy that has lasted up to date. Insulin discovery became a milestone in the history of diabetes management, which turned out to be a landmark event in twentieth-century medicine. Despite the fact that more than 3500 years have past since the first notice regarding diabetic symptoms described in Egyptian papyrus dated 1550 years B.C., diabetes mellitus remains an incurable disease.

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The crystal structure of insulin was obtained for the first time in the year 1926 by Jacob Abel, and in the mid fifties the amino acid structure of the insulin particle was defined by Frederick Sanger, initiating further studies devoted to the development of synthetic insulin. Commercial animal (beef or pork) insulin production begun in the year 1923 and these preparations were used for more than half a century until the early 80s when the development of genetic molecular techniques brought about new opportunities in insulin production [1–4]. Since then recombinant DNA technology has been used to develop insulin analogues with improved kinetics profile and different absorption time in order to more accurately approximate the endogenous insulin secretion and improve metabolic control [5].

The effect of insulin on cells can be either metabolic or mitogenic. The physiologic effects of insulin action are not homogenous and differ in the time of the effect appearance. Beside influencing glucose metabolism, insulin exerts other biological functions taking part in such complicated processes as proliferation, differentiation, and cell apoptosis. Insulin also plays the role of growth factor by, among other things, fulfilling a regulative role in gene expression and protein biosynthesis. At low concentrations its intercellular activity leads to a very quick appearance of metabolic effects, whereas at higher concentration the effects, including growth promotion and development, occur with latency [6–8].

Insulin activity is driven through specific insulin receptors [6]. It also binds to structurally related receptors for insulin-like growth factor type 1. The receptors mentioned above present 80% homology in the B-subunit. The insulin molecule and IGF-1 molecule itself have 40–50 % homology [8–11].

Insulin affinity to the IGF-1 receptor is 1000-times lower when compared to the insulin receptor and 1000-times lower when compared to the affinity presented by IGF-1. The IGF-1 receptor plays no important role in insulin activity while physiological concentrations of insulin occur. Stimulation of the IGF-1 receptor might be important at the time when there are higher than physiological concentrations of insulin observed or when there are structurally changed insulin particles present, as it appears in insulin analogues [8, 13].

Despite the improvement in insulin formulations, its administration is still subcutaneous and has many limitations. These are mainly the consequences of alternations in the pharmacodynamic and pharmacokinetic properties of subcutaneously injected insulin that enters systemic circulation and is distributed in the same concentration to the liver as well as to all other peripheral tissues instead of physiological, direct secretion to the portal system, where 70% of insulin is retained. Subcutaneous administration of human insulin is limited by the tendency to associate into dimmers and hexamers that cannot be absorbed through the capillary wall and the multifarious insulin absorption rate from subcutaneous tissue, dependent on the body area chosen for injection, which makes it difficult to predict and hampers the choice of adequate time of insulin injection before the meal ingestion.

Due to the introduction of insulin preparations to common usage, diabetes is no longer considered a mortal disease, and along with life expectancy, the duration of the disease has been prolonged. Unfortunately, because of perennial hyperglycaemia, complications occur, especially cardiovascular ones, being the cause of disability and premature death emerging among diabetic patients.

Therefore, in order to achieve improved glucose control scientists' efforts lean toward inventing the most physiological means of insulin administration, providing constant basal insulin concentration and it's prandial elevation, because this is the only way to keep an optimal metabolic control and simultaneously optimal flexibility of treatment providing freedom to carry out daily activities and improving quality of life [6].

With the use of recombinant DNA technology these were novel insulin molecules, insulin analogues with altered physicochemical, biological, and pharmacodynamic properties mimicking endogenous insulin action designed to improve substantially glycaemic control. The modification of the insulin molecule can either delay or shorten its absorption time.

The preferred site for structural modification is the B26-B30 region because it is not critical for insulin receptor recognition in order to modify the pharmacokinetic action of the insulin particle, but the c-terminal end of the insulin B chain seems to be important in insulin binding to the IGF-1 receptor [2, 6, 10].

The rapid-acting insulin analogues possess structural changes of the insulin particle causing instability of hexamers after subcutaneous administration, and fast conversion of hexamers into monomers that are absorbed, almost immediately, into the blood stream.

Rapid-acting analogues are represented by the following three 3 preparations: insulin lispro (Humalog®; Eli Lilly), insulin aspart (NovoRapid®; Novo Nordisk), and insulin glulisine (Apidra®; Sanofi-Aventis) [14–16].

Long-acting insulin analogues have the molecule of insulin modified in a way to stabilize the hexamer compound in order to absorb the preparation into the circulation with a delay.

This group of insulin analogues is represented by the following 2 preparations: insulin GlyA21ArgB31ArgB32 (Glargine, Lantus, HOE 901; Sanofi-Aventis) and insulin LysB29 (N-tetradecanoyl) des (B30) (Detemier, Levemir; Novo Nordisk) [15–18].

Because changing the insulin structure may fundamentally alter its metabolic as well as mitogenic potency, in recent years concern has been raised over the safety of insulin analogues, especially because an increase in the tumour promoting activity of insulin AspB10 in *in vivo* studies on animal model has been observed. Mitogenic activity has also been observed *in vitro* studies performed on mice embryo pancreatic β-cell lines and human breast cell lines.

AspB10 was produced by the modification of the amino acid chain in the area responsible for IGF-1 receptor affinity, and when compared to human insulin the insulin analogue was characterized by 3.5-times higher affinity to the insulin receptor and 9-times higher affinity to the IGF-1 receptor as well as 9-times increased mitogenic potency. It has been noticed that AspB10 activates the insulin receptor as well as the IGF-1 receptor and presents increased mitogenic potency and 2–4-times increased metabolic activity when compared with human insulin. It was initially thought that the mitogenic effect of this insulin analogue is caused mainly by higher affinity to the IGF-1 receptor. Confirmation of this hypothesis was obtained from a study on rat aortic smooth muscle cells where insulin AspB10 stimulated the IGF-1 receptor with an affinity comparable to the IGF-1 particle [24–26].

Insulin analogues provide diabetic patients with advantageous therapeutic properties, but insulin administration is a life-long treatment and any modification of the insulin molecule can lead to an altered insulin action profile and abnormal interaction with the insulin and IGF-1 receptor, that is why it is very important that the use of insulin analogues does not cause an increase in safety risks.

Insulin analogues mitogenic potency *in vitro* study

There has been a study performed by Kurtzhals et al. aimed to evaluate the mitogenic potential of 4 insulin analogues (lispro, aspart, detemir, and glargine) with the use of human osteosarcoma cells (Saos/B10) possessing large amounts of IGF-1 receptors and small amounts of insulin ones. In this *in vitro* study there was each of the insulins' affinity to the insulin and the IGF-1 receptor as well as dissociation time from the receptors and mitogenic potency examined [22].

In comparison to human insulin, the affinity of insulin lispro and insulin aspart to the insulin receptor was slightly increased, and the affinity of long-acting preparations to the insulin receptor was decreased by half.

In contrast to insulin aspart, having an affinity to the IGF-1 receptor similar to human insulin, insulin lispro had a 1.5-times increased affinity.

When compared to human insulin, insulin glargine had an affinity to the IGF-1 receptor 6.5-times increased and insulin detemir had the affinity 15-times decreased.

The dissociation time from insulin receptor for both insulin lispro and insulin aspart was comparable to that of human insulin. When compared to human insulin there was a faster dissociation of insulin glargine (2-times) and insulin detemir (slightly) observed.

Despite the increased affinity of insulin lispro to the IGF-1 receptor, when compared to human insulin, stimulation of growth processes by insulin lispro and insulin aspart was decreased but mitogenic potency of those preparations was the same.

Glargine mitogenic action proved to be 8-times increased when compared to human insulin, while insulin detemir presented 250-times decreased mitogenic potency [22].

Insulin glargine was the only one of the mentioned above preparations that presented a significantly increased affinity to the IGF-1 receptor. However, based on recently performed *in vitro* studies regarding different cell lines, the increased mitogenic potency was observed only in the studies regarding human osteosarcoma cells (Saos/B10) [28].

Insulin glargine

Glargine (Lantus, HOE 901) is, until now, the only long-acting insulin analogue having no pronounced plasma peak that after a single daily injection exerts a glucose lowering effect for 24 hours. Production of the insulin is performed with the use of a DNA recombination method and results from elongation of the c-terminal end of the insulin B chain by adding 2 arginine residues at positions 31 and 32 and substituting asparagine residue with glycine in the A chain at position 21 [29–31].

Insulin glargine mitogenic potency *in vitro* studies

Bahr et al. performed one of the first *in vitro* studies with the use of cardiomyoblasts (H9C2) and rat cardiomyocytes characterized by over-expression of IGF-1 receptors and simultaneously low expression of insulin receptors. There was no difference in activity toward cardiomyocytes as well as maximum metabolic activity between insulin glargine and human insulin noticed in this study [32].

To asses the potent mitogenic activity of insulin glargine through insulin receptor signalling, Bert et al. conducted a study on transfected rat fibroblasts presenting over-expression of human insulin receptor and low expression of IGF-1 receptor. There was a similarity between human insulin and insulin glargine in terms

of receptor affinity, activity of early signal transduction, and mitogenic action observed. However, the higher number of insulin receptors could mask the mitogenic potency mediated through the IGF-1 receptor [28].

Kurtzhal et al. compared insulin glargine to human insulin in order to estimate the affinity to the IGF-1 receptor and the mitogenic potency in a study on purified receptors from transfected cells lines with the use of the human osteosarcoma cell Saos/B10; an increased insulin glargine mitogenic potency and IGF-1 receptor affinity was observed [22].

Skeletal muscle cells obtained from type 2 diabetic patients and from healthy people were used in the study by Ciaraldi et al. to assess the affinity of insulin glargine and human insulin to insulin receptor and to IGF-1 receptor as well as the supposed mitogenic and metabolic potency of insulin glargine in comparison to human insulin and IGF-1. Similarly, as was proven in the studies described above, the mitogenic potency and metabolic activity of insulin glargine and human insulin were comparable [33, 34].

The main causes of mortality in the group of diabetic patients are cardiovascular complications. Taking into account the information from the above studies indicating increased affinity of insulin glargine to IGF-1 receptor and, additionally, a tendency toward the use of increasing insulin doses among type 2 diabetes patients, mainly due to insulin resistance, it is important to consider whether treatment with high doses of insulin is a safe therapy.

Endothelium cells and smooth muscle cells of blood vessel walls express both insulin and IGF-1 receptors. Throughout the years there has been an ongoing debate regarding the role of elevated insulin concentration as a cardiovascular independent risk factor. Nowadays, the idea of the positive influence of insulin therapy on the vascular system through, inter alia, improvement of lipid control, endothelium function, and anti-inflammation properties predominates. Endothelium dysfunction and loss of endothelium-dependent vasodilatation are considered the most significant factors in the pathogenesis of diabetic angiopathy and atherosclerosis progression [35–42].

Chisalita et al. evaluated insulin receptors and IGF-1 receptors of human endothelium cells localized in small and large blood vessels in terms of gene expression, ligand binding potency, receptor activation, and biological effect caused by insulin glargine and IGF-1.

The outcomes showed that the IGF-1 receptor gene expression and ligand binding were increased in comparison to insulin receptor. The study indicated that insulin glargine, in comparison to human insulin, had a 10-times higher affinity to the IGF-1 receptor, but this feature was presented only in the study performed on human osteosarcoma cells where insulin glargine affinity to the IGF-1 receptor was 6-times higher [13].

In recent communications, proliferation and apoptosis phenomenon have been indicated to have great significance in the development and stability of atherosclerosis plaque.

Staiger et al. estimated the influence of human insulin and insulin glargine on the proliferation and apoptosis of human endothelium cells and human coronary artery smooth muscle cells and proved that neither of the insulins stimulates DNA synthesis or augments apoptosis in the coronary artery cells. It must be noted that the studied coronary artery cells were obtained from healthy people, and it is possible that people with coronary artery disease could present different outcomes [46, 47].

Because insulin glargine, compared to human insulin, has a 6-times higher affinity to the IGF-1 receptor, it is recognized as a breast cancer tumour pathogenesis element. Staiger et al. studied the mitogenic potency of insulin glargine in comparison to human insulin on human breast cancer cell lines (MCF-7) and on normal human epithelial breast cell lines (MCF-10). There was no significant difference found in the mitogenic potency between insulin glargine and human insulin neither in normal epithelial cell lines nor in human breast cancer cell lines. This was the first study performed on carcinoma cell lines since the study on the human osteosarcoma cells [47].

There has been a suggestion that the rate of amount of receptors for IGF-1 to amount of insulin receptors has a key meaning for cell sensitivity to insulin mitogenic action. Defected cells that have undergone malignant transformation, characterized by over-expression of IGF-1 receptor and a low amount of insulin receptors, present an elevated mitogenic response to insulin glargine, and human osteosarcoma cells Saos/B10 can serve as an example of such a situation [48].

Insulin glargine mitogenic potency *in vivo* studies

A preclinical, toxicological study on mice and rats was performed by Stammberger et al. in order to assess the carcinogenic potency of insulin glargine. There was no higher rate of breast cancer nor any other carcinogenic effect observed in the rodents examined in the study [49].

There was no direct negative influence of insulin glargine observed on the reproductive system or foetus growth in the study of Hoffman et al. conducted in rats and rabbits. When high doses of insulin glargine were administered, a higher number of miscarriages was observed among the studied animals, but it was hypoglycaemia not the carcinogenic effect of insulin glargine that was thought to be the most probable cause of the phenomenon [50].

Despite the outcomes of the studies, suggesting that human insulin and insulin glargine exert their mitogenic action through the IGF-1 receptor, it is unlikely that therapeutic concentrations of human insulin and insulin glargine *in vivo* may lead to mitogenesis of untransformed cells trough IGF-1 receptor activation.

Moreover, there is a body of evidence that insulin receptor might be involved in evoking the mitogenic effect. In accordance with the observations of Shymko et al., confirmed by Hansen et al., the main determinant of selectivity for metabolic or mitogenic signal transduction is the time of insulin to insulin receptor binding [27, 51, 52] . Insulin analogues that have longer dissociation time than native insulin are characterized by increased mitogenicity. A slightly faster dissociation time of insulin glargine in comparison with human insulin was observed in the majority of studies. Comparing insulin glargine with insulin AspB10 presenting increased mitogenic activity, the latter was characterized by a significantly slower dissociation and prolonged phosphorylation of intercellular protein. It is worth noting that the long action of insulin glargine is connected to alternations in the pharmacokinetic properties of the insulin particle, not to prolonged binding to the receptor [26, 28, 51–55].

Conclusions

Insulin glargine has raised special scientific interest because of its potential risk of mitogenicity, but this proved to be true only for human osteosarcoma cells Saos/ /B10. The outcomes of studies performed on cell lines other than cancer and on animals do not present increased mitogenic activity or mitogenic potency of insulin glargine in comparison to human insulin [49, 56].

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