Assessment of Genetic Damage in Diabetic Rats Treated with Insulin Glargine

KARAM A. AMEIN, Ph.D.*; MOUSTAFA M. HAMDY, M.D.*; RANIA A. ABD EL-EMAM, M.Sc.* and FIKRY H. OSMAN, M.D.*
The Department of Pharmacology, Faculty of Medicine* and The Department of Genetics, Faculty of Agriculture**, Assiut University

Abstract

Changes in insulin structure may alter the way it interacts with insulin and insulin-like growth factor-1 receptors. Possible associations between the use of the long-acting insulin analog, glargine, and an increased risk of cancer have been widely examined. Strong evidence indicates a role for exogenous insulin or analogs in promoting cancer growth in diabetic patients. The clinical relevance of this pro-cancer effect of insulin in diabetic patients, however, is still unclear. In this study, the genotoxic and cytotoxic potential of insulin glargine (5, 12.5 and 25 I.U/kg, S.C. daily for 2 weeks) was evaluated against the nicotinamide (NA-230mg/kg) and streptozotocin (STZ-65mg/kg) induced somatic and germinal cells defect using a battery of in vivo cytogenetic assays such as the micronucleus, chromosome aberration, mitotic index and sperm abnormality test in male Wistar rats. The obtained results demonstrated that insulin glargine significantly reduced the diabetes-induced genetic damage and cell proliferation changes in somatic cells. Moreover, the administration of insulin glargine reduced the diabetes-induced genetic damage in germinal cells. The results suggest that insulin glargine is not genotoxic or cytotoxic compound and its use does not present a carcinogenic.

Key Words: Diabetes – Insulin glargine – Chromosomal aberrations – Micronuclei – Carcinogenicity – sperm abnormalities.

Introduction

DIABETES Mellitus (DM) is a serious and growing health problem worldwide and is associated with severe acute and chronic complications. Both diabetes and cancer are prevalent diseases whose incidence is increasing globally [1]. The association between cancer and diabetes has been investigated extensively and most, but not all studies, found that DM is associated with an increased risk of several types of cancer [1].

Oxidative stress can be associated with type II DM and reactive oxygen species produced during this stress may cause DNA damage [2]. Moreover, individuals with DM have reduced antioxidant defense capacity [3].

It has been reported that DNA damage and repair play a major role in neoplastic transformation, because mutations in DNA repair genes (e.g. BRCA1 and BRCA2) can be directly related with cancer and the efficacy of DNA repair may determine the susceptibility to carcinogenesis [4]. The oxidative damage to the germinal cells is considered to be the leading cause for infertility and other congenital and developmental defects [5]. This oxidative DNA damage and the decreased efficacy of DNA repair can contribute to the genetic damage in diabetics and, in consequence, to cancer [6].

The pharmacotherapy of diabetes includes administration of insulin and/or oral hypoglycemic drugs. New long-acting insulin formulations such as glargine results from two modifications of human insulin, substitution of glycine at position A21, and addition of two arginine molecules at the C terminal of the B chain [7].

Recently, a series of widely publicized epidemiologic analyses examined a possible association between insulin use and/or use of the long-acting insulin analog glargine and an increased risk of cancer [8-11]. Insulin glargine may have a disparate impact on cancer risk through its binding to insulin-like growth factor-1 (IGF-1) receptors [12].

The majority of cancer cells express insulin and IGF-1 receptors; the A isoform of the Insulin Receptor (IR) is commonly expressed. The A receptor isoform can stimulate insulin-mediated mitogenesis, even in cells deficient in IGF-1 receptors [13].
Multiple signaling pathways are activated after insulin receptors or IGF-1 receptors interact with their ligands. The growth-promoting consequences of receptor stimulation are more generally mediated by the Ras-Mitogen-Activated Protein Kinase (MAPK) pathway (the ras pathway), which promotes cell growth and differentiation by regulation of gene expression [14]. Once activated, these signaling pathways may stimulate multiple cancer phenotypes including proliferation, protection from apoptotic stimuli, invasion, and metastasis, potentially enhancing the promotion and progression of many types of cancer cells. Insulin-like growth factor-1 has more potent mitogenic and anti-apoptotic activities than insulin [15] and could act as a growth stimulus in pre-neoplastic and neoplastic cells that express insulin and IGF-1 receptors [16].

Among the battery of tests available, micronucleus test, chromosomal aberrations, mitotic activity and sperm abnormality assays are commonly employed to evaluate the drug/disease induced mutations [17].

Considering the lack of published reports describing the in vivo genotoxicity of insulin glargine, the present study aimed at exploring the likelihood of development of genetic damage in somatic and germinal cells in diabetic rat models following the administration of insulin glargine. A battery of in vivo cytogenetic assays, through several endpoints such as induction of micronuclei, chromosome aberrations and mitotic activity of bone marrow cells as well as evaluation of the effect of insulin glargine administration on sperm count and sperm shape abnormalities have been carried out.

Material and Methods

I- Drugs and chemicals:

Streptozotocin was purchased from MP Biomedicals, LLC., France while nicotinamide was purchased from Alpha Chem., India. Insulin Glargine (Lantus) Solostar® 100units/ml, was purchased from Sanofi-Aventis Deutschland Gmbh, France. Staining reagents and other chemicals used in this study were of analytical grade and procured from Loba Chemie Pvt. LTD, Mumbai, India and El Nasr Pharmaceutical chemical CO., Egypt.

II- Experimental animals:

This study was conducted on adult male Wistar albino rats. Animals were obtained from the Animal House of Faculty of Medicine, Assuit University. Their weight ranged between 180-250 grams and their age ranged between 12-14 weeks. Rats were housed in groups in clean capacious cages under standard laboratory conditions including good aerated room with suitable temperature (25 ± 5°C), maintained at good light. They were fed with standard rodent food and had free access to water.

III- Experimental design:

In the present study, rats were divided into the following groups; each consisting of 6 rats.

Group I: Control non-diabetic rats.

Group II: Control diabetic rats, non-treated.

Group III: Diabetic rats treated with insulin glargine, Subcutaneously (SC) in a dose of 5IU/kg body weight daily for two weeks.

Group IV: Diabetic rats treated with insulin glargine, SC in a dose of 12.5IU/kg body weight daily for two weeks.

Group V: Diabetic rats treated with insulin glargine, SC in a dose of 25IU/kg body weight daily for two weeks.

Sampling was done after 24 hours after the last dose. The doses of insulin glargine were selected as per previous reports [18].

IV- Experimental induction of diabetes:

Type 2 diabetes was induced by combination of Streptozotocin (STZ) and Nicotinamide (NAD) in adult rats. The rats administered NAD (230 mg/kg, IP) dissolved in saline 15min before STZ (65mg/kg, IP) to develop moderate and stable hyperglycemia. Streptozotocin was freshly dissolved in sterile 0.9% saline solution. The control non-diabetic rats were injected with an equal volume of sterile 0.9% saline solution. Blood glucose level was determined 72 hours after STZ injection by FreeStyle Freedom® Lite glucometer. The diabetic rats included in the study had blood glucose level >200mg/dl [19].

Treatment with insulin glargine was started on the fourth day after STZ injection (ie, after the estimation of blood glucose).

V- Experimental procedures:

I- Cytogenetic assays:

Micronucleus assay (MN):

The bone marrow MN assay was performed as the method described by Attia and his colleagues 2009. Rats were sacrificed under light ether anesthesia at the specified time after treatment with insulin glargine and both femurs were dissected out. The epiphyses were cut off and the bone
marrow cells were flushed out in conical centrifuge tubes using 1cc syringe containing hypotonic (0.56%) potassium chloride solution. The cells were allowed to stand in the hypotonic solution for 15 minutes at room temperature (or 37°C if room is cool). The suspension was then centrifuged at 400rpm for 5 minutes and the pellet was carefully suspended in 2ml of freshly prepared fixative (methanol: Glacial acetic acid, 3: 1). The cells were allowed to stand in it for 30 minutes at room temperature (or 37°C if room was cool). Centrifugation was done again at 400rpm for 5 minutes. Supernatant was discarded and another 2ml of fresh fixative was added. The process of centrifugation was repeated twice more before slide preparation [20].

**Slide preparation:**

Two smears of bone marrow were prepared from each rat. Slides were prepared by putting few drops of fixed cells on dry, clean, grease-free slides and were allowed to dry in air. After air drying, the smears were coded and stained by 5% Giemsa stain [stock Giemsa stain/distilled water] for 15 minutes. From each animal, 1000 Polychromatic erythrocytes (PCEs) were examined for Micronucleated Erythrocytes (MNEs) under 1000x magnification using an Olympus microscope [20].

**Metaphase chromosome aberrations assay:**

At the specified time after treatment, groups of rats were intraperitoneally injected with colchicine (0.5%) at 4mg/kg 90min before sacrifice [20]. The slides for chromosome analysis were prepared and stained as described earlier in the micronucleus assay. All slides were coded and scored under 1000x magnification using an Olympus microscope. Fifty well-spread metaphase plates per rat were scored for both structural and numerical aberrations (polyploidy) in bone marrow cells [300 metaphases for each group]. Cells were classified according to the most severe damage which had occurred and were placed in only one of four categories:

1 - Cells with DNA break (eg chromosomal breaks and chromatid breaks).
2- Acentric fragments.
3- Centric rings (ie ring chromosomes).
4- Polyploidy (ie cells contain multiples of the haploid chromosome number (n) other than the diploid number (ie, 3n, 4n and so on).

Evaluation of the mitotic activity of bone marrow cells by estimation of the Mitotic Index (MI).

From the same slides of metaphase chromosome aberration assay, 1000 cells from each animal were taken into consideration for the mitotic activity study, mitotic index. The mitotic index of bone marrow was evaluated by calculating the number of dividing cells in a population of 1000 cells [20].

**II- Cauda epididymal spermatozoa evaluation:**

For sperm characteristic analysis, the epididymal content of each rat was collected to estimate the frequencies of sperm count and sperm shape abnormalities. Immediately after cervical dislocation, both caudae epididymes of each animal were dissected and incisions were made. Then they were placed individually into tubes filled with 3ml of phosphate buffer saline. The tubes were placed in an incubator at 32°C for 30min to allow the sperm to actively leave the epididymes. The slides were examined by bright field microscope with an oil immersion lens according to published protocol [21]. Two hundred sperms per group were scored and the abnormalities were categorized as close as to those described by Wyrobek and Bruce, (1975). Abnormal sperms had forms readily recognizable as amorphous, without hook, headless tails, triangular and tail abnormality. Sperm count was determined under the microscope using a Neubauer hematocytometer according to the World Health Organization manual for the examination of human semen and two counts per animal were averaged [22].

**Blood glucose estimation:**

Fasting blood glucose estimation was done using the glucometer (FreeStyle Freedom® Lite glucometer, TheraSense, Inc., USA). Blood sampling was done via the tail incision method. A drop of blood collected from the tail vein was gently applied over the test zone of the glucometer and the blood glucose level was recorded immediately as mg/dL.

**VI- Data analysis:**

Statistical analysis was done using the computer software Graphpad Prism 5.0. Data were expressed as the mean ± Standard Error (SE) of the means for each group. The frequency of chromosomal aberration was computed as the number of aberration per total metaphases at each group. When two groups were compared, unpaired t-test was used. The difference was insignificant at p-value >0.05, significant at p-value <0.05. The results of the assessed parameters in different treatment groups were compared with the diabetic untreated control group and the non-diabetic control group by one way analysis of variance (ANOVA). Analyses revealing p-values <0.05 were deemed to be statistically significant.
Results

1- Effect of 2 weeks exposure with insulin glargine on the frequency of bone marrow micronuclei in NA-STZ induced diabetic rats:

The results of the micronucleus assay are presented in (Table 1). Significant increases in the frequency of MNEs were observed in all diabetic rats in comparison with the control non-diabetic rats ($p<0.05$ (unpaired $t$-test)). Treatment with insulin glargine 5IU/kg, showed a non-significant decrease in the frequency of MNEs when compared to the diabetic control group ($p>0.05$ (one-way ANOVA followed by Dunnett test for multiple comparisons)). The frequencies of MNEs induced during the diabetic phase were significantly less in comparison with the diabetic control animals in the diabetic animals treated with 12.5 and 25IU/kg of insulin glargine ($p<0.05$ (one-way ANOVA followed by Dunnett test for multiple comparisons)).

2- Effect of 2 weeks exposure with insulin glargine on the frequency of structural chromosomal aberrations in NA-STZ induced diabetic rats:

The results of the structural Chromosomal Aberrations (CAs) are presented in (Table 2). Significant increase in the frequency of total chromosomal aberrations was observed during the diabetic phase ($p<0.05$ (unpaired $t$-test)). Cells with chromosomal breaks were observed frequently in the diabetic rats and were statistically significant in comparison with the non-diabetic control group ($p<0.05$ (unpaired $t$-test)). Treatment with 5IU/kg of insulin glargine, showed a non-significant decrease in the frequency of total chromosomal aberrations when compared to the diabetic control group ($p>0.05$ (one-way ANOVA followed by Dunnett test for multiple comparisons)). Cells with chromosomal breaks were also observed in the rats treated with the previous low dose of insulin glargine and were statistically insignificantly lower in comparison with the diabetic control group ($p>0.05$ (one-way ANOVA followed by Dunnett test for multiple comparisons)). Cells with chromosomal breaks and total chromosomal aberrations were statistically significantly lower in comparison with the diabetic control group ($p<0.05$ (one-way ANOVA followed by Dunnett test for multiple comparisons)) in the animals treated with insulin glargine at a dose of 12.5IU/kg. Treatment of diabetic animals with 25IU/kg of insulin glargine showed a significant reduction in the frequencies of total chromosomal aberrations, rings and breaks in comparison with the diabetic control group ($p<0.05$ (one-way ANOVA followed by Dunnett test for multiple comparisons)).

3- Effect of 2 weeks exposure with insulin glargine on the induced mitotic arrest in NA-STZ induced diabetic rats:

Mitotic Index (MI) data recorded in the bone marrow cells at metaphase stage are also presented in (Table 2). Drastic inhibition in the mitotic activity of bone marrow cells was recorded in all diabetic rats when bone marrow cells were sampled during the diabetic phase ($p<0.05$ (unpaired $t$-test)) when compared with the non-diabetic control group. Mitotic indices were significantly elevated in the diabetic groups treated with 5, 12.5 and 25IU/kg of insulin glargine when compared with the diabetic control group ($p<0.05$ (one-way ANOVA followed by Dunnett test for multiple comparisons)).

4- Effect of 2 weeks exposure with insulin glargine on the frequency of numerical chromosomal aberrations (polyploidy) in NA-STZ induced diabetic rats:

The results of the numerical chromosomal aberrations are presented in (Table 3). Significant increase in the frequency of numerical chromosomal aberrations was observed during the diabetic phase ($p<0.05$ (unpaired $t$-test)) when compared with the non-diabetic control group. Treatment with 5IU/kg of insulin glargine, showed a significant decrease in the frequency of numerical chromosomal aberrations when compared to the diabetic control group ($p<0.05$ (one-way ANOVA followed by Dunnett test for multiple comparisons)). Treatment of diabetic animals with both 12.5 and 25 IU/kg of insulin glargine also showed a significant reduction in the frequencies of numerical chromosomal aberrations in comparison with the diabetic control group ($p<0.05$ (one-way ANOVA followed by Dunnett test for multiple comparisons)).

5- Effect of 2 weeks exposure with insulin glargine on the frequency of sperm count and sperm morphology in NA-STZ induced diabetic rats:

The results of sperm count and sperm shape abnormalities are presented in (Table 4). Administration of NA-STZ significantly increased the occurrence of sperm shape abnormalities and reduced the sperm count compared to the control non-diabetic animals ($p<0.05$ (unpaired $t$-test)]. Administration of 5IU/kg of insulin glargine elevated the sperm count although this elevation was insignificant in comparison with diabetic control group ($p>0.05$ (one-way ANOVA followed by Dunnett test for multiple comparisons)). Reduction in the sperm shape abnormalities was also observed although this difference was also statistically insignificant when compared with the diabetic control...
group \( p > 0.05 \) (one-way ANOVA followed by Dunnett test for multiple comparisons). Administration of 12.5 and 25IU/kg of insulin glargine significantly reduced sperm shape abnormalities and elevated sperm count in comparison with diabetic control group \( p < 0.05 \) (one-way ANOVA followed by Dunnett test for multiple comparisons).

Fig. (1): Microscopic image of bone marrow at metaphase stage showing MNPCE.

Fig. (2): Microscopic images of bone marrow at metaphase stage showing breaks (B), acentric (ace) and ring (R) chromosomes.

Fig. (3): Microscopic images of epididymal sperm showing normal sperm (1), headless (2), hookless (3), amorphous (4) and abnormal tails (5-7).
### Table (1): Frequencies of micronucleated polychromatic erythrocytes (MNPCEs) in bone marrow of non-diabetic and diabetic rats 24h after the last treatment with the indicated doses of insulin glargine. Frequencies expressed as mean ± SE.

<table>
<thead>
<tr>
<th>Groups and chemicals (IU/kg)</th>
<th>MN frequencies after 2 weeks of exposure (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetes</td>
<td>1.8±0.16</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3.5±0.22*</td>
</tr>
<tr>
<td>Diabetes+Insulin glargine (5IU)</td>
<td>3.1±0.16*</td>
</tr>
<tr>
<td>Diabetes+Insulin glargine (12.5IU)</td>
<td>1.8±0.16b</td>
</tr>
<tr>
<td>Diabetes+Insulin glargine (25IU)</td>
<td>0.6±0.21ab</td>
</tr>
</tbody>
</table>

* p<0.05 vs non-diabetic control (unpaired t-test).

a: p<0.05 vs non-diabetic control.

b: p<0.05 vs diabetic control (one-way ANOVA followed by Dunnett test for multiple comparisons).

### Table (2): Frequencies of different types of structural chromosomal aberrations and mitotic index in bone marrow of non-diabetic and diabetic rats 24h after the last treatment with the indicated doses of insulin glargine. Frequencies expressed as mean ± SE.

<table>
<thead>
<tr>
<th>Types of structural chromosomal aberrations (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups and chemicals (IU/kg)</td>
</tr>
<tr>
<td>Breaks</td>
</tr>
<tr>
<td>Non-diabetes (2 weeks exposure)</td>
</tr>
<tr>
<td>Diabetes (2 weeks exposure)</td>
</tr>
<tr>
<td>Diabetes + Ins. glargine (5IU)</td>
</tr>
<tr>
<td>Diabetes + Ins. glargine (12.5IU)</td>
</tr>
<tr>
<td>Diabetes + Ins. glargine (25IU)</td>
</tr>
</tbody>
</table>

* p<0.05 vs non-diabetic control (unpaired t-test).

a: p<0.05 vs non-diabetic control.

b: p<0.05 vs diabetic control (one-way ANOVA followed by Dunnett test for multiple comparisons).

### Table (3): Frequencies of numerical chromosomal aberrations (polyploidy) in bone marrow cells of non-diabetic and diabetic rats 24h after the last treatment with the indicated doses of insulin glargine. Frequencies expressed as mean ± SE.

<table>
<thead>
<tr>
<th>Groups and chemicals (IU/kg)</th>
<th>Polyploidy frequencies after 2 weeks of exposure (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetes</td>
<td>0.2±0.006</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.52±0.02*</td>
</tr>
<tr>
<td>Diabetes+Insulin glargine (5IU)</td>
<td>0.12±0.05*</td>
</tr>
<tr>
<td>Diabetes+Insulin glargine (12.5IU)</td>
<td>0.08±0.04</td>
</tr>
<tr>
<td>Diabetes+Insulin glargine (25IU)</td>
<td>0.07±0.03</td>
</tr>
</tbody>
</table>

* p<0.05 vs non-diabetic control (unpaired t-test).

a: p<0.05 vs non-diabetic control.

b: p<0.05 vs diabetic control (one-way ANOVA followed by Dunnett test for multiple comparisons).

### Table (4): Frequencies of sperm count and sperm shape abnormalities of non-diabetic and diabetic rats 24h after the last treatment with the indicated doses of insulin glargine (2 weeks exposure). Frequencies expressed as mean ± SE.

<table>
<thead>
<tr>
<th>Groups and chemicals (IU/kg)</th>
<th>Sperm count after 2 weeks of exposure (X10^6 /cauda epididymis) (mean ± SE)</th>
<th>Total sperm shape abnormality after 2 weeks of exposure (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetes</td>
<td>75.8±2.7</td>
<td>1.0±0.5</td>
</tr>
<tr>
<td>Diabetes</td>
<td>41.8±2.19*</td>
<td>13.0±1.05*</td>
</tr>
<tr>
<td>Diabetes+Insulin glargine (5IU)</td>
<td>45.3±1.85*</td>
<td>11.8±1.07*</td>
</tr>
<tr>
<td>Diabetes+Insulin glargine (12.5IU)</td>
<td>54.5±2.99*</td>
<td>8.8±1.01*</td>
</tr>
<tr>
<td>Diabetes+Insulin glargine (25IU)</td>
<td>60.8±1.79*</td>
<td>6.3±0.88*</td>
</tr>
</tbody>
</table>

* p<0.05 vs non-diabetic control (unpaired t-test).

a: p<0.05 vs non-diabetic control.

b: p<0.05 vs diabetic control (one-way ANOVA followed by Dunnett test for multiple comparisons).
Discussion

Diabetes mellitus itself is not responsible for a high mortality and morbidity among diabetic patients. They are caused mainly by its complications, first of all coronary heart disease, which are consequences of oxidative stress associated with diabetes [3,23]. Some reports suggest also that diabetes can be associated with cancer, but the mechanism underlying this association is unclear [24]. A previous study demonstrated that type II diabetes mellitus was linked with an elevated level of oxidative DNA damage, the increased susceptibility to mutagens and the decreased efficacy of DNA repairing enzymes [25]. Oxidative DNA damage may contribute to cancer promotion and progression, therefore, it can be considered as an element of the link between diabetes and cancer [26].

This study was conducted using a group of in vivo cytogenetic assays for evaluation of the possible genotoxic and cytotoxic potential of insulin glargine in diabetic rats, through several endpoints using the rat bone marrow. These are effective and sensitive short-term in vivo bioassays that utilize cytological damage as an end-point in detecting and screening chemical agents that induce chromosomal damages and rearrangement in vivo [27]. In vivo cytogenetic methods are preferred to in vitro tests because of metabolic activation and detoxification of compounds that occur in the intact animals [28,29].

The diabetogenic agent STZ is a potent alkylating agent known to directly methylate DNA with subsequent induction of DNA strand breaks, chromosomal aberrations and DNA damage in mammalian cells in vitro [28,30,31]. Streptozotocin is also demonstrated to be carcinogenic in rats, mice and hamsters [30].

Certain in vivo genotoxicity studies have shown that STZ significantly elevated the MN in the mouse bone marrow cells [32,33]. In addition, an in vivo study carried out by Vikram et al., (2007) showed a marked increase in the MN and DNA damage in the bone marrow cells of rats treated with STZ [34]. Moreover, the study conducted by Attia et al., (2009) showed a significant increase in the frequency of MN, total chromosomal aberration and reduction in mitotic indices in the rats treated with STZ [20].

In agreement with the above-cited reports, the present study showed that exposure to STZ caused significant increase in the MN frequencies, total chromosomal aberration and reduction in mitotic indices as compared to the values obtained with control non-diabetic. The genetic damage observed in the rats with experimental diabetes, could be explained by many physiological changes caused by the pancreatic β-cell destruction induced by streptozotocin. This damage may trigger inflammatory processes, as well as hyperglycemia-induced oxidative stress, that may be sufficient to increase the genetic damage [35]. Oxidative stress is associated with increased free radical production and as a consequence, with increased genetic damage [36,37].

The data of the present study demonstrated that insulin glargine is not genotoxic or cytotoxic when injected into diabetic rats at a dose 5, 12.5, 25IU/kg for 2 weeks. The obtained results from the micronucleus, chromosomal aberration and mitotic index assays demonstrated reduction in the parameters in comparison with the control diabetic group. To the extent of our knowledge, this present study is the first study which evaluates the possible genotoxic and cytotoxic potential of insulin glargine using a battery of in vivo cytogenetic assays.

Moreover, the present study demonstrated that administration of insulin glargine reduced the diabetes-induced genetic damage in germinal cells as presented by reduction in sperm shape abnormalities and elevation in sperm count. To the extent of our knowledge, and by searching the literature, no previous studies were conducted to evaluate the effects of insulin glargine on sperm count and morphology. These results can be explained by the good glycemic control and reduction in the hyperglycemia-induced oxidative stress caused by the diabetic state.

In line with the results of this present study, Stammberger et al., (2002) evaluated the possible carcinogenic potential of insulin glargine by conducting lifetime carcinogenicity studies in rodents (rats and mice). The study had comprehensively demonstrated that insulin glargine did not cause mammary tumors in rodents. No other neoplastic changes that may affect humans were detected. The study revealed neoplastic changes that were not related to treatment such as an increased incidence in malignant fibrous histiocytoma at the injection site, which was associated with the permanent irritation of local connective tissue due to the acidic pH of the injected insulin glargine [18].

Certain in vitro genotoxicity studies have shown that the ability of insulin glargine to stimulate cellular growth generally correlates with its ability to bind to the IGF-1 receptor, but prolonged interaction with either receptor also appears necessary for stimulation of mitotic activity [38].
Kurtzhals and his colleagues used the human osteosarcoma cells to compare receptor affinities and mitogenic potencies of the insulin analogues in current clinical use, and found that insulin glargine has a six-to eightfold increase in receptor affinity and mitogenic potency compared with human insulin [39]. Sanofi-aventis had previously observed a similar increase in mitogenic activity in osteosarcoma cells [40].

In another study, colorectal, breast and prostate cell lines showed proliferative changes and increased resistance to apoptosis in response to exposure to pharmacological doses of insulin glargine, but not to human insulin [41].

There are multiple and complex mechanisms potentially responsible for the mitogenic effects of insulin. Insulin may bind and activate the related IGF-1 receptor, which has a more potent mitogenic and transforming activity than the insulin receptor. Moreover, insulin decreases IGF-1 binding proteins (IGF-BP 1 and IGF-BP2) [42]. This will result in increased free IGF-1, the biologically active form of the growth factor. Many cancer cells have an increased IR content [43]. The IR may be expressed in two different isoforms, A and B [44]. In malignant cells, the A isoform (IR-A) expression is predominant, [45,46] and its activation elicits more mitogenic than metabolic effects [45]. By binding to the over-expressed IR-A, insulin may favor cancer progression and facilitate the growth of tumors [45].

Insulin mitogenic activity might be enhanced at the cellular level by post-receptor molecular mechanisms, including insulin (or its synthetic analogs) residence time on the receptor and the intracellular up-regulation of the insulin mitogenic pathway. The AMP activated protein kinase (AMPK), mammalian target of rapamycin (mTOR), and insulin-signaling pathway represent three interrelated components of a complex mechanism controlling cell responses to nutrients, and their dysregulation may favor malignant cell proliferation in response to hyperinsulinemia [47].

All these in vitro experimental conditions, however, may not necessarily reflect an in vivo situation; therefore it is difficult to compare the results of these previous studies with the data obtained in the in vivo experiments.

Insulin glargine is partially degraded at the injection site, yielding two bioactive products known as M1, which lacks the diarginine residues at B31 and B32, and M2, which has additional deletion of the threonine at B30. Both products retain the glycine substitution for asparagine at A21. These are therefore closely similar to, but not identical with, human insulin [48,49] and their mitogenicity appears to be low [18].

M1 and M2 fully retain the same metabolic properties as human insulin, but in contrast to glargine, they do not differ from human insulin in affinity for IGF-1 receptor (IGF-1R) and mitogenesis [50]. Lucidi and his coworkers demonstrated that type 2 diabetic subjects treated with glargine, the therapeutic dose of insulin glargine resulted in biotransformation to M 1, which constituted >90% of circulating insulin, with only a minor presence of the originally injected glargine in plasma (<10%) [51].

Other previous studies indicated that in vivo, glargine concentration in plasma is negligible and well below that shown in vitro to demonstrate greater affinity for IGF-1R [50,51] and greater mitogenesis [53]. It is unlikely that there is an increased risk of cancer in humans following administration of insulin glargine, at least via IGF-1R stimulation. Therefore, the findings of in vitro studies on mitogenesis using glargine as such [54] are of limited informative value for interpretation of the results of glargine administration in vivo.

The authors of this present study hypothesize that these observations suggest that insulin glargine behaves to some extent as a prodrug, generating bioactive breakdown products both at the site of injection and within the circulation. It follows that insulin glargine may be less mitogenic in vivo than in vitro.

Conclusion:

On the light of all of these results, the authors of this study can conclude that insulin glargine use is not a genotoxic or a cytotoxic compound when used in vivo. Together with the in vivo studies that demonstrate equivalence between human insulin and insulin glargine with respect to IGF-1 receptor binding and mitogenic activity, these results indicate that insulin glargine is unlikely to pose a cancer risk in humans.

Acknowledgements:

The authors acknowledge the financial support received from Assuit University. We are grateful for the Department of Pharmacology and Histology, Faculty of Medicine, Assuit University and every kind hand which helped and participated in accomplishment of this work.
References


الملخص العربي

التغييرات المحدثة في هيكل الأنسولين قد تغير الطريقة التي يتفاعل بها مع مستقبلات الأنسولين ومستقبلات عامل النمو 1 الذي يشبه الأنسولين. وقد درست على نطاق واسع احتمالات بين استخدام الأنسولين طويل المفعول غلجن، زيادة خطور الإصابة بالسرطان. يشير دليل قوي إلى وجود دور للأنسولين الخارجي أو النظير في تعزيز نمو السرطان في مرضى السكري. ومع ذلك فإن الأهمية السريرية لهذا التأثير الموالي للسرطان بالنسبة للأنسولين في مرضى السكري، لا يزال غير واضح. في هذه الدراسة، تم تقديم إمكانات السمية الوراثية والسامة للأنسولين غلجن (0.5 و 1.2 وحدة بواية/كيو، حصل تحت الجلد يوميا لمدة أسبوعين) ضد الجراثيم المحتمة بالتيكيناميد (STZ-65mg) والسترونتريسيسين (NA-230mg) في كل من الخلايا الجسدية والجرثومية باستخدام مجموعة من المقاييس الوراثية في الجسم الحي مثل النمو الصغير، الاتجاهات السريرية ومؤشر الإحساس من اعتبار شروط الحفاظ المنوي في ذكر فرنان وستار.

أظهرت النتائج أن الأنسولين، غلجن، أدأ إلى انسحاب كبير أو استبدال احصائي في التغيرات التي يسببها مرض السكري في الأضرار الوراثية وتكرار الخلايا في الخلايا الجسدية. وعلاوة على ذلك، فإن إعطاء الأنسولين، غلجن، أدأ إلى خفض القرش الوراثي الناتج عن مرض السكري في الخلايا الجسدية. وتشير النتائج إلى أن الأنسولين، غلجن، ليس مرتكب سام للجينات أو للخلايا واستخدامه لا يشكل خطرًا مسرطنًا.