



Hyperglycemia Induction in HepG2 Cell Line

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Abstract

Background: Hyperglycemia is one of the important features of diabetes. In cell culture studies different methods are used to mimic the hyperglycemia condition. In this study we investigate response of human liver cancer cell line (HepG2) to high insulin, high glucose, and high insulin/ high glucose medium exposure.

Methods: HepG2 cells were settled in DMEM+0.1% FBS or DMEM free-serum medium with high concentrations of d-glucose (30 mM) and/or insulin (1 μM) for 24h after an overnight starving in serum-free medium. The level of hyperglycemia was estimated in the supernatants via GOD-POD method.

Results: Serum-free medium with high insulin/ high glucose concentration made the higher level of hyperglycemia in HepG2 cells.

Conclusions: Our study interduced high insulin/ high glucose treatment as the best way to induction hyperglycemia.

Keywords: Hyperglycemia, HepG2, Insulin, Glucose, diabetes.

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obtained from Sigma-USA. Glucose-Std kits were obtained from Pars azmoun -IRI.

HepG2 cells were cultured in DMEM-F12 supplemented with 10% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin in a 37°C humid atmosphere with 5% CO₂. The cells were seeded onto six-well plates at 1.2×10^6 cells per well to reach 80% confluence. To induce insulin resistance, HepG2 cells were exposed to a high concentration of D-glucose (30 mM) and/or high insulin (1 μM) in DMEM + 0.1% FBS or serum-free medium for 24 h after an overnight serum-starving condition. Afterwards, the supernatant was collected and the Glucose-Std kit, which uses the glucose oxidase-peroxidase (GOD-POD) method, was used to estimate the level of hyperglycemia.

Results

HepG2 cells were treated according to Table 1 for 24 h after an overnight starving in serum-free medium. In this experiment, treatment with D-glucose (30 mM) and insulin (1 μM) in serum-free medium increased the level of hyperglycemia (Table 1).

Table1: The glucose level of supernatant in different culture conditions.

Treatment	The glucose level of supernatants (mM)	
	DMEM free-serum	DMEM+0.1% FBS
Insulin	72	64
Glucose	688	673
Insulin+ Glucose	702	688

*DMEM: Dulbecco's modified Eagle's

** FBS: Fetal bovine serum

Discussion

Hyperglycemia or high blood sugar is a condition in which an excessive amount of glucose circulates in the blood plasma. This is generally a blood sugar level higher than 11.1 mM (200 mg/dl). A subject with a consistent range above 7 mM (126 mg/dl) is generally considered to have diabetes, which can cause organ damages due to glucose toxicity.^{6,7}

Many mechanisms have been proposed for glucose toxicity as follows:

- In adipose tissue and skeletal muscle following increasing glucosamine levels, intracellular adenosine triphosphate (ATP) is depleted and the glucose transporter type 4 (GLUT4) membrane transport of GLUT4 is suppressed.⁸
- In the liver, the regulatory mechanism of glucose transporter type 2 (GLUT2) membrane transportation is disturbed. Therefore, glucose cannot be a limiting factor for glucose metabolism.⁹
- The elevated glucose levels lead to an increase in serine phosphorylation and a decrease in tyrosine phosphorylation of the insulin receptor through

Introduction

Diabetes is one of the most common metabolic diseases in the world and has caused deaths and disabilities in many countries.¹ Recent studies in America have shown that the prevalence of diabetes has increased from 144% in 1980 to 151% in 2009. Type 2 diabetes mellitus (T2DM) includes more than 90% of all diabetes cases.² T2DM often occurs at an old age because of obesity, physical inactivity, unhealthy diet, and genetic predisposition. In T2DM, the blood sugar level in the body is increased, leading to insulin resistance and other complications.^{3,4}

Hyperglycemia, define as an elevated blood glucose level, is one of the important complications of T2DM. It occurs due to a malfunction or secretion disorder of insulin. Hyperglycemia causes further damage to the beta cells of the pancreas, followed by more insulin resistance. Thus, a vicious cycle called glucose toxicity occurs in the body.⁵

In cell culture studies, hyperglycemia circumstances are usually induced to mimic diabetic conditions. In this study we have examined the different ways of hyperglycemia induction in HepG2 cells to find the best method.

Materials and Methods

HepG2 cell lines were purchased from the Iran national cell bank, Pasteur Institute. Dulbecco's modified Eagle's medium F12 (DMEM-F12) was purchased from Gibco-UK. Fetal bovine serum (FBS), penicillin, and streptomycin were

phosphatidylinositol 3-kinases/protein kinase B (PI 3-k/ AKT) pathway.¹⁰

- Glucose toxicity causes oxidative stress leading to active inflammatory pathways, which are followed by exacerbation of diabetic symptoms.¹¹

In T2DM, three of the following phases may occur: 1-hyperglycemia, followed by 2-hyperinsulinemia, and then 3-hyperglycemia, which may overlap with hyperinsulinemia for some time. It has been shown that high insulin, high glucose, and high glucose/high insulin conditions can damage tissues.¹²⁻¹⁴

In this study, we examined the different methods to induce a hyperglycemic condition in order to mimic a diabetic condition. According to our results, a higher glucose level is caused by the treatment of HepG2 cells with high amounts of insulin and glucose simultaneously in DMEM medium without FBS. Previous studies have suggested the same method to induce hyperglycemia in HepG2 cells.^{10,15,16} However, most of the studies conducted induced hypoglycemia using a high glucose medium only. Some examples include the following conditions: exposing neonatal primary cultured cardiomyocytes to 25 mM glucose for different time period in which the maximum affect was observed from 24 h to 48 h of high glucose stimulation;¹⁷ incubation of cardiomyocytes cells with 33 mM glucose for 36 h;¹⁸ treatment of mouse primary peritoneal macrophage with 25 mM glucose for 18 h;¹⁹ and high glucose/high insulin exposure decreases not only insulin-stimulated GLUT4 translocation in skeletal muscle but also insulin receptor and AKT phosphorylation.²⁰ It appears that a high glucose/high insulin condition mimics the usual diabetic circumstances in which the body increases insulin uptake to raise glucose levels.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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