

D-Glucose-Induced Cytotoxic, Genotoxic, and Apoptotic Effects on Human Breast Adenocarcinoma (MCF-7) Cells

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Abstract

Introduction: Glucose is a simple sugar that plays an important role in energy production in biological systems. However, it has been linked to many long-term health problems including the risk of heart disease and stroke, erectile dysfunction in men and pregnancy complications in women, and damage to the kidneys, nerves, eye and vision. Also, the underlying mechanisms of diabetic complications are poorly understood.

Methods: In the present study, D-glucose-induced cytotoxic, genotoxic, and apoptotic effects were studied using MCF-7 cells as an *in vitro* test model. Cell viability was determined by MTT assay. Genotoxic damage was tested by the means of alkaline single cell gel electrophoresis (Comet) assay. Cell apoptosis was measured by flow cytometry assessment (Annexin-V/PI assay).

Results: The results of MTT assay indicated that D-glucose significantly reduces the viability of MCF-7 cells in a dose and time-dependent manner. Similar trend was obtained with the trypan blue exclusion test. Data obtained from the Comet assay indicated that D-glucose causes DNA damage in MCF-7 cells in a dose-dependent manner. The flow cytometry assessment (Annexin V FITC/PI) showed a strong dose-response relationship between D-glucose exposure and annexin V positive MCF-7 cells undergoing early apoptosis.

Conclusion: Taking together, these data provide clear evidence that D-glucose induces cytotoxic, genotoxic, and apoptotic effects on MCF-7 cells. This finding represents the basis for further studies addressing the pathophysiological mechanisms of action of glucose overdose.

Keywords: D-glucose; MCF-7 cells; Cytotoxic; DNA damage; Apoptosis

Introduction

Glucose (C₆H₁₂O₆) also known as D-glucose, dextrose, or grape sugar is a simple monosaccharide found in plants. It is the major source of energy for most cells of the body systems. Glucose, fructose, and galactose are three dietary monosaccharides that are absorbed directly into the bloodstream during digestion. Although glucose plays an important role in energy production in our body, a previous report indicated that intermittent high glucose induces apoptosis due to activation of cellular oxidative stress in human umbilical vein endothelial cells [1]. Reactive oxygen species (ROS) are considered to be important mediators of several biological responses, including cell proliferation and extracellular matrix deposition. Glucose auto-oxidation, non-enzymatic glycation, the formation of advanced glycation end products, and the overproduction of ROS by mitochondria are potential sources of hyperglycemia-induced oxidative stress [2,3]. Han and his collaborators demonstrated that high D-glucose concentrations (15, 20, and 25 mM) induced production of hydrogen peroxide and lipid hydroperoxide in PTCs cultured cells through dependent on mitochondrial ROS and NADPH oxidase [4]. Previous studies with cultured neuronal cells indicated that elevated glucose levels induce dysfunction and apoptosis, possibly through the formation of ROS [5].

However, several other reports have pointed out different results from experiments evaluating the adverse effect of glucose at high level of exposure. A report by Sakuma et al. indicated that high glucose inhibits apoptosis in coronary artery smooth muscle cells by up-regulating anti-apoptotic proteins [6]. Other studies using endothelial cells (EC) demonstrated that a high-glucose concentration induces proliferation

and survival in human umbilical vein EC [7,8], human dermal microvascular EC [9], aortic EC [10], and retinal EC [11]. These conflicting studies may be explained, at least in part, by the differences in species, tissues of origin, or experimental conditions. Although there is enough scientific evidence showing the effects of glucose in normal cells, little is known about the molecular mechanisms of glucose overdose in cancer cells. Therefore, the present study was designed to test the cytotoxic, genotoxic, and apoptotic effects of D-glucose using human breast adenocarcinoma (MCF-7) cells as a test model.

Materials and Methods

Cell culture

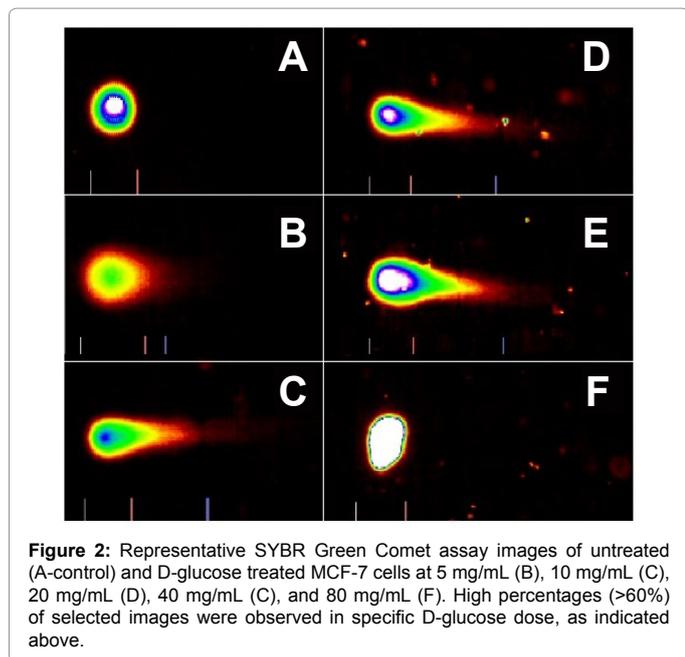
The human breast adenocarcinoma (MCF-7) cells, purchased from the American Type Culture Collection -ATCC (Manassas, VA), were thawed by gentle agitation of their containers (vials) for 2 minutes in a water bath at 37°C. After thawing, the content of each vial was transferred to a 75 cm² tissue culture flask, diluted with RPMI 1640

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Received March 06, 2014; Accepted April 22, 2014; Published April 25, 2014

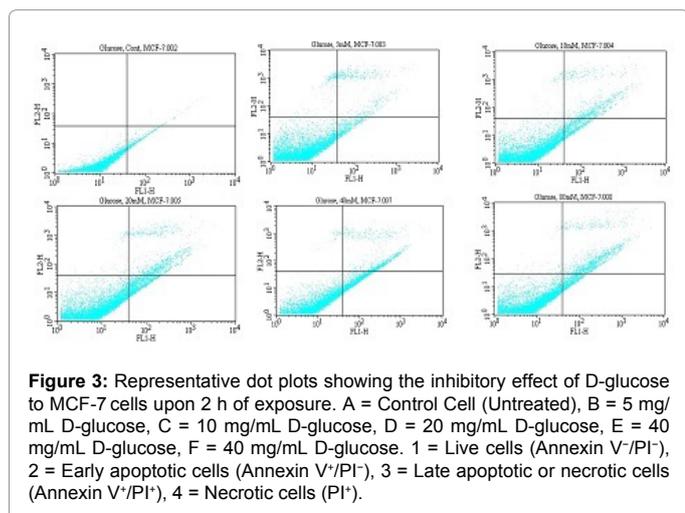
Citation: Tchounwou CK, Yedjou CG, Farah I, Tchounwou PB (2014) D-Glucose-Induced Cytotoxic, Genotoxic, and Apoptotic Effects on Human Breast Adenocarcinoma (MCF-7) Cells. J Cancer Sci Ther 6: 156-160. doi:10.4172/1948-5956.1000265

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and other factors related to glucose metabolism including insulin and IGFs may contribute to breast cancer development [18]. Glucose may play a direct role in the development of breast cancer by favoring the selection of malignant cell clones [19]. For example, a study by Warburg showed that neoplastic cells use glucose for proliferation [19]. Increased metabolism of glucose toward the pentose phosphate pathways is one of the central metabolic characteristics of malignant tissues [20]. A large-scale epidemiological study of 21 modern countries that track morbidity and mortality (Europe, North America, Japan and others) revealed that sugar intake is a strong risk factor that contributes to higher breast cancer rates, particularly in older women [21].

Consistent with high concentrations (10-80 mg/mL) of glucose used in the present study, previous reports indicated that high glucose inhibit the proliferation, migration and *in vitro* angiogenic capacity of bone marrow-derived endothelial progenitor cells, and alter the regenerative potential of mesenchymal stem cells [22,23]. High glucose induced nuclear factor kappa B mediated inhibition of endothelial cell migration. In contrary to our finding, several other studies using endothelial cells (EC) demonstrated that high-glucose concentration increases cell growth and survival in human umbilical vein EC [7,8], human dermal microvascular EC [9], aortic EC [10], and retinal EC [11]. These conflicting studies may be explained, at least in part, by the differences in species, tissues of origin, or experimental conditions.



Next, we determined whether the glucotoxicity is associated DNA damage in MCF-7 cells; by the means of alkaline single cell gel electrophoresis (Comet) assay. We found that D-glucose has a strong genotoxic potential and is able of causing DNA damage in cancer cells. Our results demonstrated that D-glucose induces genotoxic effects to MCF-7 cells in a dose-dependent fashion, suggestive clear evidence that D-glucose may be a potent DNA damaging agent against breast cancer when used at high doses. There is limited scientific data in the literature explaining how D-glucose exposure induces DNA damage in cancer cells. Hence, further studies are needed to establish the genotoxic mechanism on the basis of the genetic damage induced by D-glucose. Thus, we showed that high glucose concentrations induce DNA damage in MCF-7 cells. Similarly, other studies in our laboratory showed that the size, shape and distribution of DNA within the comet correlate with the extent of DNA damage in human promyelocytic leukemia cells when treated with arsenic trioxide [24].

2.3%), (20.0 ± 5.1)%, (27.3 ± 6.4)%, and (21.2 ± 3.7)% in 0, 5, 10, 20, 40, and 80mg/mL D-glucose, respectively (Table 1).

Furthermore, we examined whether D-glucose treatment induces apoptosis of MCF-7 cells. To accomplish this research objective, we performed Annexin V/propidium iodide (PI) staining that allows the discrimination of viable cells (Annexin V-/PI-), early apoptotic (Annexin V+/PI-), and late apoptotic or necrotic cells (Annexin V+/PI+).

Discussion

The adverse effects of high glucose concentrations on various cancer cell types have been known for some time. Nevertheless, there remains considerable doubt about the cellular and molecular mechanisms of action of D-glucose in MCF-7 cells. In the present study, we first demonstrated that D-glucose exerts a concentration-dependent dual effect upon 2 h of exposure: it causes differentiation or proliferation of MCF-7 cells at low concentrations and cell death at relatively high concentrations. As seen in figure 1, D-glucose slightly increases the proliferation of cancer cells at physiologic or low concentrations (5 mg/mL) and gradually decreases the viability of cancer cells at high concentrations (10-80 mg/mL) in a dose and time-dependent manner.

Consistent with low or physiologic concentrations of glucose used in the present study upon 2 h, previous research indicated that glucose

Sample ID	Treatment	Annexin V/PI negative Cells or Live Cells (Mean ± SD)%	Annexin V/PI Positive Cells or Apoptotic cells (Mean ± SD)%
A	0 mg/mL	96.5 ± 1.1	3.5 ± 1.1
B	5 mg/mL	89.0 ± 0.5	11.0 ± 0.5
C	10 mg/mL	84.5 ± 2.3*	15.5 ± 2.3*
D	20 mg/mL	80.0 ± 5.1*	20.0 ± 5.1*
E	40 mg/mL	72.7 ± 6.4*	27.3 ± 6.4*
F	80 mg/mL	80.8 ± 3.7*	19.2 ± 3.7*

Table 1: Summary data of annexin V assay obtained from the flow cytometry. Human breast adenocarcinoma (MCF-7) cells were cultured in the absence or presence of D-glucose for 2 h as indicated in the Materials and Methods. Values are shown as means ± SDs of 3 replicates per experiment. *P < 0.05 versus compared with control group.

V⁺/PI⁺). We observed that D-glucose induces apoptosis of MCF-7 cells (Figure 3). The obtained results show that the percentage of apoptotic cells was dependent on D-glucose concentration with maximal level of apoptotic cell death at 40 mg/mL (Table 1). In line with our current finding showing that high glucose induces apoptosis of MCF-7 cells, previous laboratory studies demonstrated that high glucose concentrations disturb cell cycle, induce DNA damage, delay endothelial cell replication, and cause excessive cell death in cultured human endothelial cells [25,26]. Other studies have pointed out that high glucose selectively triggers apoptosis in cultured endothelial cells [27,28]. In contrast to its effect on MCF-7 cells as seen in the present study, high glucose has been reported to inhibit apoptosis in coronary artery smooth muscle cells by up-regulating anti-apoptotic proteins [6]. Thus, the molecular events linking high glucose with the apoptotic machinery of cancer cells may be far more complicated than being realized. Cell death is thought to take place at least by two ways that include apoptosis and necrosis.

Apoptosis is an active and physiological mode of cell death that is believed to be mediated by active intrinsic mechanisms, although extrinsic factors can contribute [29-32]. Generally, it shows non-inflammatory morphological changes of cell shrinkage which include cytoplasmic and nuclear condensation, chromatin condensation (pyknosis), ordered DNA fragmentation (karyorrhexis), blebbing of the plasma membrane, formation of apoptotic bodies (cellular fragmentation of membrane-bound fragments) and exposure of surface molecules such as phosphatidylserine (PS) on the plasma membrane to facilitate complete phagocytosis of apoptotic cells [33,34]. In contrast, necrosis is an uncontrolled cell death that is characterized by progressive loss of cytoplasmic membrane integrity, rapid influx of Na⁺, Ca²⁺, and water, resulting in cytoplasmic swelling and nuclear pyknosis [34-36]. The latter feature leads to cellular fragmentation and release of lysosomal and granular contents into the surrounding extracellular space, with subsequent inflammation [37,38]. A recent pharmacological study in our laboratory demonstrated that *Vernonia amygdalina* (Africa medicinal plant) induces growth arrest and apoptosis accompanied by secondary necrosis cell death of breast cancer (MCF-7) cells [39]. A 2010 scientific report demonstrated that the mechanism underlying glucose-induced β -cell death involves the upregulation of Fas receptors, which can interact with the constitutively expressed FasL on neighboring β -cells. Fas-FasL interaction leads to cleavage of procaspase-8 to caspase-8. Activated caspase-8, the most upstream caspase in the Fas apoptotic pathway, promotes caspase-3 activation and DNA fragmentation [40].

Conclusion

We have demonstrated in the present *in vitro* study that relevant concentrations of D-glucose at low doses (5 mg/mL) slightly increase the proliferation of cancer cells upon 2 h of exposure while on the other hand, high doses (10-80 mg/mL) of D-glucose significantly ($P < 0.05$) reduce the viability of MCF-7 cells, induce DNA damage and cause apoptosis of MCF-7 cells with a maximum dose-dependent response at 40 mg/mL. Taking together, these data provide clear evidence that D-glucose overdose is a potential inducer of cytotoxic, genotoxic, and apoptotic effects in tumors cells such as MCF-7 cells. This finding represents the basis for further studies addressing the pathophysiological mechanisms of action of glucose overdose.

Acknowledgements

The research described in this publication was made possible by a grant from the National Institutes of Health (NIH-NIMHD Grant No. G12MD007581) at Jackson

State University. The authors presented this work at the American Association on Cancer Research 102nd Annual Meeting held in Orlando, FL. April 02-06, 2011.

No authors have any conflict of interest relevant to this work. CT and CY have performed the experiment and drafted the manuscript that was reviewed by all authors. IF has assisted in performing the statistical analysis and data interpretation. PT has supervised the experiment and reviewed the manuscript for submission.

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