



RESEARCH ARTICLE

In vitro evaluation of glutathione implementation on oxidative DNA damage and oxidant status in high glucose conditions

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Glutasyon uygulamasının yüksek glukoz ortamında oksidatif DNA hasarı ve antioksidan sistem üzerine etkilerinin *in vitro* olarak değerlendirilmesi

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Öz

Amaç: Bu çalışma, yüksek oranda glukoz ilave edilen BHK-21 hücre serisinde antioksidan özellikleri bilinen glutatyonun hücrelerde olası oksidatif DNA hasarı (8-hidroksi-2-deoksiguanozin) ve antioksidan sistem üzerine etkilerini ortaya koymak amacıyla planlandı.

Gereç ve Yöntem: Bu amaçla, BHK-21 hücre serisinde *in vitro* koşullarda düzenli pasajları yapılarak (%5 FBS, %10 horse serum, %1 L-Glutamin, %1 penisilin/streptomisin içeren RPMI 1640 besi yerinde ve %5 CO₂ ve % 95 nem ve 37oC'de) inkübe edildi. MTT hücre canlılık testleri yapılarak glutatyonun kontrol grubuna göre ve glukozun IC50 değeri belirlendi. Hücreler pleytlere, 2x10⁶ hücre olacak şekilde ekildi. Kontrol ve deneme grupları ve bu gruplar arasında çaprazlama olarak, çalışma grupları (glukoz; (285 mM), glutatyon (250 µM) hazırlandı. Yirmi dört saatlik inkübasyonu takiben tripsine edilen hücreler, dondur/çöz yöntemiyle parçalanarak analize hazırlandı. Elde edilen hücre kültürü lizatında; oksidatif DNA hasarı, TAS, TSO ve OSI değerleri ELISA ile spektrofotometrik olarak ölçüldü.

Bulgular: 8-OHdG düzeyleri, glukoz uygulanması ile önemli oranda arttığı, HG+GSH grubunda kontrole göre anlamlı olarak arttığı saptandı (p≤0.05). Sadece GSH verilen grup ise kontrolden farksız olarak bulundu. TAS bakımından, GSH uygulanan gruplarda kontrole fark bulunmazken, HG +GSH verilen grupta ise kontrole göre önemli artış tespit edildi (p≤0.05). TOS ve OSI ise HG+GSH uygulanan gurupda kontrole göre önemli bir artış gözlemlendi (p≤0.05).

Öneri: Çalışmadan elde edilen sonuçlara göre yüksek glukoz uygulanan hücrelere uygulanan glutatyonun hücresel düzeyde bu dozlarda koruyucu etkisi gözlenmemiştir. Ancak glutatyon uygulanan guruplarda, uygulanan glutatyon dozlarının hücreler üzerine toksik doz olmadığı da belirlenmiştir.

Anahtar kelimeler: DNA hasarı, glutatyon, hücre kültürü, TAS, TOS

Abstract

Aim: This study aimed to show the effects of glutathione, recognized by its antioxidant specialties, on the potential DNA damage (8-hydroxy-2-deoxyguanosine) and the antioxidant system changes upon its implementation in BHK-21 cells cultured with high glucose.

Materials and Methods: BHK-21 cell line was regularly surpassed *in vitro* conditions (5% FBS, 10% horse serum, 1% L-Glutamine, 1% penicillin/streptomycin in RPMI 1640 medium, and 5% CO₂ and 95% humidity and 37oC) incubated. The control group determined glucose's IC50 value based on the viability tests executed on MTT cells. Cells were seeded in plates as each would have 2x10⁶ cells. The control, the test, and the crossbreed test (glucose; (285 mM), glutathione (250 µM)) groups were prepared. After 24 hours of incubation, trypsinized cells were designed for analysis through vitrification. In the lysate of the cell culture that was procured, Oxidative DNA damage, TAS, TSO, and OSI were measured by the spectrophotometric system with ELISA.

Results: It was observed that 8-OHdG levels increased significantly with glucose application. Moreover, the increase in the HG+GSH group was more significant when compared to the control group (p≤0.05). No difference with the control group was found only in the group where GSH was applied. As for TAS, whereas any difference was observed in GSH used groups, the increase in the HG+GSH group was significant compared to the control group (p≤0.05). that were the same as the control group. TOS and OSI considerably increased in HG + GSH implemented groups as to the control group (p≤0.05).

Conclusion: According to the results, no protective impacts of glutathione at the cellular level in the doses mentioned above were observed on high-dose glucose implemented cells. On the other hand, it was revealed that the applied amounts of glutathione in the process did not cause any toxic effects.

Keywords: DNA damage, glutathione, cell culture, TAS, TOS





Introduction

Hyperglycemia, the most critical indicator of Diabetes Mellitus (DM), causes vascular, renal, retinal, or neuropathic disorders in the long term and acute metabolic complications (Yaycı et al 2021). It is thought that it may be important in terms of revealing the effects of glutathione administration in a current issue such as diabetes, especially in the treatment of the disease and in the regulation of its prognosis.

Increased free radicals formed in a hyperglycemia environment cause loss of membrane integrity, structural or functional changes in proteins, and genetic mutations by interacting with lipids, proteins, and nucleic acids. Organisms own some enzymatic and non-enzymatic antioxidant defense systems to cope with the effects of these harmful radicals. (Abou-Seif and Youssef 2004, Andican and Burçak 2005, ADA 2011). Glutathione (GSH), an antioxidant agent, contains a thiol group and is widely found in almost every tissue of mammals to defend against oxidative stress and is considered a potent biomarker for redox imbalance in cells. (Franco et al 2007). GSH, also called the primary antioxidant system, can protect cells from free radicals and harmful oxidation effects. (Huang and Hu 2011, Yur et al 2013). In addition, many studies have been conducted to show that GSH levels are reduced in experimental, Type I and II and experimental diabetes. (De Mattia et al 1998, Rizvi and Zaid 2001, Sekhar et al 2011).

This study investigates GSH application's effects on kidneys in a high glucose environment *in vitro*. It also aimed to examine the impacts of glutathione application as an essential antioxidant agent on the antioxidant system and possible oxidative DNA damage (8-hydroxy-2-deoxyguanosine).

Material and Methods

Cell culture

The Baby Hamster Kidney (BHK) -21 cell line was used in the study. BHK-21 cells in RPMI-1640 (Capricorn) containing 5% FBS (Capricorn), 10% horse serum (Capricorn), 1% L-Glutamine (Capricorn), 1% penicillin / streptomycin (Capricorn) and 5% CO₂ was incubated at its growth culture in 95% humidity and 37°C.

MTT cytotoxicity test

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability tests were performed to determine IC₅₀ values of compounds. The proliferative doses of thymoquinone and lycopene were determined. For this purpose, BHK-21 cells were seeded at 2×10⁶ cells/well in 96-well plates and incubated overnight at 37°C. After exposing the described doses of compounds for 24 h, the

medium of wells was discarded. MTT (0,5mg/ml in sterile PBS) solution (10% of completed medium) was added to each well and incubated for three h at 37 °C. At and of the incubation time, MTT medium was discarded and added to lysis solution (1%Triton-X, 10% 0,1 mol/ l HCl, 89% Isopropanol) to each well for solubilization of the formazan crystals. The absorbance of each well was measured at 570 nm by using a microplate reader. GraphPad Prism 8 software (San Diego, CA) analyzed inhibition and increasing growth in cells. Each experiment in the MTT assay was performed at least four times.

Biochemical analysis

Cells were treated with glucose, glutathione, and Glucose+Glutathione combinations for the value and time at which IC₅₀ values were determined. Since IC₅₀ values were obtained at 24 hours, cells were collected through trypsinization at the end of 24 hours. Following the collection process, the cells obtained by lysis by freeze/thaw method and the 8-OHdG level (DNA Damage ELISA kit- Catalog Number: ADI-EKS-3501, Enzo Life Science, USA) total oxidant (Rel Assay Diagnostics, Turkey) and full antioxidant capacity (Rel Assay Diagnostics, Turkey) was measured spectrophotometrically. The OSI level was determined using the data obtained.

Statistical analysis

Descriptive statistics for the features considered were expressed as mean, standard deviation, minimum and maximum value. ANOVA tests were performed to determine whether there was a difference between the groups regarding these characteristics (SPSS 22.0). In the calculations, the statistical significance level was taken as 5%.

Results

The data obtained as a result of the study were evaluated statistically and summarized in Table 1 and Table 2.

The control group was accepted as 100 % alive, and the results of the MTT % viability test at the end of 24 hours in BHK-21 cell lines, which were administered glucose at various doses, and glucose + GSH were applied, are summarized in Figures 1 and Table 1.

MTT results were obtained by administering different doses of glucose and GSH (250 μM), and these doses were evaluated. It was found that cell viability increased with each dose after GSH was added (Figure 1).



	Control	Glucose (50 mM)	Glucose (100 mM)	Glucose (250 mM)	Glucose (350 mM)
100		85,45	79,06	72,46	27,04
GSH(250µM)	105,34	94,703	91,92	84,47	64,36

Group	8-OHdG (ng/ml)	TAS (mmol Trolox-equiv./lt)	TOS (µmol H ₂ O ₂ equiv./lt)	OSI
Control	5.255±0.817 ^a	1.209±0.188 ^a	2.362±0.404 ^a	1.957±0.174 ^a
High Glucose (HG)	7.643±1.088 ^b	1.091±0.141 ^b	3.317±0.689 ^{ab}	3.065±0.613 ^{ab}
Glutathione (GSH)	5.583±0.991 ^a	1.157±0.213 ^{ab}	3.074±0.123 ^a	2.722±0.514 ^a
HG+GSH	7.622±1.1352 ^b	1.904±0.031 ^b	3.559±0.139 ^b	3.932±0.162 ^b

a, b: The difference between group averages with different letters on the same row is statistically significant

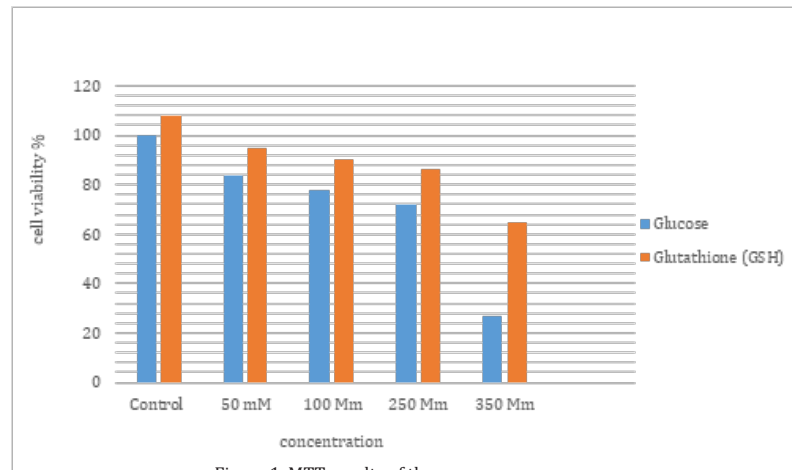


Figure 1. MTT results of the groups

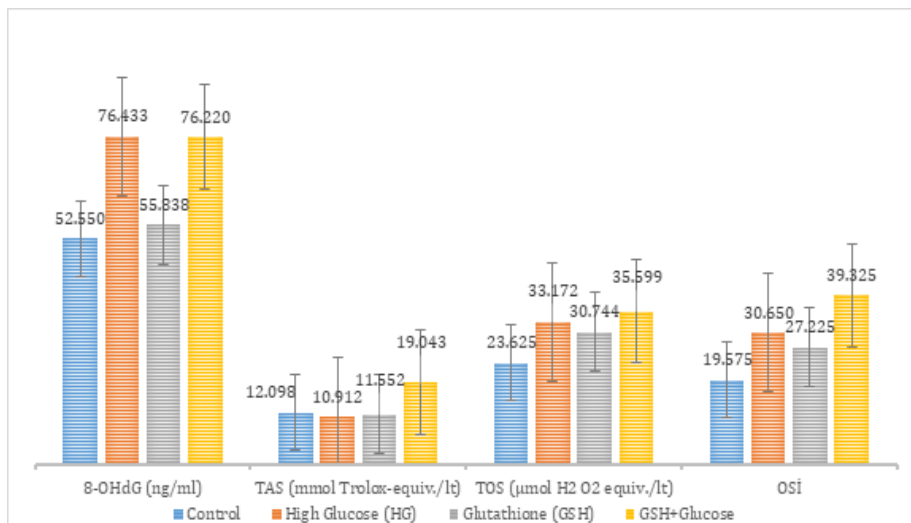


Figure 2. 8-OHdG, TAS, TOS and OSI values





It was found that 8-OHdG levels increased significantly with glucose administration and increased considerably in the G+GSH group compared to the control group ($p \leq 0.05$). The group given only GSH was no different from the control group. Interestingly, it was significantly higher in the HG+GSH group than in all other groups in TAS ($p \leq 0.05$) (Figure 2).

TOS levels and OSI values increased significantly in the HG+GSH group compared to the control ($p \leq 0.05$) (Figure 2). It was found that the GSH (250 μ M) dose applied in this study was not effective.

Discussion

Diabetes Mellitus is an endocrine and metabolic disease that occurs due to absolute or functional insulin deficiency and is characterized by impairment in fat and protein metabolism, especially carbohydrate metabolism (Vincent et al 2004). With the progression of the disease, the risk of developing specific complications such as retinopathy, renal failure, neuropathy, and atherosclerosis increases. (Memişoğulları and Bakan 2004). In the oxidative stress hypothesis, the imbalance between reactive oxygen species (ROS) and the rate of formation of free radicals and antioxidant defense capacity causes chronic complications of diabetes. In other words, hyperglycemia causes oxidative stress. In diabetic patients with increased levels of oxidative stress indicators such as lipid hydroperoxides, conjugated dienes, thiobarbituric acid reactive substances (TBARS), and isoprostanes, the decrease in the number of antioxidant parameters such as vitamins E and C, glutathione, SOD, catalase, glutathione peroxidase indicates that oxidative stress may have an essential role in the pathogenesis of chronic complications of diabetes (Gutteridge 1995).

Lipids found in mitochondria and endoplasmic reticulum membranes, and plasma are the main targets of peroxidation and ROS attacks. Free radicals/antioxidants are in balance in ordinary healthy people. In diabetes, this balance is disrupted in favor of free radicals (Memişoğulları 2005). This causes complications of diabetes. If we can make these antioxidant mechanisms more active or increase this impaired balance in favor of antioxidants, we can deal with the intricacies of diabetes. Antioxidants inactivate oxidants with four different agencies (Cherubini et al 2005, Yur et al 2013).

Many researchers have shown that oxidative stress causes DNA damage due to experimental diabetes and *in vitro* studies. In diabetic cases, 8-OHdG, an indicator of oxidative DNA damage, is increased in tissues and body fluids (Andican and Burçak 2005, Park et al 2001). In another study, lycopene, which has antioxidant effects, exerts DNA protective effects by inhibiting comet formation and reducing 8-OHdG levels (Huang and Hu 2011). Lower and

medium levels of ROS are generally recognized as inducing mitosis and having beneficial effects in cell growth, while excessive ROS can cause DNA strand breaks, DNA mutations, and DNA double-strand aberrations, further leading to oxidative stress. The sulfhydryl group in GSH is essential in maintaining the status of DNA repair and expression in the nucleus (Lv et al 2019, Bailey et al 2020). In the process of ribonucleic acid reduction, GSH acting as a donor of hydrogen catalyzes the reduction of ribonucleic acid to deoxyribonucleic acid, which plays a contributory role in DNA synthesis (Zhang et al 2016, Tian et al 2021).

Studies have reported that GSH levels in diabetes are significantly lower than in healthy individuals (Abou-Seif and Youssef 2004). Glutathione reductase, serum glutathione peroxidase, and catalase activity decrease diabetes. Komosinska-Vassev et al 2005, and Ueno et al 2002 reported that an adequate supply of GSH can prevent or delay kidney and nerve function in diabetes by protecting against oxidative stress.

In their study, Yaycı et al (2021) investigated the effect of GSH application on caspase systems in the kidney cell line where high glucose was applied, and GSH administration somewhat reduced apoptosis due to high glucose. Still, it did not cause a significant change in oxidative DNA damage.

This study evaluated TAS, TOS, and OSI index as parameters indicating the oxidative state and 8-OHdG levels as an indicator of oxidative DNA damage. It was found that 8-OHdG levels increased significantly with glucose administration and increased significantly in the HG + GSH group compared to the control ($p \leq 0.05$).

The group given only GSH was no different from the control group. Interestingly, it was significantly higher in the HG+GSH group than in all other groups in TAS ($p \leq 0.05$).

TOS levels and OSI value increased significantly in the HG + GSH group compared to the control ($p \leq 0.05$). It was found that the GSH (250 μ M) dose applied in this study was not effective.

According to the results obtained in the study, no protective effect of glutathione applied to cells with high glucose was observed at these doses at the cellular level. However, it was also determined that glutathione was not toxic to cells at these doses, as observed only in glutathione-applied groups. Studies show that the administration of GSH in various doses and routes has a vital role in preventing diabetes-related complications.

As a result of the protective application of GSH (1g/100g) in the development of diabetic renal failure and neuropathy, the 8-OHdG excretion excreted in the urine is suppressed





(Ueno et al 2002), and intravenous GSH administration increases the GSH / GSSG ratio in the erythrocyte and total glucose intake (De Mattia et al 1998), Yur et al (2013) reported that GSH administration has beneficial effects on pancreatic cells.

In patients with diabetic nephropathy, GSH and Vitamins E and C are more beneficial and strengthen each other's effects (Kuchake and Upasani 2013). In the treatment of diabetes, it is recommended to use antioxidants or antidiabetics with antioxidant properties and antidiabetics to cope with oxidative stress (Memişoğulları 2005). Studies show that GSH administration is beneficial against oxidative and free radical damage (Richie 1992, Nagata et al 2001).

Conclusion

As a result, it was determined that oxidative DNA damage, TOS, and OSI index increased in high glucose treated BHK-21 cells compared to control, and oxidative parameters did not change in the glutathione-applied group. It was observed that glutathione administration with high glucose did not alter the oxidative DNA damage, TAS, TOS, and OSI values compared to the control but increased in the GSH +Glucose applied groups. It was concluded that the administration of glutathione and glucose in the BHK-21 cell line causes an increase in oxidant parameters. The application of different doses of glutathione and more detailed studies are suggested to be planned considering the application time and parameters.

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This study was presented orally at the 28th National Biochemistry Congress and was published as a summary text in the congress book.

Conflict of Interest

The authors did not report any conflict of interest or financial support.

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Analysis and / or Interpretation: Fatmagül Yur
Literature Review: Fatmagül Yur
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Critical Review: Semiha Dede, Sedat Çetin, Ayşe Usta

Ethical Approval

This study was carried out with the permission of the Van Yuzuncu Yıl University Animal Researches Local Ethic Committee (Decision No: 2022/03-01) report.

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