The Effects of Taurine on Cell Viability in BHK-21 Cell Culture

Neslihan TAŞÇENE¹, Burcu Menekşe BALKAN², Görkem KISMALI³, Tevhide SEL³, Veli GÜLYAZ¹

¹Foot and Mouth Disease Institute
²Mehmet Akif Ersoy University Faculty of Veterinary Medicine, Biochemistry Department
³Ankara University Faculty of Veterinary Medicine, Biochemistry Department

Corresponding Author:
Assistant Prof. Dr. Burcu Menekşe BALKAN
Mehmet Akif Ersoy University,
Faculty of Veterinary Medicine,
Department of Biochemistry,
Burdur, Turkey Postal code: 15030
E-mail: burcualpaslan@yahoo.com

Abstract
The aim of this study is to investigate the effects of taurine treatment on cell viability and proliferations in BHK-21 cells. In this study, BHK-21 cells were seeded at a density of 50.000/ml per well in 96 well plates in medium composed of Glasgow-Minimum Essential Medium (GMEM). The cells were either left untreated (control) or treated with different concentrations of taurine for 24 h. XTT cell viability assay was performed, LDH activity and glucose level measured spectrophotometrically. Addition of 0,1mM – 15mM taurine increased but 30mM–70mM taurine concentration decreased cell viability and proliferations. A significant difference in medium LDH activity was observed between control and treated cells. Higher concentration of taurine (30mM-70mM) significantly increased the medium LDH activity. Significantly decreased the medium glucose level was found at high cell viability. These results suggest a role for taurine as a cytoprotective role in the BHK-21 cell in dose-dependent manner.

Keywords: Taurine, BHK-21, XTT, LDH
1. INTRODUCTION

Taurine (2-amino Ethan sulfonic acid) is a non-protein Sulphur derived amino acid which has antioxidant activity, synthesized from methionine and cysteine. Taurine is found in very high concentration on mammalian tissue and plasma. It has important effects on many biological processes such as development of central nervous system and retina, regulation of calcium metabolism and membrane stabilization (Aruoma et al., 1988).

Taurine found in high concentration in the leukocytes (20-50 mM), followed by nervous system and retina (Georgia et al., 2003). Although taurine is considered a semi-essential amino acid for humans, it is essential in some animals such as fox and felidae (Takashi et al., 2014).

Taurine inhibits the generation of reactive oxygen species. Because taurine contains a sulfonic acid rather than carboxylic acid, it does not form an aldehyde from hypochlorous acid. Instead it forms a relatively stable chloramine compound. Taurine is an antioxidant that specifically mediates the chloride ion and hypochlorous acid concentration as a result it protects the body from potentially toxic effects of aldehyde release (Birdsall, 1998). Hypochlorite is an oxidant molecule which activates the tyrosine kinase signal cascade thus leads to the formation of inflammatory mediators. Hypochloric acid causes the formation of polymorph nuclear leukocytes and eosinophils and it is also bactericidal effects. But excessive production of hypochlorite causes oxidative stress (Weiss et al., 1982).

Taurine shows antioxidant activity by preventing the generation of oxidants. Taurine disrupt the sequence of events that follow the signaling cascade initiated by toxins that leads to changes in calcium movement, and the subsequent superoxide generation. Taurine provides these through reactive oxygen species inhibition and intracellular calcium concentration stabilization (Wu et al., 2005).

Neutrophils and monocytes have high level of taurine and hypochloric acid. This hypochlorite reacts with taurine to form taurine-chloramine complex which is much more toxic than hypochlorite. When the taurine-chloramine complex occurs, it reduced the hypochlorite related oxidative stress (Idrissi et al., 2012). Georgi et al. (2003) show that in addition to its anti-inflammatory effects, taurine-chloramine complex also inhibits NO (Nitric Oxide) and TNF-α (tumor necrosis factor alpha) production.

Taurine is an important osmoregulator. It involves in cell volume regulation together with other low molecular-weighted compounds (Kim et al., 2007). Taurine, betaine, myoinositol, sorbitol and glycerophosphorylcholine (GPC) are the most relevant intracellular osmolytes (Pasetes-morales and Schousboe, 1997).

Recent years, many studies were performed about potential effects of taurine in diabetes treatment. These studies have indicated that taurine plays a significant role in overcoming insulin resistance and other risk factors in animal models of Type 1 and Type 2 diabetes (NandhiniandAnuradha, 2002; Haber et al., 2003; Franconiet al., 2004; Franconi et al., 2006; De la Puerta et al., 2010; Ito et al., 2012).

Taurine has an insulin secreting effects on pancreatic B cells. By regulating insulin signaling pathway and post receptor events, taurine, supports important functions (De la Puerta et al., 2010).

Taurine is known to have anti-diabetic effects by stimulating the insulin secretion and lowering blood the glucose level. Taurine also stimulates glucose intake in heart tissue (De la Puerta et al., 2010). Tomoka et al, (2004) suggest that 20 mM taurine administration inhibits apoptosis by preventing the formation of the Apaf-1/caspase 9 apoptosome in primary cardiomyocytes.

Although there are not much study, in-vitro and experimental studies shows that even with low dose taurine administration, beneficial effects were observed on thromboocyte aggregation, retinopathy and nephropathy. In other study (Kim et al. 2007), it was revealed that taurine has role on fetal development. Especially it protects fetus from harmful effects of diabetes.
In present data; the effects of different doses of taurine well known antioxidant agent, on cell viability and proliferation on BHK-21 cells investigated. For this purpose, in addition to cell viability test; cytotoxicity parameter LDH activity and metabolic activity parameter glucose level was measured.

2. MATERIAL AND METHOD
Baby Hamster Kidney (BHK-21) cells were routinely grown in low-glucose GMEM (Glasgow Minimum Essential Medium) buffered with%10 FCS (Fetal Calf Serum),%10 TPB (Triptose Phosphate Broth), %0,01 Penicillin, Streptomycin sulphate, Neomisin. 50.000 cell/ml, 100 ul GMEM medium were added in 96 well plates and maintained in an 5% CO₂ incubator at 37°C, for 24 hours. After this incubation medium was removed. Thereafter, different concentration of taurine (0,1mM, 1mM, 2,5 mM, 5 mM, 10 mM, 15 mM, 30 mM, 40 mM, 50 mMve 70 mM) was added to each well and incubated again for 24 hours. Taurine was not added to the control group.

To measure the effects of taurine on cell proliferation, XTT test was performed. The absorbance rate of each well optical density (OD value) was measured spectrophotometrically at 450 nm by ELISA (Tecan) reader. LDH activity and glycose levels were measured with auto analyzer.

3. STATISTICAL ANALYSIS
Statistical comparison between the treated and control groups were performed using one-way ANOVA with post hoc Duncan test. P-values (P<0, 05) At least 3 independent test perform for each group.

4. RESULTS
Although 0,1mM-15mM taurine concentrations increased cell viability and proliferation, 30mM-70mM taurine concentration caused significant decrease. (Cell viability %60, %43, %31, and %28 respectively) (Table 1, Figure 1). The difference in medium LDH activity between control and taurine groups was significant.

**Table 1.** Results of XTT Cell Viability test in different doses of Taurine applied BHK-21 cells

<table>
<thead>
<tr>
<th>mM Taurine</th>
<th>XTT cell viability %</th>
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<tr>
<td>0</td>
<td>100</td>
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<tr>
<td>0.1</td>
<td>120</td>
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<tr>
<td>1</td>
<td>130</td>
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<tr>
<td>2.5</td>
<td>140</td>
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<td>5</td>
<td>120</td>
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<td>10</td>
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LDH activity increase significantly (p<0,001) in high doses of taurine (30mM, 40mM, 50Mm, 70mM) applied group compare to control group (%114, %130, %119 and, %129) (Table 2). This result indicates that high doses and increasing doses of taurine have cytotoxic effects on BHK-1 cells.
Figure 1. Images of different doses of Taurine applied BHK-21 cells

Positive control (No Taurine applied cells), Negative control (SDS applied cells)

Table 2. LDH Levels in different doses of Taurine applied BHK-21 cells

A significant decrease (p<0.001) in glucose levels was observed in high cell viability percentages. (When cells viability increases 117%, glucose levels decrease 46%)(Table 3). This reduction occurs because when cell viability decrease, cells are not able to use glucose.
5. DISCUSSION

Chen et al. (1998) reported that taurine causes proliferation and differentiation of fetal brain cells. Application of 100-6400 μM taurine in the medium to neuronal cell cultures has been observed to result in increased cell DNA synthesis and increased cell count. As a result of this study, it was found that taurine plays a role as a neurotrophic factor in fetal brain proliferation and differentiation during human brain development (Mattuet al. 2012).

Ren et al. (2008) investigated the effect of taurine on nitric oxide and protein kinase c alpha (p-PKCalpha) in angiotensin II -induced neonatal rat cardiac cell cultures (CFb) and the influence of taurine on the proliferation of CFb cells.

An inhibitory effect on cell proliferation and collagen synthesis was observed in CFb cells treated with 40-160 mM Taurine (This effects has been associated with inhibition of p-PKCalpha expression).

Taurine has also been reported to be effective on bone metabolism. Seol-Hee et al. (2007) reported that taurine increased cell proliferation in human osteoblast cells (HOB). As a result of 10 mM and 20 mM taurine applications in HOB cells, cell viability increased and LDH activity decreased. Similar to the finding in HOB cells, Shivaraj et al (2012) and Mandujano et al. (2014) observed an increase in cell proliferation in low dose taurine applications (10uM-2,7mM) in neural stem cells.

Contrary to other findings, Taurine has also been reported to reduce cell viability. 20Mm- 160 mM taurine application in human hepatocellular carcinoma (HHCC) HepG2 cells (Shuo et al., 2015) and 5-50 mM taurine application in HSC-T6 cells (Mandujano et al., 2014), inhibited cell proliferation as dose-dependent manner. The decrease in cell viability was reported to be independent of apoptosis (Mandujano et al., 2014).

There are a lot of studies about taurine regulates glucose levels in the case of hyperglycemia. A study by Santos-Silva et al. (2015) reported that taurine promotes glucose entry into the cell.

Lapson et al (1983), show that taurine exerts hypoglycemic effects by enhancing insulin action. Taurine also facilitates the interaction of insulin with its receptor (Maturo and Kulakowski, 1988). On the other hand, taurine enhances glycogen synthesis, glycolysis and glucose uptake in the liver and heart of adult rats. These effects were dependent of insulin concentration (Higo et al., 2008).
6. CONCLUSION
Present results indicated that taurine has dose depended cytoprotective and antiproliferative role in BHK-21 cells. The cytoprotective and antiproliferative effects were observed in similar taurine doses but in different cell lines.
LDH activity, which is an indicator of cytotoxicity, was increased at cell viability decreased of taurine doses. In all taurine groups glucose consumption was increased in BHK-21 cells. With this study, baseline values for further in vivo and in vitro studies were established. Clinical trials investigating the effects of taurine on metabolic pathways in various cell lines are required. In addition to this, there is a need for molecular level studies including cancer cell lines.

7. ACKNOWLEDGEMENT
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8. REFERENCES


