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RESEARCH ARTICLE

Anti-Proliferative Effect of Melamine on Human Colon Adenocarcinoma Cells

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ABSTRACT

Melamine is an organic chemical used primarily as a plastics stabilizer and fire retardant. Also, it was used to fraudulently elevate the protein content of animal feed and dairy products, which led to infant kidney diseases and pet deaths. In this study, melamine was studied for its effects on growth in the human colon adenocarcinoma cells (Caco-2). Different concentrations of medium-dissolved melamine were incubated for 24 h with Caco-2 cells and tested for its anti-proliferative potential using MTT assay. Half-maximal inhibitory concentration (IC_{50}) of melamine was calculated from dose-response curve and found to be 14636 μg/L in the cells. The results demonstrated that the dose dependent melamine decreased cell proliferation and direct anti-proliferative effect in Caco-2 cells.

Keywords: Anti-proliferative, Caco-2, Melamine, MTT

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Melaminin İnsan Kolon Adenokarsinom Hücreleri Üzerindeki Anti-Proliferatif Etkisi

ÖZ

Melamin, öncelikle plastik dengeleyici ve yangın geciktirici olarak kullanılan organik bir kimyasaldır. Hayvansal yem ve süt ürünlerinin protein içeriğini suni şekilde yükseltmek için kullanılmasıyla bebeklerde böbrek hastalıklarına ve hayvan ölümlerine yol açmıştır. Bu çalışmada, melaminin insan kolon adenokarsinom hücrelerinin (Caco-2) proliferasyonu üzerine etkileri araştırılmıştır. Farklı konsantrasyonlarda çözülmüş melamin Caco-2 hücreleri ile 24 saat inkübe edildi ve MTT testi kullanılarak anti-proliferatif potansiyeli test edildi. Melaminin yarı maksimal inhibisyon konsantrasyonu (IC_{50}), doz-cevap eğrisinden hesaplandı ve hücrelerde 14636 μg/L olarak bulundu. Sonuçlar doza bağımlı olarak melaminin Caco-2 hücrelerinin proliferasyonunu azalttığını ve Caco-2 hücrelerine karşı anti-proliferatif etkiye sahip olduğunu göstermiştir.

Anahtar Kelimeler: Anti-proliferatif, Caco-2, Melamin, MTT

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INTRODUCTION

Melamine (2, 4, 6-triamino-1, 3, 5-triazine) is an organic base chemical becoming headline news recently after the occurrence of an outbreak of nephrolithiasis and acute kidney injury among infant and young children due to consumption of milk adulterated with melamine to fraudulently elevate its protein content in China. (Chan et al. 2008, Skinner et al. 2010). Also, in 2004 in Asia and in 2007 in North America, melamine-contaminated pet food resulted in renal failure outbreaks in dogs and cats (Guan and Deng 2016). Though melamine has a low acute toxicity with a high LD_{50} in animals, it causes chronic renal toxicity including formation of kidney stones and kidney injury when chronic ingestion of melamine-adulterated food and feed in excessive doses (Dalal and Goldfarb 2011, Hau et al. 2009). Many researchers have focused on the effect of melamine-related renal failure, but there is little information about the toxic effects of melamine to other tissues and cancers (Choi et al. 2010, Han et al. 2011, Hsieh et al. 2012). Therefore, it is necessary to investigate the possible anti-proliferative effects of melamine. The aim of the present study was to investigate the anti-proliferative effects of melamine on Human Colon Adenocarcinoma Cells (Caco-2).

MATERIALS and METHODS

Cell Culture

Caco-2 cells (ATCC® HTB-37™) were maintained in the Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich®) supplemented with 10% fetal bovine serum (FBS, Sigma), 100,000 U/L penicillin and 10 mg/L streptomycin (Thermo Fisher Scientific, USA) in a humidified incubator with 5% CO_2 and at 37°C. The cells for experiments were seeded at $1x10^3$ cells/well in 96 well plates (BD Falcon, Rockville).

Cell Viability Assay

Melamine (Sigma-Aldrich®) were dissolved in DMEM and diluted to working concentrations (100, 50, 12.5, 6.25, 3.124, 1.563 and 0.781 μg/ml) with fresh medium. After serial dilution of melamine, cell viability was estimated at 24 h by MTT (3- [4, 5-dimethylthiazole-2- yl]-2, 5-diphenyltetrazolium bromide; thiazolyl blue) assay to obtain IC_{50} concentrations of melamine as described by Ulukaya et al. (2008). Briefly, after 24 h incubation with melamine, the medium was aspirated and MTT was added to reach a final concentration of 0.5 mg/mL for 4 h. Formazan crystals formed by viable cells were dissolved in Dimethyl Sulfoxide (DMSO, Merck). Cell viability was analyzed using the SpectraMax i3x Multi-Mode Microplate Reader (Molecular Devices, USA) at 540 nm wavelength. Triton X-100 (0.1%) was used as positive control and the cell cultivation medium was used as negative control (nontoxic, untreated). The cell viability (growth) was calculated according to the nontoxic control, which was considered to be %100 viability. Positive control was set to 0% viability. The degree of inhibition of melamine treated cells was expressed as a percentage of control cells (untreated). The half maximal inhibitory concentration (IC_{50}) value representing the concentration of melamine that is required for 50% inhibition was calculated using a statistical program.

Statistics

IC_{50} was determined using the Software GraphPad PRISM (version 7).

RESULTS

MTT assay was carried out to determine anti-proliferative effect of melamine on Caco-2 cells. Figure 1 demonstrates the growth inhibition curves of melamine at different concentrations. IC_{50} value of melamine was calculated and founded to be 14636 μg/L (Table 1). This result referred to the anti-proliferative effect of melamine.

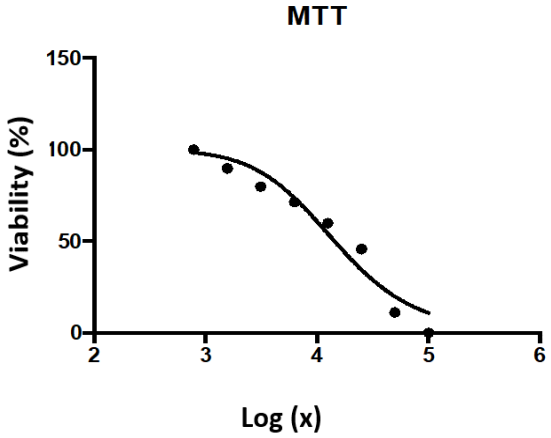

Figure 1. The inhibition curve of melamine at eight different concentrations.

Table 1. IC_{50} levels (μg/L) of melamine, according to the MTT

Log(Inhibitor) vs. normalized response -- Variable slope	
Best-fit values	
IC_{50}	14636
HillSlope	-1.224
$logIC_{50}$	4.165
Std. Error	
$LogIC_{50}$	0.06791
HillSlope	0.216
95% Confidence Intervals	
IC_{50}	9983 to 21458
HillSlope	-1.753 to -0.6956
$logIC_{50}$	3.999 to 4.332
R square	0.9547

DISCUSSION

There are a wide range of chemicals available in the market. It is important to investigate whether these chemicals have toxic effects on humans and animals (Bilir et al. 2018, Florento et al. 2012). The *in vitro* cytotoxicity test is one of the most important consideration to evaluate bioactive compounds. The cytotoxic responses of various cell types to various chemicals ere assessed using cell based assays, such as MTT, lactate dehydrogenase enzyme test. MTT is colorimetric test to use for the measurement of cell proliferation (cell growth rate), cytostatic effects or cytotoxic effects. It is commonly used for the assessments of cytotoxic compounds like anticancer drugs, toxic agents, and other pharmaceuticals (Aslantürk 2017, Florento et al. 2012, Mervin et al. 2016). A change in cell number causes a concomitant change in the level of formazan crystals produced by cellular reduction of the MTT, demonstrating the degree of cytotoxicity induced by the chemicals. IC_{50} is the concentration of the tested chemical that lead to the death of 50% of the cells, might be used to predict the degree of cytotoxicity (Florento et al. 2012).

Melamine is an organic chemical causing nephrotoxicity in human and animals. Numerous study on the toxicity of melamine focused on renal damage. However, there are a few research on the cytotoxicity of melamine (Liu et al. 2014). It has been shown that melamine caused apoptosis of the normal rat kidney (NRK)-52e cells via excessive production of cellular Reactive Oxygen Species and the activation of p38 MAPK (mitogen-activated protein kinase) signaling pathway, leading to cellular apoptosis (Guo et al. 2012). In another study, melamine revealed IC_{50} values of 1.89 and 2.07 mg/mL in NRK-52e cells and 293T cells (Normal human kidney cell lines), respectively (Sun et al. 2016). Radko et al. (2010) reported that after 48 h of exposure of melamine, EC_{50} values for FaO (Rat hepatoma cells) and L6 cells (Rat skeletal muscle cells) were 6.4 and 8.2 mM, respectively. In other study, it has been reported that the 24-h IC_{50} values of melamine in Madin-Darby canine kidney epithelial cell line (MDCK) and human renal adenocarcinoma cell line (ACHN) were 4792 and 2792 μg/ml, respectively (Choi et al. 2010). In the present study, after 24-hour exposure of melamine, IC_{50} value for Caco-2 cells were 14636 μg/L. Cell proliferation was suppressed by melamine in a dose-dependent manner in this study. The cytotoxicity of melamine in cancer cell lines such as Caco-2, FaO, ACHN was more potent than in normal cells such as L6 cells, MDCK, NRK-52 (Choi et al. 2010, Guo et al. 2012, Radko et al. 2010, Sun et al. 2016).

CONCLUSION

There are no studies on the anti-proliferative activity of melamine on human colon adenocarcinoma cells before. In the present study, it was examined the anti-proliferative activity of melamine on these cells. The cells appeared to be sensitive to melamine. The anti-proliferative effect of melamine was found to be moderate in this cell line.

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