Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 3 Issue 9 September 2019

Mini Review

The Genotoxic Effects of Cadmium and Temozolomide on Hela Cells

Ying-Liang Liu* and Michael A Edelbrock

Department of Biology and Animal Science, The University of Findlay, College of Sciences, USA

***Corresponding Author:** Ying-Liang Liu, Department of Biology and Animal Science, The University of Findlay, College of Sciences, USA.

Received: August 16, 2019; **Published:** August 19, 2019

Abstract

Cadmium is a heavy metal that is carcinogenic to humans. Literature suggests that cadmium is not acting alone but rather is a co-carcinogen. This study investigated whether the combination of cadmium and temozolomide act in combination with one another to increase cell genotoxic stress. Cadmium and temozolomide can cause apoptosis if cells are exposed at high concentrations. Hela cells, a human cell line, were treated with those agents at sub-lethal concentrations. The genotoxic effects of these agents were evaluated using the Comet Assay. Concentrations of cadmium used were 0, 1 µM whereas the concentrations used for temozolomide were 0, 0.05, 0.2, 0.5, and 1 µM. Hela cells were exposed to those agents at the same time and were incubated for one day. Cells were then isolated and Comet Assay was performed. Slides with cells were stained with ethidium bromide so it can be observed under the fluorescent microscope. Tail moment was measured (N=50 for each treatment) as an indicator of DNA damage. Average tail moments were 0.959 for control, 2.046 for TMZ treated, and 1.939 for both cadmium and TMZ treated. A t-test was used to determine statistical difference (P=0.05). Although both agents exerted DNA damage when compared to control (0 µM Cd and 0 µM TMZ), no significant difference between cadmium and temozolomide treatment was measured.

Keywords: Cadmium; Temozolomide; Hela Cells

Background and Methods

Hela cell is a type of cancer cell that is widely used for many scientific researches today. A growing cell line of Hela cells were seeded in 6-wells culture plates after several passages. The cells were incubated for 24 hours and then treated with various concentrations of cadmium and temozolomide. Cadmium is a metal element that has shown to be carcinogenic to human body whereas temozolomide is a prodrug that gets converted into an active alkylating metabolite which expresses cytotoxic effects on the cell genome. The cells were then incubated for another 24 hours prior to cell processing. After the incubation period, the cells in each well were isolated by trypsin and centrifuged in 1.5 mL tubes. Cells were mixed with 0.5% low melting agarose and placed on premade slides dipped in 1.5% agarose. Coverslips were put on the slides and allowed to solidify in the fridge for at least 10 minutes. Coverslips were then removed and the slides were treated with lysing solution for 75 minutes. Next, the slides were treated with denaturing solution for 15 minutes. Then the slides were placed in the alkaline buffer solution in the electrophoresis tank for 40 minutes before power was turned on. The power was set at 28 volts and the slides underwent electrophoresis for 20 minutes. After electrophoresis, the slides were washed with 0.4 M Tris solution for 15 minutes and then placed in 95% ethanol for 10 minutes. Lastly, the slides were allowed to dry for 24 hours before being stained with ethidium bromide. This procedure is called the Comet Assay and it is used for detecting DNA breaks. There were 10 combinations of cadmium and temozolomide treatment (including controls). Each slide was examined under the fluorescent microscope and 50 cell images were taken for each slide.

Discussion

The comparisons between samples' tail lengths are presented by graphs 1 to 4. Each graphs has three controls: sample 1 was treated with no Cd and no TMZ; sample 2 was treated with 1 micromolar Cd only; and sample 7 was treated with 1 micromolar TMZ only. Student T-test was performed and alpha was set as 0.05. Graph 1 compares sample 3 (treated with 1 micromolar of both Cd and TMZ) to sample 7 with a T-test of 0.41 which is insignificant. Graph 2 compares sample 4 (treated with 1 micromolar Cd + 0.5 micromolar TMZ) to sample 8 (treated with 0.5 micromolar TMZ only) with a T-test of 0.018 which is significant. Graph 3 compares sample 5 (treated with 1 micromolar Cd + 0.2 micromolar TMZ) to sample 9 (treated with 0.2 micromolar TMZ only) with a T-test of 0.10 which is insignificant. Graph 4 compares sample 6 (treated with 1 micromolar Cd + 0.05 micromolar TMZ) to sample 10 (treated with 0.05 micromolar TMZ only) with a T-test of 0.0007 which is significant.

Picture 1 shows normal cells without any DNA trail as most of the DNA content stayed within the cell because it is too big to pass through the membrane. In picture 2, there were some damage to the DNA and it was able to pass through the membrane during electrophoresis, leaving a trail. This trail was quantified as tail length and moment that was reported in results. Picture demonstrated severe DNA breaks which left a longer trail [1,2].

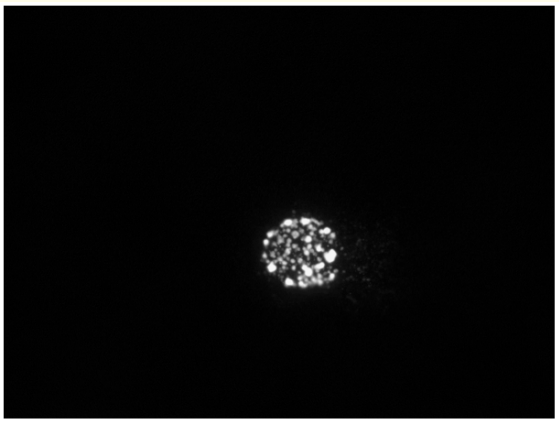

Figure 1

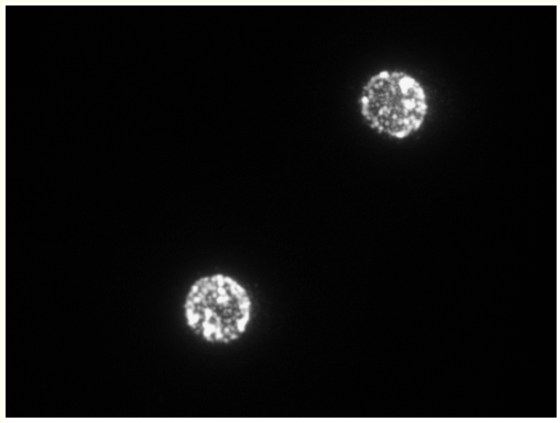

Figure 2

Conclusion

Although the combination of both agents exerts more DNA damages compared to either agents alone, the T-test did not demonstrate significance for half of the comparisons (as seen in graph 1 and graph 3.

Bibliography

1. Guillamet E., *et al*. "In vitro DNA damage by arsenic compounds in human lymphoblastoid cell line (TK6) assessed by alkaline Comet assay". *Mutagenesis* 19.2 (2004): 129-135.

2. Singh NP., *et al*. "A simple technique for quantitation of low levels of DNA damage in individual cells". *Experimental Cell Research* 175 (1998): 184-191.

Volume 3 Issue 9 September 2019