

# O'Neil, Loni A. and O'Neil, M. Todd

## Antigenotoxic Effects of N-acetylcysteine on Benzo[a]pyrene induced DNA Damage in HepG2 Cells as measured by the Alkaline Comet Assay

Faculty Mentor: Kim L. O'Neill, Microbiology

A major factor involved in the risk of developing cancer with the probability of enhancing or suppressing carcinogens is dietary composition. A chemoprevention approach to cancer means preventing this disease by the administration of one or several compounds. For a number of years, N-acetylcysteine (NAC) has shown in studies to be an effective chemopreventive agent, but there have also been some studies disproving the effectiveness of NAC.<sup>1,2</sup> This is because NAC has different functions in different cell types. Because of this, we decided to test the effectiveness of NAC against DNA damage in human HepG2 cells and to determine the effectiveness using the Alkaline Comet Assay. Our hypothesis is that NAC will be effective in either preventing or repairing DNA damage caused by B[a]P.

HepG2 cells are human hepatoblastoma cells that consist of an abundance of smooth endoplasmic reticulum, where cytochrome P-450 enzymes are involved in the xenobiotic metabolism.<sup>3</sup> Because of this, cytochrome P-450 enzymes play an important role in the metabolism of chemical carcinogens.<sup>4</sup> The biotransformation reactions caused by cytochrome P-450 enzymes metabolizing different toxins, chemicals, and foods are classified as either phase I or phase II enzymes.<sup>4</sup> Phase I enzymes catalyze the formation of reactive intermediates that are carcinogens. Phase II enzymes neutralize reactive compounds. Phase II enzymes are much more effective and safe in promoting tumor inhibition whereas phase I enzymes often promote tumor incidence.<sup>4</sup> NAC is thought to promote cytochrome P-450 enzymes to react to produce phase II enzymes which is why we decided to use HepG2 cell line.

The Alkaline Comet Assay can quantify DNA strand breaks. In this assay, cells are suspended, lysed, and electrophoresed to separate the damaged DNA from the nucleus, forming a comet tail. We decided to pre-treat cells with NAC, then treat them with NAC accompanied with Benzo[a]pyrene (B[a]P). B[a]P is a known carcinogen which damages the DNA.

We seeded HepG2 cells at 1,000,000 cells/ml in T25 flasks and allowed the cells to attach for 24 hours. We then treated the cells for 24 hours with varying concentrations of NAC (25  $\mu$ M, 50  $\mu$ M, 100  $\mu$ M, and 200  $\mu$ M), with positive and negative controls. The cells were then treated 25  $\mu$ M benzo[a]pyrene and the same concentration of NAC from the previous treatment. We incubated the cells for another 24 hours. We then evaluated the effects of benzo[a]pyrene on the DNA using the Alkaline Comet Assay.

A sufficient amount of accurate results have thus not been produced, but we do expect to have complete, viable results within a few short months. We still hypothesize that NAC will be effective in either preventing or repairing DNA damage caused by B[a]P.

The majority of the obstacles that we faced in our research proved to be minor details that turned out to be major mistakes in the experiment. These minor details consisted of: cell viability,

culture contamination, instrument accuracy and collaboration, and human error. It was very helpful to more understand all the planning and trials that go on for any research that is performed. Overall, it has been a very helpful experience.

Literature;

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