

Stevens, Amanda

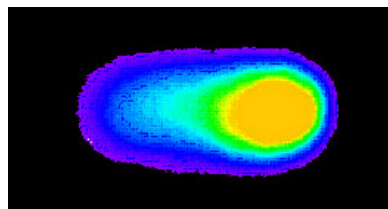
An Exploration of the Genotoxic Effects of Ellagic Acid on Human HepG2 Cells and Its Potential Impact on Cancer Prevention

Faculty Mentor: Kim O'Neill, Microbiology and Molecular Biology

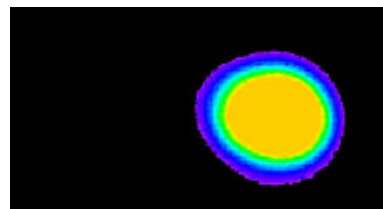
Over the past few years, cancer prevention has become a more important focus for research in the fight against cancer. Recent studies suggest that perhaps as much as 70-80 % of all cancers could be prevented by diet alone. Ellagic acid is a compound found in foods like raspberries, strawberries, and apples, and is thought to be important for cancer prevention. Benzo[a]pyrene is a potent environmental carcinogen detected in substances such as cigarette smoke, cooked foods, and fossil fuel combustion. Studies involving rodents exposed to Benzo[a]pyrene (B[a]P), whose diets included ellagic acid (EA), showed a decrease in tumor formation as compared to rodents whose diets did not include ellagic acid.¹

It is believed that cancer can be caused by damage to DNA, which leads to mutations that can interfere with normal cell functions. Dysregulation of normal cellular functions over a long period of time can cause a cancer cell to keep growing when it should shut itself off and die. B[a]P is a large molecule that binds tightly to DNA once activated, and although the nature of the EA/B[a]P interaction is not certain, it is possible that EA changes the B[a]P molecule to prevent it from binding and causing damage to DNA. This would explain why EA prevents B[a]P-induced cancer in rodents; less DNA damage means a smaller chance for cancer-causing mutations.

My project was to treat a human cell line (HepG2) with B[a]P and varying levels of EA, then quantify the amount of resulting DNA damage to cells. The hepatoblastoma cell line HepG2 is ideal because it has retained much of the enzyme activity found *in vivo* (in the body), especially that of specific enzymes involved in the metabolism of B[a]P. I used the Comet Assay to quantify DNA damage. Once a large molecule like B[a]P binds to DNA, the cell repairs itself by excising the entire B[a]P-bound section, then it fills in the gaps. If many B[a]P molecules were bound then cut out, and the two DNA strands were separated, several short DNA fragments would result. In the Comet Assay, a cell's DNA is run through an electric field, which causes the shorter fragments to travel faster than the longer, heavier ones. Then the DNA is stained and observed with a microscope. If there were many strand breaks, the cell will look like a comet, the tail representing the faster-moving (short) strands. If the cell had very little DNA damage, all the DNA fragments would be the same size and would move at the same rate, and under the microscope the DNA would look circular.



DNA damage



No DNA damage

The results from this project were surprising. I hypothesized that EA would prevent DNA damage from B[a]P, since it prevented tumors in rodents. However, initial results showed a slight *increase* in damage upon treatment with EA in addition to B[a]P. Further studies showed that this was not the trend, and that sometimes EA seemed to increase damage, but sometimes it decreased it. I concluded that EA only very slightly inhibits B[a]P-induced DNA damage when prevention actually occurs, and thus EA's anticancer activity is more likely by another mechanism.

Three items of note led me to my final conclusions. First, there was no overall pattern in damage observed for increasing doses of EA. The larger doses didn't always show more or less damage; sometimes the intermediate doses showed the most prominent effect, but no dose demonstrated consistent results. Second, the amount of damage above or below the B[a]P controls was small, and not statistically significant. When EA increased damage, the increase was very small, and when it decreased damage, again it was only by a small amount. Third, I am absolutely confident that the changes were not due to careless human error. I had been proficient in the Comet Assay for several months before beginning this project, and all procedures were done in an unusually precise manner. I also ran several replicates of the experiment – about eight comets in all – each of which took 30+ hours.

Although I would have been more pleased to report a significant finding about EA and its effects on B[a]P-induced DNA damage, scientific research means that not all hypotheses will be correct. My research question, 'Does ellagic acid directly inhibit DNA damage caused by Benzo[a]pyrene?' can be answered: 'Not necessarily.' Perhaps *in vivo* conditions could somehow activate EA to prevent B[a]P damage, but that's something to explore in a different experiment.

The experience I've gained from this project has been vital to my major, and will help in my future schooling and career. I was able to intern over the summer in a cancer research facility because of my experience with this project and others, and I'll be able to attend the graduate school of my choice in part because of my laboratory experience. I would like to express my appreciation to ORCA, Dr. O'Neill, and my fellow undergraduates, without whom this project would not have been possible.²

References:

1. Lesca P. Protective effects of ellagic acid and other plant phenols on benzo[a]pyrene-induced neoplasia in mice. *Carcinogenesis*. 1983 Dec;4(12):1651-3.
2. Many thanks to Devin D. Twitchell and Cameron P. Richards.