The Effect of Nicotine on DNA Damage

Biology

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Abstract

The purpose of this project is to examine the effect of nicotine damage on banana DNA. DNA will be extracted from a banana and then nicotine will be added into the DNA. This procedure will be repeated with a serial dilution. The observations made and the amount of DNA left will indicate the amount of damage. If the nicotine concentrations are increased, then the amount of damage will increase. The hypothesis is supported and accepted because the amount of DNA left in the tube significantly decreased when a higher concentration of nicotine was added.
Review of Literature

Every year about 440,000 people die from smoking cigarettes\(^{(1)}\). Nicotine, a highly addictive substance, is found in cigarettes. Recent studies show that nicotine may be a genotoxicity on DNA. People always talk about how cigarettes are bad for one’s health, but does DNA genotoxicity ever come up in the conversation?

Genotoxicity means the amount of damage a genotoxin (poisonous substance) can cause to a DNA molecule. Genotoxicity could also cause genetic mutations; genetic mutation occurs when a gene is damaged or changed in a way to alter the genetic message being carried\(^{(2)}\). Nicotine would be a chemical mutagen; chemical mutagen changes the bases in DNA in many ways. One way would be to mimic the correct nucleotide bases in a DNA molecule, but remove parts of the nucleotide (such as the amino group on adenine), and add hydrocarbon groups to various nucleotides. This causes the bases to pair up incorrectly during DNA replication\(^{(3)}\).

DNA or Deoxyribonucleic Acid is a nucleic acid that is like a blueprint, it contains genetic information that is used in the development of all living organisms\(^{(4)}\). A DNA molecule is made up of two chains composed of chemical compounds called nucleotides\(^{(5)}\). The chains twist upwards like a winding staircase.
forming what is known as the double helix. Each nucleotide consists of three units: sugar molecules or deoxyribose, a phosphate group, and one of the four nitrogen containing compound bases. The four bases are adenine (A), guanine (G), thymine (T), and cytosine (C) \(^{6}\). Deoxyribose is in the center of the nucleotides with a phosphate group on one side and a base on the other. The phosphate group is linked to the adjacent deoxyribose and they form the parallel side rails of the ladder. The bases face inwards toward one another forming the rungs of the ladder.

There are four types of DNA damage, all four bases in DNA can be modified at various positions, mismatches of the normal bases because of failure to proofreading during DNA replication, breaks in the backbone, and crosslinks that can be formed between bases \(^{7}\). The agents that could cause DNA damage are UV rays, oxygen radicals, chemicals found in chemotherapy, and hydrocarbons; including some found in cigarette smoke \(^{8}\). Failure to repair the DNA produces a mutation.

Nicotine is a substance responsible for addiction to tobacco and supposed to contribute to tobacco carcinogenesis \(^{9}\). “Certain genes in the human body have been said to be at risk of nicotine dependence, one of these genes is called CHRNA5 or alpha-5
nicotine dependence” (10). If exposed to nicotine the genes have a two fold increase risk for nicotine dependence (11).

In this experiment DNA from a banana will be extracted and then different concentrations of nicotine will be added to it. The different concentrations will be 10mM, 0.1mM (1/100), and then 0.01 mM (1/1000). Observations will be made and compared to the unmodified DNA and that will help determine the amount of damage done to each sample.

Notes

1. “DNA Repair”
2. Ibid
3. Ibid
5. DNA The Thread of Life.
6. “DNA Repair”
7. Ibid
8. Ibid
9. Stern, N., and A. Hochman
10. Studies Identify DNA Regions Linked to Nicotine Dependence
11. Ibid

Purpose

The purpose of this project is to determine if nicotine has damaging effects on DNA. It will also be determined if nicotine concentration amounts will damage DNA.
Hypothesis

If the concentrations of nicotine increase, then the DNA damage will increase.

Experimental Design

Independent variable: Different concentrations of nicotine

Degrees of variation: Serial dilution of nicotine solution

Dependent variable: Amount of DNA left

Control Group: Unmodified DNA

Constants: Type of nicotine, type of banana, powdered buffer, blue monster DNA stain, isopropyl alcohol, and cell blaster.
## Materials

<table>
<thead>
<tr>
<th>Materials</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Bananas</td>
<td>Nicotine</td>
</tr>
<tr>
<td>Powdered buffer</td>
<td>Cell blaster</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>Blue monster DNA stain</td>
</tr>
<tr>
<td>Graduated test tubes</td>
<td>Nylon fast flow filter</td>
</tr>
<tr>
<td>Glass extraction rod</td>
<td>Measuring spoons</td>
</tr>
<tr>
<td>Knife</td>
<td>Glasses</td>
</tr>
<tr>
<td>Plastic cups</td>
<td>Beakers</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
</tr>
</tbody>
</table>
Procedure

1. Get a beaker and add 120 milliliters of distilled water.

2. Add 1 ¼ teaspoon of the powdered buffer. Use the glass extraction rod to mix the buffer until it completely dissolves.

3. Add 1 teaspoon of Cell Blaster, Mix gently. Chill the buffer on ice.

4. Get a banana and slice chunks, put the chunks in the glass cup. Use the knife and mash the chunks.

5. Get a beaker and place 15 milliliters of the mush in it. Then mix in 30 milliliters of the chilled buffer. Swirl gently for at least 2 minutes.

6. Get another beaker and place the nylon fast flow filter over it and pour the contents of the beaker with the mush and buffer in. Let it filter for at least 30 seconds or until the fluid stops dripping.

7. Pour into a plastic cup 2 milliliters of water and 2 nicotine tablets. Let the tablets dissolve. Then make a serial dilution.

8. Transfer 5 milliliters of the extraction mixture into a graduated test tube.

9. Carefully deposit 5 milliliters of the isopropyl alcohol into the graduated test tube. Then put one drop of blue monster DNA stain into the graduated test tube. Measure the amount of DNA.

10. Pour 0.5 milliliters of nicotine into the DNA and then wait five minutes.

11. Record the amount of DNA left in the solution and compare it to unaffected DNA.
Making the Powdered Buffer

Chilling the Powdered Buffer on Ice

Mixing the mashed Banana chunks and powdered buffer together.

Pouring the Banana and buffer mixture into the nylon fast flow filter.
Pouring the filtered buffer into the test tube

Pouring the isopropyl alcohol into the test tube
Results

The amount of DNA left in the test tubes containing a nicotine concentration of 10mM had less left than the DNA in the control. This was determined by measuring the amount of blue DNA stain. Test tubes containing a 0.1mM (1/100) concentration and 0.01mM (1/1000) concentration contained more DNA left than the 10mM concentration. The 10mM concentration only had 0.06 milliliters of DNA left, while the 0.1mM had 0.15 milliliters of DNA left and the 0.01mM had 0.14 milliliters left. The control had 0.34 milliliters of blue DNA stain.
Results

Control 0.01mM 0.1mM 10mM

10mM of nicotine
### Amount of Spread of Blue DNA Stain (in milliliters)

<table>
<thead>
<tr>
<th>Nicotine</th>
<th>Trial One</th>
<th>Trial Two</th>
<th>Trial Three</th>
<th>Trial Four</th>
<th>Trial Five</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mM</td>
<td>0</td>
<td>0.1</td>
<td>0.05</td>
<td>0.05</td>
<td>0.1</td>
<td>0.06</td>
</tr>
<tr>
<td>0.1(1/100) mM</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.15</td>
<td>0.2</td>
<td>0.15</td>
</tr>
<tr>
<td>0.01(1/1000) mM</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.14</td>
</tr>
<tr>
<td>Control (no nicotine)</td>
<td>0.27</td>
<td>0.33</td>
<td>0.37</td>
<td>0.37</td>
<td>0.36</td>
<td>0.34</td>
</tr>
</tbody>
</table>
Nicotine Damage Measured from Blue DNA Stain Spread

The Effect of Nicotine on DNA Damage

The Amount of DNA left (mL)

Trials

1 2 3 4 5

Control
0.01mM
0.1mM
10mM
Conclusion

In conclusion, nicotine can cause DNA damage. The higher the nicotine concentration the lower the amount of DNA left, which means it is concentration dependent. A higher nicotine concentration has more strength to damage DNA, while the serial dilutions have less nicotine. Therefore, it damaged less DNA. In the data collected 10mM the highest nicotine concentration had only 0.06 milliliters of DNA left in the test tube. 0.1mM (1/100) had 0.15 milliliters of DNA left and 0.01 (1/1000) had 0.14 milliliters of DNA left.

The highest concentration 10mM had less DNA than the lower concentrations. This data lead to the conclusion that if the nicotine concentrations increase then the amount of DNA damage will increase. The hypothesis is supported and accepted.
Statistical Analysis

A t-test was run to compare each experimental group with the control.

10 mM  $p = 0.0001$

0.1 mM  $p = 0.0015$

0.01 mM  $p = 0.0007$

In each case, the results were highly significant.

Further Investigations

To further investigate this experiment the DNA could be tested in Vivo. One could use a human cell line to investigate the response to nicotine in different concentrations. To examine the DNA damage one would use a gel electrophoresis. Another way to further investigate this experiment would be to draw blood from cigarette smokers and non-smokers. Then one would isolate the DNA and then compare the DNA damage.
Application

This experiment can be applied to daily life by informing cigarette smokers or tobacco users about the effects of nicotine on their DNA. It will warn them that nicotine could cause serious damage and harm. Cancer and possible genetic mutations may affect their children (next generation). Therefore, more people may stop smoking after learning the affects of nicotine. This experiment can also be applied to lowering the amount of smoking deaths each year. The current death toll is about 440,000 deaths each year.
Bibliography


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