Abstract

Hookah has recently been popularized in the United States as alternative to smoking tobacco using cigarettes. Since consuming hookah involves pulling smoke through water before inhaling it, there is a preconception that the potentially dangerous water-soluble tar components are trapped in the water, thus making the vapors safer. We performed a DNA nicking assay to test mutagenic properties of hookah smoke to compare them to cigarette smoke. The DNA nicking assay involves incubating various concentrations of hookah smoke solutions with bacterial DNA to measure the level of cuts in the sugar backbone of the bacterial DNA. The amount of DNA nicking by hookah solutions was orders of magnitude less than the nicking that has been detected with cigarette smoke. Additionally, UV spectroscopy revealed that some of the smoke components did remain trapped in the water. Though more research remains to be conducted, the results of this and similar studies could be used to explain differences in the rates of cancer and other tobacco-related diseases between countries or regions in which one delivery method is preferred or more prevalent/dominant than the other.

Keywords: hookah, cigarettes, mutagenic, smoking, DNA nicking

Introduction

The act of smoking tobacco has been performed by people of all cultures and backgrounds for about 2,000 years now (A Brief History of Smoking, 2013). There are many ways to smoke, be it through pipes, cigarettes, cigars, or bongs that are more modern, e-cigarettes, vaping pens, or hookahs. Hookahs are not exactly new, as they have been in use in the Middle East for the last 400 years, but the use of hookahs to smoke tobacco is an emerging trend here in the United States, especially among adolescents and young adults (Barnett and Livingston, 2017). Part of the reason for this could be that smoking tobacco using a hookah is seen as a safer alternative to smoking tobacco through a traditional cigarette. A hookah is a water pipe designed for the use of smoking tobacco in a different way than usual. With a hookah water pipe, the tobacco is heated via burning of charcoal that sits on top of the tobacco. This occurs in a bowl positioned at the top of the apparatus. Perforated aluminum foil separates them, and the charcoal and tobacco are never in direct contact. Then, the smoke released from the tobacco due to the heat from the charcoal is passed through the long shaft into the vase at the base of the hookah that contains water. The water cools the smoke before the smoker inhales it though a hose attached to the top of the base of the hookah. It was thought that passing the tobacco smoke through the water before inhaling it would eliminate the health hazards of tobacco, and this is one of the reasons hookah is believed to have been invented. This perception, however, is false (Hookah smoking and its risks 2018).

Many years of research and data accumulation has provided proof that regular exposure to first and secondhand cigarette smoke leads to a multitude of respiratory diseases and more than a dozen different types of cancer (U.S. Department of Health and Services 2014). Smokers also have an increased risk for strokes, blindness, amputations, and reproductive problems. The toxins in cigarette smoke cause damage through mechanisms such as DNA nicking and DNA methylation, which either change our genes or change how they are expressed, respectively (Lee and Pasouva, 2013). DNA nicking occurs when the integrity of the double stranded DNA is compromised, prompting our body’s natural repair mechanisms to try to fix the problem. However, the repair is not always successful, and can easily lead to mutations in our DNA, meaning that the instructions that code for the proper, functioning proteins in our bodies now contain errors. The mutations, consequently, lead to all the aforementioned diseases and health problems. DNA nicking has previously been confirmed to occur as a result of cigarette smoke (Stone et al., 1995). This study aims to both positively detect DNA nicking from hookah smoke and to quantitatively compare the levels of nicking between the two different tobacco delivery methods.
Methods

Smoke Sample Acquisition. Acquisition of hookah smoke residue was done in a controlled laboratory setting at California State University, Stanislaus. In a fume hood, I set up a traditional hookah apparatus and connected the end of the hose to a cellulose filter. I used small traditional charcoal briquettes to heat a common brand of mint-flavored hookah tobacco. Every thirty seconds, 30 mL of air was pulled through the hookah and then through the filter before being vented into the fume hood. This represents what a smoker inhales. Then, the smoke trappings on the cellulose filters were removed by soaking the filter in a small jar of water for 24 hours. Next, the filters were removed from the jar and the smoke components were dried down using a Centrivap vacuum, and then suspended in a small amount of water again (to get a more concentrated solution) to be tested for absorbance using a UV-Vis spectrophotometer. Additionally, the water in the hookah base after the smoke acquisition session was also tested for absorbance.

DNA Nicking Assay. A DNA nicking assay with PUC-18 (supercoiled DNA plasmid) was performed. A supercoiled plasmid, which is simply a plasmid twisted around itself multiple times, is incubated with an agent which is suspected to damage DNA. If DNA damage does occur, the plasmid will become much more flexible. This freedom to twist will allow the plasmid to uncoil into a more relaxed form. By using a supercoiled plasmid, even a single nick in the sugar backbone of the DNA will cause a significant change in the surface area of the plasmid that is available to interact with an agarose gel.

Various solutions were created for this assay. Each gel included two control solutions. The first control solution was DNA that was not incubated, and the second was DNA which was incubated only with TE buffer. PUC-18 was also incubated with glucosamine, which is known to nick DNA, to provide a standard solution. The experimental solutions were composed of DNA incubated with varying concentrations of the tar solutions. Once the solutions were created, they were incubated at 37 °C for four hours, before being loaded onto a 0.8% agarose gel. The electrophoresis took place over two hours at a potential of 80 volts and 0.04 amps of current. Then, using ImageJ software, the bands on the gel were digitized and related to concentration to determine the response to increasing concentration of the tar solutions.

Results

Hookah smoke solution samples obtained from the filters show absorbance at around 280 nanometers (Fig. 1), as did the aqueous solutions taken directly from the hookah base after the smoke acquisition session (Fig. 2).
smoking hookah is overwhelmingly less dangerous than
smoking cigarettes.

The positive detection of DNA nicking by hookah
smoke implies that regular smoking of tobacco, using a
hookah water pipe could lead to mutations in the smoker’s
DNA that might result in cancer and other diseases later in
life. For people who are classified as “light” cigarette
smokers, smoking under a pack per day, the risk of
developing malignant cancer is anywhere from double to
quadruple that of non-smokers (Schane et al., 2010). One
cigarette, on average, takes only 20 puffs of smoke to
finish, while the average hookah smoking session exposes
a person to about 200 puffs of smoke (Hookahs 2018).
Therefore, the average hookah session is roughly the
equivalent of smoking 10 cigarettes. Even though some of
the cancer- causing toxicants are trapped in the water of
the hookah vase, smoking tobacco though a hookah water
pipe can potentially increase a smoker’s risk of cancer by
similar magnitudes as smoking tobacco through cigarettes
does.

This could lead to a public health issue in our society,
especially if hookah continues to increase in popularity.
Surveys conducted in recent years across the US that gauge
high school and college students’ perceptions of cigarettes
versus hookah show that most people have more favorable
outlooks toward hookah than they do towards cigarettes.
For example, a survey done in 2015 assessed the cognitions
of a random sample of middle and high school students in
Florida. The study found that these adolescents had far
more positive cognitions towards hookah use that cigarette
use. About 87% of them answered that they would smoke
hookah if their best friend offered them some, while only
about 40% would do the same if offered a cigarette. In
addition, about twice as many answered that hookah does
make young people look cool than answered that smoking
cigarettes does. Additionally, about 68% thought that
quitting hookah would be easy, while only about 32%
thought that quitting cigarettes would be easy (Barnett
and Livingston, 2017).

In the end, the individual behavior of the smoker
determines to what extent the effects of this habit will have
on his or her health. If a cigarette smoker goes through a
pack a day, he or she will be inhaling more toxins than a
hookah smoker who has one hookah session every
weekend. Still, research that aims to quantify and qualify
the health risks associated with the inhalation of tobacco
smoke through the many different smoking apparatuses
available in our society today is as important and valid as
ever. Furthermore, once concrete knowledge is obtained, it
is necessary to find effective and efficient ways to
disseminate this knowledge throughout the general public,
so that they can make informed decisions about the act of
smoking tobacco.
Future Work

Potential next steps to take further include characterization of the chemical components of hookah by mass spectrometry, as well as exploring methods to detect radicals in the hookah smoke.

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References