

Dying for a Smoke: The Apoptotic Effects of Cigarette Smoke on the Spleen

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Each year, nearly 1 in 5 Americans will die from it¹; similarly, more than 90% of the debilitating lung cancer cases are direct result of it². What could be the causal agent behind these gruesome statistics? None other than cigarette smoke itself! It is well known that smoking cigarettes damages many organs of the body; it causes a plethora of pathological disorders and lowers the general well-being of smokers. Throughout scientific literature, cigarette smoke is known to be responsible for a multitude of diseases, including several types of cancer, cardiovascular disease, and respiratory disease.

Currently, it is presumed that a modulation in apoptosis could be one of the factors responsible for the disease states commonly associated with cigarette smoke. Apoptosis is commonly known as programmed cell death. It refers to the morphological features of programmed cell death, which is characterized by cell shrinkage, nuclear condensation, membrane blebbing, fragmentation into membrane bound apoptotic bodies, and membrane changes that eventually lead to phagocytosis of the affected cell.³ However, it is important to realize that apoptosis should not be given a negative connotation; in fact, the cells in our bodies need to undergo apoptosis in order to

maintain homeostasis. Billions of cells die every day! For instance, red blood cells experience a rate of cell death in the order of about 250×10^9 /day⁴. Similarly, during development apoptosis helps sculpt parts of the body; it is through apoptosis we have fingers instead of webbed hands! However, when the balance of cell death and cell growth is disturbed, serious consequences can be seen. This is where cigarette smoke comes into play; by modulating the processes of apoptosis, it can disrupt the body's normal balance, thereby paving the way to disease.

A study conducted on the apoptotic effects of cigarette smoke on lung tissue helps to illustrate the dire consequences of its use on the body's homeostasis. In this study, which evaluated bronchiolar and alveolar epithelium, it was determined that the control animals revealed an apoptosis index of only 5.4% whereas the index was raised to 84% in treated animals⁵. Therefore, the exposed animals experienced higher rates of cell death than the control animals. However, some researchers describe cigarette smoke to have a pro-apoptotic action (meaning it causes apoptosis) whereas others state it has anti-apoptotic effects (meaning it does not induce apoptosis, but rather prevents it from happening). After a thorough literature research, I have realized that perhaps the modulation of apoptosis by cigarette smoke could be organ dependent; meaning it could induce apoptosis in one organ but suppress it in another.

¹ Centers for Disease Control and Prevention., (2006) Health Effects of Cigarette Smoking, National Center for Chronic Disease Prevention and Health Promotion. Available from: http://www.cdc.gov/tobacco/data_statistics/Factsheets/health_effects.htm

² Albermann, K., Lang, S., Zeidler, R., (2007) Nicotine and apoptosis, *Apoptosis* 12: 1927-1943

³ Denton, M., Dong Z., Mikhailov, V., et al (1999) Apoptosis: Definition, Mechanisms, and Relevance to Disease, *Am J Med* 107: 489-506 This journal article is a good source in regards to the background of apoptosis, which a person from any field may benefit from reading.

⁴ Lockshin, R., Zakeri, Z. (2007) Cell death in health and disease, *Journal of Cellular and Molecular Medicine* (Online Accepted Articles).

⁵ Albermann, K., Lang, S., Zeidler, R., (2007) Nicotine and apoptosis, *Apoptosis* 12: 1927-1943

Similarly, my literature review has led me to conclude that, although there is a plethora of studies examining the effects of cigarette smoke on lung tissue, there are not enough which examine the effects of cigarette smoke on the thymus or spleen, both important organs to the immune response. The immune response is important against fighting infections and helping to maintain the body's homeostasis. Therefore in my research project, I hope to test the effects of cigarette smoke on splenic tissue and examine its impact on important immune cells (B and T lymphocytes). Past studies have found that cigarette smoke has led to higher frequencies of DNA strand breaks in peripheral lymphocytes⁶. However, instead of examining peripheral lymphocytes I hope look at the cells of the spleen, which is an organ that contains both T and B lymphocytes. Due to the fact that cigarette smoke can cause unmanageable stress to the cell (perhaps in the form of DNA damage), which leads to apoptosis, I hypothesize that there will be a statistically significant increase in apoptosis of the cells of the spleen.

In this experiment, twenty Swiss Webster mice were exposed to the smoke of a standard research cigarette, from the University of Kentucky, once a day, fifteen puffs per cigarette each day for duration of nine months. To ensure for comparable data, an equal number of mice were used as controls. At the end of the smoking period, the mice were weighed and sacrificed. Important organs such as the brain, uterus, lung, liver, thymus, thyroid, and spleen from both the experimental and control mice were collected. The tissues from these organs were embedded and sectioned at four micrometers in thickness.

⁶ Lai, Z., Ng S., Silverstone, A., (2006) Effects of Prenatal Exposure to Cigarette Smoke on Offspring Tumor Susceptibility and Associated Immune Mechanisms, *Toxicological Sciences* 89: 135-144

Slides of the lung, uterine, and spleen from cigarette smoke-exposed and non-exposed mice were TUNEL stained. Although the other organs were stained as well, the organ in focus for my research project is the spleen, due to its direct immunological impact. Therefore the data that I collect will come from the control and experimental splenic tissue that is TUNEL stained. TUNEL stain (Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling) is the most commonly used method of detecting apoptosis in histological sections.⁷ Basically, one of the key features of apoptosis is the degradation of DNA, which results in strand breaks within the DNA. These strand breaks are important in the TUNEL process, for apoptotic cells are identified using terminal deoxynucleotidyl transferase to add biotin-dUTP to the strand breaks⁸. After the addition of biotin-dUTP, further reactions may be initiated, which will ultimately allow for the strand breaks to appear dark brown. This allows for easier identification with light microscopy.

These TUNEL stained splenic tissue slides will be observed under a compound light microscope at 400X. Pictures of the images seen will be recorded using a Nikon digital camera and scored for apoptosis. When scoring for apoptosis, I will have to create a randomized sampling method. To ensure this occurs, I will use a grid of 2.5 cm x 2.5 cm and place it at random spots on the image; 5 random spots will be counted at the outside border of the image of the splenic tissue and 5 random spots will be counted in the middle. TUNEL staining allows for easier identification of the apoptotic cells, because the cells that are dying will have stained

⁷ Gavrieli, Y., Sherman, Y., Shmuel A., (1992) Identification of Programmed Cell Death In Situ via Specific Labeling of Nuclear DNA Fragmentation, *The Journal of Cell Biology* 119: 493-501

⁸ Gavrieli, Y., Sherman, Y., Shmuel A., (1992) Identification of Programmed Cell Death In Situ via Specific Labeling of Nuclear DNA Fragmentation, *The Journal of Cell Biology* 119: 493-501

brown nuclei. However, apoptosis is not the only situation in which sufficient DNA strand breaks may occur; necrosis (an unordered and accidental form of cell death)⁹ will also label positively. To solve this problem, I plan to do a morphological analysis of the section to be counted in conjunction with TUNEL staining. Therefore, if a cell is TUNEL positive, I will look to see if it displays these following characteristics of apoptosis: nuclear blebbing, cell shrinkage, cytoplasmic blebbing, nuclear condensation, membrane bound fragments, and detachment from surrounding cells. Necrosis does not display those characteristics. Therefore, if a cell stains TUNEL positive and it can be categorized under the morphology of apoptosis, I can be assured of the validity of the results. For this reason, I will utilize a biochemical and morphological analysis of the data.

More deaths are caused each year by tobacco use than by all deaths from human immunodeficiency virus (HIV), illegal drug use, alcohol use, motor vehicle injuries, suicides, and murders combined¹⁰. As it is shown, the health risks associated with cigarette smoke can impact a person's well-being and ultimately, their livelihood. Therefore, it is of the utmost importance to research the issues associated with cigarette smoke, and to elucidate the causes and links behind its disease association. I hope by exploring the effects of cigarette smoke on the spleen, I can find more information about the immunological impacts that are a direct result of smoking.

⁹ Kanduc, D., Mittelman, A., Serpico R., (2002) Cell death: Apoptosis versus necrosis, *International Journal of Oncology* 21: 165-170

¹⁰ Centers for Disease Control and Prevention., (2006) Health Effects of Cigarette Smoking, National Center for Chronic Disease Prevention and Health Promotion. Available from: http://www.cdc.gov/tobacco/data_statistics/Factsheets/health_effects