What is the mechanism of 2,4-D?

Abstract

In 2000, Schreinemachers published a paper showing an increased risk for certain types of cancers such as reticulum cell sarcoma, prostate, and pancreatic cancers in areas with high usage of the herbicide 2,4-Dichlorophenoxyacetic acid (2,4-D). However, she did not suggest a mechanism of action. Based on data from traditional mutagenesis and carcinogenesis experiments, 2,4-D is not considered mutagenic or weakly mutagenic and its carcinogenic potential is unclear. The purpose of this research project is to elucidate the mechanism by which the herbicide 2,4-D is involved in carcinogenesis. We hypothesize that epigenetic changes may be responsible. Klein *et al* developed a method to study epigenetic changes due to chemical exposure. It uses Chinese hamster ovary cells as a model. Chinese hamster ovary cells will be exposed to 2,4-D and the growth rate and mutation rate will be determined. Subsequently polymerase chain reaction (PCR) will be used to determine the specific epigenetic changes in the cells.

Process/Methods

The herbicide 2,4-Dichlorophenoxyacetic acid (2,4-D) is used in wheat production. It prevents the growth of broadleaf plants and therefore reduces weed growth in wheat fields increasing yields. In addition, 2,4-D is also used as a household herbicide for killing weeds in laws and gardens, in particular dandelions. 2,4-D acts as plant hormone causing uncontrolled and disorganized plant growth causing it to die (The Nature Conservancy, 2001). However, the exact mode of action is not known. In 2014, Song published a paper shedding light on the mode of action of 2,4-D. She explained that the herbicide works to mimic auxin at the molecular level inducing abnormal growth by inhibiting specific target sites inside the biochemical/physiological pathways of the plant, resulting in the catastrophic and lethal consequences. She suggests that the auxin receptors, transport carriers, and transcription factors are the key pieces to find the mode of action of 2,4-D.

In 2000 and 2003 Schreinemachers published two papers studying the association of the use of 2,4-D in agriculture and the occurrence of cancers and birth defects on the general population. Schreinemachers collected data on the occurrence of birth defects and cancers and used wheat acreage as a measure for 2,4-D exposure in counties in North Dakota, Minnesota, Montana, and South Dakota. The data showed an increased risk for birth defects such as lung malformation and abnormal formation of fingers and toes and certain types of cancers particularly prostate and pancreatic cancers (Schreinemachers 2000 & 2003). Schreinemachers found that increased wheat acreage correlated with a high risk of experiencing the side effects of the herbicide (Schreinemachers 2000, 2003).

In general, 2,4-D is considered a General Use Pesticide (GUP) in the U.S. It is classified as slightly toxic (Class III) by oral exposure and highly toxic (Class I) by eye exposure. Acute toxicity is minimal considering LD50 ranging from 666 mg/kg/day all the way to 16.3 g/kg/day (Exotoxnet PIP, 2,4-D, 1996). The side effects of the herbicide were problems such as, loss of bladder control, incoordination, and weak reflexes (Exotoxnet PIP, 2,4-D, 1996). Available data suggests adverse affect on reproduction and development only occurs at high dosage and therefore no adverse effects on reproduction and development in humans is expected at most commonly used exposure levels (Exotoxnet PIP, 2,4-D, 1996). According to several studies summarized by the extension toxicology network, the herbicide is not mutagenic because 2,4-D has not damaged any DNA in human lung cells. Therefore, 2,4-D is classified as a weak mutagen. Its potential as a carcinogenic compound is unclear from the available data because of conflicting results.

We are interested in finding the mechanism by which 2,4-D causes cancer and birth defects. Data shows that it is most likely not mutations (Exotoxnet PIP, 2,4-D, 1996). Therefore, we hypothesize that an epigenetics mechanism may be involved. Epigenetics is the changes within an organism by modification of gene expression, without changes within an organism's genetic code. If the genetic code was altered it would be classified as mutagenic. To elucidate the mechanism, we will use a method developed by Catherine Klein at NYU. She studied the effects of arsenic. Like 2,4-D, arsenic is known to cause tumors, but like 2,4-D is also a weak mutagen in traditional mutagenic testing. Klein *et al* used modified Chinese hamster ovary (CHO) cells

called G12 to study epigenetics changes caused by arsenic compounds. The toxicology lab at VCSU was able to obtain the G12 CHO cells. I will use these cells and the same methodology as described in the paper by Klein to see if 2,4-D is able to modify the epigenome. It would be extremely interesting to find the mechanism especially because of the extensive usage of 2,4-D throughout North Dakota.

Timeline/Benchmarks

This project will take place over the course of a year. I will start the project in the fall semester of 2016 and conclude the project in the end of spring semester of 2017. During the fall semester I will performing the mutagenesis assays. Each essay will take about 3-4 weeks (fall semester). I will repeat the experiments three times to be able to perform statistical analysis. In the spring semester I hope to isolate and screen the independent 2,4-D induced mutants (first 8 weeks spring semester). Subsequently, I will screen the mutants for methylation status (second 8 weeks spring semester)

Budget

6-Thioguanine (6TG): $35.20/100 mg

Hypoxanthine Aminoprotein Thymidine (HAT) Supplement: $36.99/100 ml bottle

60 mm culture dishes: $114.50 /600 dishes

90 mm culture dishes: $124.10 /300 dishes

F12 medium: $62.80/6 bottles

5% Fetal Bovine Serum: $622.20/500 ml. For 5 liters of medium

Any material costs above the provided amount of $250 will be covered by the Science Department.

Dissemination

I will present the findings of this research project during the Valley City State University scholarship symposium in spring of 2017. I will also be presenting at the Biomedical research conference at Minnesota State University in Moorhead in 2017. If funds are available, I would like to present at the annual meeting of Environmental Mutagenesis and Genomics Society in September of 2017.

Educational Objectives

The objective of this project is for me to grow as a student and enhance my experimental and research skills. This project will allow me to become more advanced in research and analysis and become familiar with advanced lab tools and techniques. I plan on attending PA school to become a Physician Assistant, and this project will provide me with the required research and lab skills necessary for admission into the program. I am interested in learning the process of finding the mechanism that is causing the deformities within the cells. I know it's not as easy as just looking at different cells, it has to do with trial and error, adding different chemicals and different amounts of those chemicals to find that mechanism. I am excited to be part of elucidating the mechanism by 2,4-D affect cells and possibly cause cancers in humans

Integrative Nature of Work

This project integrates my knowledge of chemistry and biology, which are both extremely important subjects for me to understand as it is part of the knowledge I need to become a Physician Assistant. Diseases are a malfunction of cellular processes and can be caused by chemical and biological phenomena. Therefore, a thorough understanding of the function of the cells from a biological and chemical perspective is essential. This project will allow me to further expand my knowledge of organic chemistry, biology, and cells and integrating my knowledge. I will be able to expand on my knowledge of the technology of the tools utilized in the lab. Most of all, this project will allow me to learn the ins and outs of research. It will teach me the proper way to do research and analyze my findings in order to present them in a way that is easily understood. It will teach me the research methods that I wouldn't necessarily learn in the classroom. I know I will be using the research skills in my future career. This project will also provide data for further research and for the next generation of students to continue the research.

References

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