The Effects of Metolachlor Exposure in THP-1 Alveolar and Monocyte and Macrophage Cellular Functions

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Effects of Metolachlor Exposure on THP-1 Alveolar Monocyte and Macrophage Cellular Functions

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Introduction

• Analysis of Metolachlor
  ▫ History
  ▫ Chemistry
  ▫ Application
  ▫ Environmental Fates and Concerns
• Experimental Design (Objective I, II & III)
  ▫ Procedure
  ▫ Expectations
• Acknowledgments
Metolachlor: History

• Pre-Emergent Broad Spectrum Herbicide (1976)
  ▫ Used to control broadleaf plants and weeds
  ▫ Primarily agricultural or feed crops
    • Corn, Soybeans and Sorghum
  ▫ Alternatively
    • Ornamentals, trees, shrubs, cotton, peanuts, etc.
Metolachlor: Chemistry

• Chemical Activity
  ▫ Inhibits long-chain fatty acid synthesis
  ▫ Unintended consequences uncertain

• Character
  ▫ Primarily an odorless, clear to amber colored liquid
  ▫ Can be found in granular forms
  ▫ 21 known degradates (3)
Metolachlor: Chemistry

• Aliases
  ▫ Trade Names
    • Bicep®, CGA-24705®, Dual®, Pennant® and Pimagram®
  ▫ Alone or Herbicidal Cocktail (3)
    • Combined with atrazine, cyanazine and fluometuron
Metolachlor: Application

- Historically
  - 60 million lbs. in U.S. annually (2)
- Recommended Application
  - Ground application
  - Aerial, irrigation and chemigation
- Prohibited Applications
  - Greenhouses and enclosed areas
  - Peaty, sandy or loamy soils
  - Fruit bearing trees or vines
  - Grazing areas
Metolachlor: Environmental Fates

- **EPA Classifications**
  - **Soil**
    - Persistent to Moderately Persistent
    - Mobile to Highly Mobile
  - **Ground Water**
    - Primary source of exposure
    - Considerable contamination to ground water – found in over 20 states
      - 0.08 – 850 ppb found in various water sources (0.078-849.03 μg/L)
  - **Air Contamination**
    - Volatilized – Ontario watershed findings ~5ng/L
    - Dust Contamination - ~50% of 39 homes in Iowa study had measurable levels
      - Improper PPE pre and post application
Metolachlor: Health Concerns

- **Toxicity (EPA)**
  - Generally low level of toxicity in acute tests
  - Toxicity Category III (oral and inhalation routes)
  - Toxicity Category IV (eyes or skin)
- **Animal testing - High levels of exposure**
  - Dogs - Low birth and body weight
  - Rabbits- Increased liver and kidney size
  - Rats - Carcinogenic- liver nodules and carcinomas in females
- **Humans (New Jersey) - Correlation**
  - Low birth weights (2010)
Metolachlor: Health Concerns

• Symptoms of Metolachlor Poisoning
  ▫ High levels of exposure
    • Eye or skin irritation, cramps, shortness of breath, weakness, sweating and diarrhea
  ▫ Prolonged exposure
    • Anemia, hypoxemia, convulsions and jaundice
Experimental Questions

• Effects on human alveolar leukocytes
  ▫ Inhibition of normal cellular function
  ▫ Provocation of an erratic function

• Specific Function
  ▫ How will metolachlor effect cells?
    • Phagocytosis?
    • Apoptosis?
    • Necrosis?
Experimental Outline

• Objective I: Monocyte/Macrophage Phagocytosis Assay
  ▫ Measuring the effects of Metolachlor on human alveolar monocytes and macrophages via flow cytometry
• Objective II: Apoptosis Assay
  ▫ Measuring possible effects of Metolachlor on the apoptotic pathway of monocytes and macrophages
• Objective III: Reactive Oxygen Species (ROS) Assay
• (Will not be discussed)
Objective I: Phagocytosis Basics

- THP-1 Cells
  - Human monocytic lineage
  - Derived from a 1 year old human male with acute leukemia patient
  - Immunohistochemistry
Objective I: Phagocytosis Basics

- Monocytes
  - Develop in bone marrow and migrate to various body tissues
  - Alveolar Monocyte
    - Immune Defense
      - 1st line of contact (sentinel cells)
    - Phagocytosis
    - Inflammation
Objective I: Phagocytosis Basics

- Macrophages
  - Mature monocytes
  - Phagocytosis
  - Cytokine secretion
  - Migration – lymph nodes (acquired immunity)
Objective I: Phagocytosis Tools

- **LPS**
  - Lipopolysaccharide
    - Activates cellular function
- **PMA**
  - Phorbal Myristate Acetate
    - cellular activation (differentiation)
- **FITC labeled latex beads**
  - Fluorescein isothiocyanate tags and Rabbit IgG
Objective I: Phagocytosis Design

- Cells maintained in media @ 37° C
- Control Group vs LPS Group (~1 x 10^6)
  - Metolachlor exposures at 50ppb, 100ppb, 500ppb and 1,000ppb along with a positive and negative control
  - Three time trials
    - 24, 48 and 72 hours
- Differentiated trials
  - FITC labeled beads
    - Added 24 hours from completion of trial
    - Cells subjected to assay treatment
    - Cells washed and suspended in assay buffer
Objective I: Flow Cytometry

- Cells subjected to flow cytometer
  - Cells are funneled through one at a time
  - Laser passed through cell
    - Fluorescent tag
  - Forward and side scattered light
  - Recorded data
  - Allows the determination of FITC presence
    - Presence indicates the cells with normal fxn
    - Standard level comparison
Objective II: Apoptosis Basics

● Apoptosis is the process of highly regulated cellular death
  ▫ ~ 50-70 billion cells die everyday in an adult human
  ▫ (of ~ 37.2 trillion)
  ▫ Apoptosis promotes normal development
    • Homeostasis
    • Counterpoint to cell proliferation
    • Can remove any unwanted or damaged cells

● Necrosis is the process of premature cell death
  ▫ Caused by outside factors
    • Severe damage, toxins, infections
    • Inflammatory response that can block phagocytic fxn
    • Can damage surrounding tissues
Objective II: Apoptosis Basics

• Phosphatidylserine
  ▫ An important phospholipid found in cells
  ▫ Oriented towards the cytosolic side of cellular membrane

• Initiation of apoptosis
  ▫ PS is acted upon by flippase
  ▫ Reverse orientation and signal macrophages
Objective II: Apoptosis Basics

- Apoptosis assay
  - Exploitation of the presence phosphatidylserine
  - PS binds to Annexin-V stain
    • Annexin-V is conjugated with a fluorochrome
  - Propidium Iodide
    • Reacts with nucleic acid
    • Leaky cell membranes
      • Necrosis or late apoptosis
- Florescence is detectable via flow cytometry
Objective II: Apoptosis Design

- Cells maintained in media in incubator 37° C
- Control Group vs LPS Group (\(\sim 1 \times 10^6\))
  - Metolachlor exposures at 50ppb, 100ppb, 500ppb and 1,000ppb along with a positive and negative control
  - Three time trials
    - 24, 48 and 72 hours
  - Annexin-V exposure (PI)
    - Cells incubated for 15 minutes in darkness, 37° C
    - Cells washed, centrifuged and suspended
    - Subjected to flow cytometer for quantitation

http://images.1233.tw/annexin-v-pi-stain/
Measures of Significance

• Data Sets
  ▫ 6 experiments per trial
    • 3 replicates
  ▫ Average Experimental Mean
    • Normalized due to cell/cell variation
    • Comparison using ANOVA
    • $\alpha=0.05$
    • P-value and significance
    • Post-hoc analysis (Tukey)
Expected Results

- It is likely that Metolachlor will affect function in these cells
  - Specifically, I think that it will impair normal function
  - Higher levels of exposure
    - Phagocytosis
    - Apoptosis - uncertain

- Previous studies, readings and initial experimental results
Accolades Thus Far

- Dr. David McClenahan
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- Dr. Darrell Wiens
- Yutao (Max) Su
Citations

6. [www.atcc.org](http://www.atcc.org) THP-1 monocytic cell line