Beneficial Effects of a Non-Steroidal Anti-Inflammatory Drug (NSAID) on Chemotherapy-Induced Behavioral Changes

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Hypothesis:

Naproxen, a nonsteroidal anti-inflammatory drug (NSAID), mitigates the behavioral changes produced by breast cancer chemotherapy agent, cyclophosphamide.
Among women, breast cancer is the most frequent cancer diagnosis— affecting 2.1 million women each year.

Median age of diagnosis = 62 years → post-menopausal females.

U.S. women have a 1-in-8 lifetime risk of being diagnosed with breast cancer. 

↑ risk due to:

- Increased life expectancy
- Menopausal hormone use
- Rising prevalence of obesity
- Improved detection through mammography screening
Background: Chemotherapy

- Chemotherapy agents are used to limit the spread and growth of cancerous cells
- **Cyclophosphamide** is a mainstay chemotherapy agent in the treatment of breast cancer
  - Targets proliferating cells
  - Alkylating agent → interferes with DNA cross-linking → cell death
  - Crosses blood-brain-barrier (BBB)
Background: Depression

- Chemo Brain” symptoms:
  - Depression
  - Anxiety
  - Difficulty with memory
  - Changes in attention

- Depression is commonly reported by breast cancer patients undergoing chemotherapy treatment
  - A cause of significant mortality in these patients
  - Impacts patient’s quality of life, adherence to treatment plans and survival
Increased peripheral inflammatory markers seen in breast cancer patients up to 6 months after the completion of chemotherapy

- 30% persist beyond 6 months

Inflammation in the CNS may contribute to:
- Mood alterations (i.e. Depression & anxiety)
- Behavioral changes
- Fatigue

Anti-inflammatory treatment may be beneficial in reducing these symptoms

- **Naproxen** is a nonsteroidal anti-inflammatory drug (NSAID)
  - Non-selective cyclooxygenase (COX) inhibitor
  - Crosses the blood-brain barrier
  - Oral administration
Experimental Design:

- Female Mice
  - C57 Bl/6 strain
  - Charles River Laboratories
  - 6-8 weeks of age upon arrival
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  - Intact or Ovariectomized (OVX)
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- Experimental variables:
  - Intact or Ovariectomized (OVX)
  - Saline or Cyclophosphamide (CP)
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- C57 Bl/6 strain
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Experimental variables:
- Intact or Ovariectomized (OVX)
- Saline or Cyclophosphamide (CP)
- Control Diet or Naproxen Diet
**Experimental Design:**

- **Phase 1:** Drug & Diet Administration
  1. Control Diet or Naproxen Diet
  2. Cyclophosphamide (CP) or Saline Injections (IP)

- **Phase 2:** Behavioral Assays
  - Activity: Locomotor Chamber
  - Depression: Tail-Suspension Test (TST)
  - Anxiety: Elevated Zero Maze (EZM)

- **Phase 3:** Analysis of Inflammatory Markers
  1. Sacrifice
  2. Plasma Collection
  3. Brain Tissue Collection
  4. Immunohistochemistry
  5. ELISA assay
**Phase 1: Diet + Administration**

- **NSAID: Naproxen**
  - Administration of Naproxen Diet (375 mg) or Control Diet

- **Chemotherapy agent: Cyclophosphamide (CP)**
  - Administration of CP (100 mg/kg i.p.) or saline
  - 5 doses over a 2-week period: one dose every 3rd day

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### Timeline

**Drug Admin. (Saline or CP)**

1, 4, 7, 10, 13, 16, 17, 18

**Behavioral Tests**

13, 16, 17

**Tissue Collection**

18
Phase 2: Behavioral Assays

- Locomotor Activity: Chamber
- 90 minute trial
- Infrared beam-sensor monitored the number of movements
- **Analysis:**
  - General Activity
Cyclophosphamide (CP) treated mice on the control diet had significantly reduced locomotor activity than the mice given saline and on control diet.

Naproxen diet “normalized” the locomotor activity.
Phase 2: Behavioral Assays

Elevated Zero Maze (EZM) → Anxiety-like Behavior

- 5 minute trial
- **Analysis:**
  - Time spent in closed arms
  - Arm entries
  - Protected head dips
- Increased time spent in the closed arms and decreased number of head dips may indicate an anxiety-like behavior phenotype
Results: Elevated Zero Maze

- Cyclophosphamide (CP) and naproxen did not influence arm entries
Results: Anxiety-like Behavior

- CP treated mice on the control diet made significantly fewer head dips compared to saline control mice on control diet.
- Naproxen diet “normalized” or “rescued” this behavior.
- Cyclophosphamide (CP) and naproxen did not influence arm entries.
Results: Anxiety-like Behavior

- CP treated mice on the control diet made significantly fewer head dips compared to saline control mice on control diet.
- Naproxen diet “normalized” or “rescued” this behavior.
Phase 2: Behavioral Assays

- Tail-Suspension Test (TST) → Depression-like Behavior
- 6 minute trial
- **Analysis:**
  - Total Time Immobile
  - Latency to 1st Immobility

- Increased time spent immobile may indicate learned helplessness – surrogate measure for depression-like phenotype
- Learned helplessness is a behavior in response to an uncontrollable and aversive stress
Results: Depression-like Behavior
Cyclophosphamide (CP) treated mice on the control diet showed a trend toward shorter latency to first immobility compared to the mice given saline and on control diet, indicating that they “gave up” and became helpless over a shorter interval.

CP treated mice on the naproxen diet did not show this trend. Instead these mice showed a trend toward longer latency to first immobility, indicating that they took longer before “giving up”
Phase 3: Inflammatory Analysis

- **Sacrifice**
  - Isoflurane Anaesthesia
  - Rapid Decapitation
- **Trunk Blood Collection**
- **Hippocampi Dissection**
- **Inflammatory Marker Analysis**
  - Analyze cytokine content (ELISA)
    - IL-1, IL-6, IL-10, TNF-α, NF-kB
  - Immunohistochemistry
Results: Inflammatory Markers
Conclusion:

- Cyclophosphamide produced significant effects in EZM and Locomotor activity test
  - CP mice on control diet made a fewer head dips indicating anxiety-like behavior. (EZM) and displayed reduced locomotor activity than the mice given saline and on control diet
- Naproxen restored baseline behaviors in EZM and Locomotor Activity tests in CP-treated mice
- CP mice on control diet had a shorter latency to 1st immobility while CP treated mice on naproxen diet displayed a longer latency to 1st immobility.
  - The trend indicates that naproxen restored or even may have improved performance in the tail suspension test in CP-treated mice
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References: